Donor-reactive cytokine profiles after HLA-identical living-related kidney transplantation

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Abstract

Background. After HLA-identical living-related (LR) kidney transplantation, only non-HLA antigen mismatches between donor and recipient may exist. We questioned whether donor-reactive responses against non-HLA antigens could be found after HLA-identical LR kidney transplantation, and wondered whether donor reactivity in the HLA-identical setting was different from the HLA-mismatched setting during immunological quiescence. Healthy individuals served as controls.

Methods. Elispot assays were performed to determine the number of alloreactive IFN-γ-producing cells (pc), IL-10 pc, granzyme B (GrB) pc and IL-13 pc from peripheral blood mononuclear cells (PBMC) of HLA-identical, HLA-mismatched LR kidney transplant recipients and healthy individuals.

Results. The frequency of alloreactive IFN-γ pc, IL-13 pc and GrB pc was higher in healthy individuals compared to both transplant patient groups. In the HLA-identical group, significantly higher numbers of donor-reactive IL-10 pc were found compared to their autologous control. These frequencies were also higher compared to the HLA-mismatched and healthy control group. The number of donor-reactive GrB pc was higher in the HLA-mismatched group than in the HLA-identical group. Donor-reactive IFN-γ pc and IL-13 pc were comparable in both transplant groups.

Conclusions. In recipients of HLA-identical LR kidney transplant, high donor-reactive IL-10-producing cells (pc) in PBMC from recipients of HLA-identical living-related kidney transplant, in combination with low donor-reactive IFN-γ pc, IL-13 pc and GrB pc, were found. This may reflect active downregulation of reactivity against non-HLA molecules.

Keywords: alloreactivity; Elispot assay; IFN-γ; IL-10; kidney transplantation; minor histocompatibility antigens; non-HLA antigens

Summary

In the present study, high donor-reactive IL-10-producing cells (pc) in PBMC from recipients of HLA-identical living-related kidney transplant, in combination with low donor-reactive IFN-γ pc, IL-13 pc and GrB pc, were found. This may reflect active downregulation of reactivity against non-HLA molecules.

Introduction

After HLA-identical LR kidney transplantation, all major HLA molecules are identical (HLA-A, B, C, DR and DQ) with the donor, and mismatches may exist only in non-HLA antigens or minor histocompatibility antigens (mHAgs). Minor HAggs are HLA-restricted peptides derived from cellular proteins that differ in amino acid sequences between donor and recipient due to genetic polymorphisms [1–3]. After HLA-identical allogeneic bone marrow transplantation (BMT), immune responses are caused by mHAg mismatches between donor and recipient [3]. Theoretically, after HLA-identical LR kidney transplantation, both mismatches in mHAgs [4] and mismatches in other non-HLA antigens [5] may induce rejection of the kidney transplant.

Cytokines are important mediators in regulating lymphocyte activation, proliferation, differentiation and survival [6]. Pro-inflammatory cytokines, such as interleukin (IL)-2, interferon (IFN)-γ and tumour necrosis factor (TNF)-α, have been postulated to promote allograft rejection. In contrast, anti-inflammatory cytokines, such as IL-4, IL-5, IL-10 and IL-13, have been associated with downregulation of the immune response [7–9]. Some studies also reported that anti-inflammatory cytokines, such as IL-4, were present during rejection after clinical heart transplantation [10,11].

Recently, studies reported a beneficial role of IL-10 after BMT and HLA-mismatched kidney transplantation [12–15]. After BMT with HLA-identical sibling donors, donor-stimulated peripheral blood mononuclear cells (PBMC) showed significantly higher numbers of IL-10-producing cells (pc) in Elispot assays and higher levels of IL-10 mRNA expression in the absence of graft-versus-host
Cytokine profiles after HLA-identical living-related kidney transplantation 2017

Table 1 (A). Characteristics of HLA-identical living-related kidney transplant recipients

<table>
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<tr>
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<th>Gender</th>
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<th>Don</th>
<th>Age (years)</th>
<th>Time after KTx (years)</th>
<th>PRA (%)</th>
<th>CsA (mg/day)</th>
<th>Tacro (mg/day)</th>
<th>MMF (mg/day)</th>
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</table>

aHLA-identical LR kidney transplantation, identical at the HLA-A, B, C, DR, and DQ loci; bID, identification number; cPat, patient; dDon, donor; eKTx, kidney transplantation; f PRA, panel-reactive antibodies; gCsA, cyclosporine A; hTacro, tacrolimus; iMMF, mycophenolate mofetil; jAZA, azathioprine; kPred, prednisone.

Disease (GVHD) compared to samples taken during GVHD [13,14]. After HLA-mismatched kidney transplantation, higher numbers of donor-reactive IL-10 pc in PBMC were found by Elispot assays in the absence of rejection compared to during acute rejection [15]. In addition, high levels of IL-10 determined in supernatants from unstimulated PBMC by enzyme-linked immunosorbent assay (ELISA) were associated with stable graft function [12].

Cytotoxic T-lymphocytes (CTL) play a crucial role in allograft rejection [16,17]. CTLs are capable of inducing apoptotic cell death via the death receptor pathway, such as FAS-FAS ligand, or via the exocytosis pathway, which involves perforin and granzyme B (GrB). The number of CTLs responding to donor antigens can be measured by determining the CTL precursor frequency (CTLpf) in limiting dilution assays [18]. After HLA-identical LR kidney transplantation, no donor-reactive responses can be measured by CTLpf. An alternative for cytotoxicity is to determine the activity of CTL by using the IFN-γ or GrB Elispot assay [19–21].

In the present study, we questioned whether we could detect donor reactivity against non-major HLA molecules after HLA-identical LR kidney transplantation. Autologous responses were used as negative controls. Furthermore, we wondered whether the donor-reactive response in the HLA-identical setting was different from the HLA-mismatched setting during immunological quiescence.

Subjects and methods

Kidney transplant recipients and healthy individuals

Both the transplant recipients and kidney donors visited our out clinic regularly to control their renal function. After informed consent, 35-ml heparinized blood was taken from both recipients and their donors of HLA-identical (n = 13) and HLA-mismatched (n = 12) LR kidney transplants, and from healthy individuals (n = 10: n = 5 male and n = 5 female blood donors). The characteristics of HLA-identical and HLA-mismatched LR kidney transplant recipients are described in Table 1A and B, respectively. From the HLA-identical recipients, one patient received his second graft and two patients their third graft (Table 1A). In the HLA-mismatched group, two patients received their second graft. After HLA-identical LR kidney transplantation, none of the patients experienced an acute rejection episode. In the HLA-mismatched group, only one patient developed an acute rejection period within 1 week after transplantation (Table 1B).

Elispot assay: IFN-γ, IL-10, IL-13 and GrB

PBMC from transplant recipients, their kidney donors and healthy individuals were isolated from heparinized blood by density gradient centrifugation using Ficoll–Paque (density 1.0777 g/ml; Amersham Biosciences, Uppsala, Sweden). PBMC were collected from the interphase, washed twice with RPMI-1640-DM (Cambrex, Verviers, Belgium) supplemented with 100 IU/ml of penicillin (Cambrex) and 100 µg/ml of streptomycin (Cambrex). Thereafter, PBMC were stored in RPMI-1640-DM containing 15% foetal calf serum (FCS) and 10% dimethyl sulfoxide (MERCK, Germany) at −140°C until use.

The phytohemagglutinin (PHA) proliferation assay was performed to control the viability of the PBMC as described before [18]. The stimulation index (SI) was calculated by the ratio of the cpm obtained in the presence of PHA to the cpm in the absence of PHA. Only results of viable cells (SI ≥ 50) were analysed in the described results.

Alloreactivity was determined in an IFN-γ, IL-10, IL-13 and GrB Elispot assay. In a 96-well round bottom plate (Nunc, Roskilde, Denmark), six replicates of 1 × 10⁵ PBMC from healthy individuals were stimulated with 100 µl of 1 × 10⁵ irradiated (40 Gy) PBMC derived from another healthy individual. In the HLA-mismatched setting,
Table 1 (B). Characteristics of HLA-mismatched living-related kidney transplant recipients

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<th>PRA (%)</th>
<th>HLA-mm</th>
<th>CsA</th>
<th>Tacro</th>
<th>MMF</th>
<th>AZA</th>
<th>Pred</th>
<th>Immunosuppressive regimen (mg/day)</th>
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Id, identification number; Pat, patient; Don, donor; KTx, kidney transplantation; PRA, panel-reactive antibodies; HLA-mm, mismatches at the HLA-A, B, and DR loci; CsA, cyclosporine A; Tacro, tacrolimus; MMF, mycophenolate mofetil; AZA, azathioprine; Pred, prednisone.

Fig. 1. Cytokine responses in culture medium alone and PHA responses in PBMC from healthy individuals (HI), HLA-mismatched (HLA-mm) and HLA-identical (HLA-id) living-related (LR) kidney transplant recipients determined in IFN-γ (A), IL-10 (B), IL-13 (C) and granzyme B (GrB) (D) Elispot assay.

1 × 10⁵ patients’ PBMC were stimulated with 100 μl of 1 × 10⁵ irradiated (40 Gy) donor-specific PBMC. Because we expected low frequencies of cytokine pc in the HLA-identical setting, 100 μl of 2 × 10⁵ patients’ PBMC was added to 100 μl of 2 × 10⁵ irradiated donor-specific PBMC to increase the sensitivity of the Elispot assays. Additionally, irradiated stimulator cells alone were incubated in culture media [if cytokine producing cells (pc) were detected, <5 spots/2 × 10⁵ PBMC in IFN-γ, IL-13 and GrB Elispot assays, and <20 spots/2 × 10⁵ PBMC in IL-10 Elispot assays were found]. To control the influence of irradiation on cytokine production, responder cells were incubated with irradiated responder (40 Gy) cells (autologous response). The autologous response was subtracted from the alloresponse. Responder PBMC in the culture medium alone were used as a negative control (Figure 1A–D). As a positive control, PBMC were stimulated with 1 μg/ml PHA (Murex Biotech, Kent, UK) (Figure 1A–D). After 40 h of incubation at 37°C and 5% CO₂, the non-adherent cells were collected, washed and resuspended in a 300 μl culture medium. The non-adherent cells were transfered in three wells of a flat-bottom 96-well plate (Nunc, Roskilde,
Table 2. Known mismatched minor histocompatibility antigens and donor-reactive IFN-γ pc, IL-10 pc, IL-13 pc, and granzyme B pc after HLA-identical LR kidney transplantation

<table>
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<tr>
<th>Gender</th>
<th>Mismatched mHAgs in the HLA-identical donor</th>
<th>HLA-restriction molecule present</th>
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<sup>a</sup>ID, identification number; <sup>b</sup>GrB, Granzyme B; <sup>c</sup>nd, not enough cells to determine for cytokines; <sup>d</sup>Mismatched mHAgs with the known HLA-restriction molecule are presented as cursive.

Danish) pre-coated with either mouse anti-human IFN-γ, IL-10, IL-13 or GrB monoclonal antibody (U-Cytech Biosciences, Utrecht, The Netherlands) and post-coated with phosphate-buffered saline (PBS) supplemented with 1% bovine serum albumin (BSA; U-Cytech Biosciences). The cells were incubated for 5 h at 37°C under 5% CO<sub>2</sub> for IFN-γ, IL-13 and GrB Elispot assays, and overnight for IL-10 Elispot assay. After incubation, the cells were lysed with ice-cold milli-Q water and washed extensively. Subsequently, the wells were incubated overnight at 4°C with 100 µl of diluted biotinylated goat anti-human cytokine (IFN-γ, IL-10, IL-13 or GrB) polyclonal antibody (U-Cytech Biosciences) followed by incubation with 50-µl phi-labelled goat anti-biotine antibodies (U-Cytech Biosciences) for 1 h at 37°C. After washing the wells, 30 µl of reagent (activator I + II, U-Cytech Biosciences), that activates phi, was added and incubated for 15 to 30 min at room temperature in the dark. The reaction was stopped by adding milli-Q water to the wells. The spots were counted automatically by using a Bioreader 3000 Elispot reader (BioSys, GmbH, Karben, Germany).

**mHAg typing after HLA-identical living-related kidney transplantation**

PBMC of the patient and the donor were typed for 11 known mHAgs: HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, HwA-9, HwA-10, UGT2B17 and HY (Table 2). From the 13 HLA-identical donor–recipient couples, 10 couples (ID1, 2, 3, 4, 6, 8, 10, 11, 12, 13) were typed for 11 known mHAgs and 3 couples (ID5, 7, 9) were not typed for HwA-10. As described, DNA from donor and recipient was isolated using the QiAamp<sup>®</sup> DNA Mini Kit [22].

**Statistical analysis**

The Kruskal–Wallis test was used to compare age and gender between healthy individuals, and HLA-mismatched and HLA-identical LR kidney transplant recipients. We used the Mann–Whitney U-test to compare time after kidney transplantation, serum creatinine, and proteinuria between HLA-mismatched and HLA-identical LR kidney transplant recipients. To compare the frequencies of cytokine pc between healthy individuals, HLA-mismatched and HLA-identical LR kidney transplant recipients, the Mann–Whitney U-test was used. The Wilcoxon signed-rank test was used to compare the number of donor-reactive cytokine pc with the number of their autologous control in PBMC from HLA-identical LR kidney transplant recipients and the frequencies of IFN-γ pc, IL-10 pc, IL-13 pc and GrB pc within PBMC from healthy individuals, HLA-mismatched LR kidney transplant recipients and HLA-identical LR kidney transplant recipients. Two sided P-values ≤ 0.05 were considered significant. For statistical analysis, SPSS 11.5 for Windows was used (SPSS, Inc., Chicago, IL, USA).

**Results**

**Clinical results**

At the time of blood collection, all patients were in a clinical stable period and none of the patients had acute rejection or infections. There was no difference in time after transplantation (P = 0.54) and panel-reactive antibodies (PRA) (P = 0.60) between both patient groups (Table 1A and B). Serum creatinine levels were comparable between the HLA-identical group and the HLA-mismatched group (P = 0.54). None of the HLA-identical and HLA-mismatched recipients had proteinuria (<0.5 g/l). The median age of the HLA-identical and HLA-mismatched recipients and healthy individuals was 49 years (range: 29–58 years), 41 years (range: 19–62 years) and 40 years (range: 26–61 years), respectively. No difference in age (P = 0.82) and gender (P = 0.61) was found between kidney transplant recipients and healthy individuals.
Alloreactivity in PBMC from healthy individuals, HLA-mismatched and HLA-identical LR kidney transplant recipients

The frequency of IFN-γ pc directed to alloantigens in PBMC from healthy individuals (median, 17 IFN-γ pc/2×10^5 PBMC; range, 0–176) was significantly higher than that in PBMC from HLA-mismatched (median, 4 IFN-γ pc/2×10^5 PBMC; range, 0–36; P = 0.03) and HLA-identical recipients (median, 1 IFN-γ pc/2×10^5 PBMC; range, 0–22; P = 0.003; Figure 2A). The number of donor-reactive IFN-γ pc was comparable between the HLA-mismatched and HLA-identical groups (P = 0.27).

The healthy control group showed low numbers of alloreactive IL-10 pc (median, 0 IL-10 pc/2×10^5 PBMC; range, 0–20), which was comparable to the number of donor-reactive IL-10 pc of the HLA-mismatched group (median, 0 IL-10 pc/2×10^5 PBMC; range, 0–142; P = 0.97, Figure 2B). Remarkably, PBMC from the HLA-identical group (median, 13 IL-10 pc/2×10^5 PBMC; range, 0–145) demonstrated higher frequencies of donor-reactive IL-10 pc compared to the number of alloreactive IL-10 pc in PBMC from the healthy control group (P = 0.03) and the HLA-mismatched group (P = 0.04).

Additionally, we analysed whether increased numbers of IL-10 pc were directed to donor antigens or to autoantigens. Therefore, we compared the number of IL-10 pc of the uncorrected donor-reactive response with the number of IL-10 pc of the autologous response. The number of donor-reactive IL-10 pc (median, 20 IL-10 pc/2×10^5 PBMC; range, 3–185) was significantly higher than the autologous reactive IL-10 pc (median, 12 IL-10 pc/2×10^5 PBMC; range, 2–40; P = 0.03; Figure 2B), suggesting that the donor-reactive response was directed to donor antigens and not reflecting autoreactivity. Also, in the other Elispot assays, the number of cytokine pc was higher after donor stimulation than in the autologous control (Figure 2A, C, D).

The number of alloreactive IL-13 pc (median, 7 IL-13 pc/2×10^5 PBMC; range, 2–22) and GrB pc (median, 8 GrB pc/2×10^5 PBMC; range, 0–20) in PBMC from healthy individuals was higher compared to the number of donor-reactive IL-13 pc and GrB pc in PBMC from HLA-mismatched (IL-13: median, 1 IL-13 pc/2×10^5 PBMC; range, 0–12; P = 0.03; GrB: median, 5 GrB pc/2×10^5 PBMC; range, 0–28; P = 0.56) and HLA-identical recipients (IL-13: median, 0 IL-13 pc/2×10^5 PBMC; range, 0–2; P < 0.001; Figure 2C; GrB: median, 1 GrB pc/2×10^5 PBMC; range, 0–4; P = 0.001; Figure 2D). The number of IL-13 pc was comparable between both patient groups (P = 0.37), but the number of GrB pc was higher in PBMC from the HLA-mismatched group compared to the HLA-identical group (P = 0.02).

In the healthy control group, allostimulated PBMC demonstrated higher numbers of IFN-γ pc compared to the numbers of IL-10 pc (P = 0.01), IL-13 pc (P = 0.07) and GrB pc (P = 0.03; Figure 2). Also, the number of IL-13 pc was higher than the number of IL-10 pc (P = 0.05).

The number of donor-reactive IFN-γ pc, IL-10 pc, IL-13 pc and GrB pc was comparable within the HLA-mismatched group (Figure 2).

It has been suggested that the balance between IFN-γ and IL-10 reflects the immune status of transplant recipients in relation to their donor graft [15]. Therefore, we compared the frequency of allospecific IFN-γ pc with the frequency of IL-10 pc. In PBMC from healthy individuals, high numbers of IFN-γ pc were observed in combination with low numbers of IL-10 pc (P = 0.01). No relation was found between the number of donor-reactive IFN-γ pc and IL-10 pc in PBMC from HLA-mismatched recipients (P = 0.64). Interestingly, PBMC from HLA-identical recipients showed low numbers of donor-reactive IFN-γ pc in combination with high numbers of donor-reactive IL-10 pc (P = 0.01; Figure 3).

Minor histocompatibility antigen typing after HLA-identical LR kidney transplantation

We analysed whether donor-reactive responses (≥1 cytokine pc) found after HLA-identical LR kidney transplantation were directed to known mismatched mHAgS between donor and recipient (Table 2). The cytomegalovirus (CMV) and Epstein-Barr virus (EBV) serological status had no influence on donor-reactive IFN-γ, IL-10, IL-13 and GrB responses.

All numbers of IL-13 pc and GrB pc were low, and consequently, no relation could be found between the number of IL-13 pc and GrB pc and the number of mHAg mismatches.

From the 13 donor–recipient combinations, 10 couples (ID1, 2, 3, 4, 6, 8, 9, 10, 11, 12) demonstrated mismatches for known mHAgS. Three couples (ID5, 7, 13) had no mismatches for mHAgS. Those three patients had no IFN-γ pc directed to donor antigens, but had significant numbers of IL-10 pc.

Five of the 10 patients had the correct HLA-restriction molecule for donor mHAg presentation to recipient T-cells [23]. One of those patients (ID2) had the highest frequency of IFN-γ pc. Unfortunately, not enough cells were available to perform the IL-10 Elispot assay. Two other patients (ID1, ID10) demonstrated high numbers of IL-10 pc in combination with significantly lower numbers of IFN-γ pc. On the other hand, two other patients (ID6, ID11) had detectable numbers of IFN-γ pc in combination with no IL-10 pc.

From the five couples (ID3, 4, 8, 9, 12) without the correct HLA-restriction molecule, four patients (ID3, 4, 9, 12) showed high numbers of IL-10 pc and one patient (ID8) had no IL-10 pc, all in combination with low numbers of IFN-γ pc (≤2 IFN-γ pc/2×10^5 PBMC).

Two patients (ID6, ID8) tested more than 3 years after transplantation had no IL-10 pc directed to donor antigens.

Discussion

In humans, mHAg disparities between donor and recipient may influence transplant outcome after BMT [23]. Also, after HLA-identical LR kidney transplantation, theoretically, mismatches in mHAgS between donor and recipient and in combination with the presence of the correct HLA-restriction molecule may trigger T-cell responses [23]. Nowadays, the number of identified human mHAgS...
Fig. 2. Alloreactivity in PBMC from healthy individuals (HI) and donor-reactive response in patients with HLA-mismatched (HLA-mm) or HLA-identical (HLA-id) living-related (LR) kidney transplant using IFN-γ (A), IL-10 (B), IL-13 (C), and granzyme B (GrB) (D) Elispot assay. The donor-reactive responses in HLA-identical LR kidney transplant recipients (dotted circles) were further analysed for their autologous responses and uncorrected donor-reactive responses.
have been expanded to a total of 14 autosomal mHAgs and 10 Y-chromosome encoded mHAgs [22]. However, it is yet not known how many mHAgs exist, but is expected to be much more [3, 24]. In our study, during immunological quiescence, we found only in 2 out of 13 patients high donor-reactive IFN-γ pc (> 5 IFN-γ pc/2 × 10^5 PBMC). We observed no donor-reactive IFN-γ pc, IL-13 pc and GrB pc in the absence of known mHAg mismatches. In contrast, high numbers of donor-reactive IL-10 pc were detected in combinations with and without known mHAg mismatches.

Recently, Perez-Garcia et al. demonstrated an increased risk for acute GVHD after HA-8 mismatched HLA-identical sibling donor allogeneic BMT [25], which could cause high T-cell responsiveness directed to donor HA-8. In our study, one couple had an HA-8 mismatch, which could be presented in HLA-A2. This patient had high numbers of donor-directed IFN-γ pc, which suggests a potential risk for graft failure, because HA-8 is also expressed on epithelial cells of the kidney [26].

IL-10 is a cytokine that has suppressive effects on the production of proinflammatory cytokines and T-cell responses [27]. Several cells produce IL-10, such as activated Th2 cells, monocytes and T-regulatory type 1 (Tr1) cells [27,28]. In our experiments, we only used non-adherent cells. Therefore, monocytes do not interfere in the presented Elispot assays. Th2 cells and Tr1 cells can be activated by donor cells [27]. Both Th2 and Tr1 cells mainly produce IL-10. Th1 cells also produce transforming growth factor (TGF)-β [29], and activated Th2 cells also produce IL-4, IL-5 and IL-13 [28]. We found high numbers of donor-reactive IL-10 pc in combination with no or low numbers of IFN-γ pc, IL-13 pc and GrB pc after HLA-identical LR kidney transplantation. Because we did not detect donor-reactive IL-13 pc, we assume that Tr1 cells play an important role in those patients. Unfortunately, we have not determined TGF-β.

After BMT with HLA-identical sibling donors, Tr1 cells may play a role via the secretion of IL-10 in preventing GVHD [13,14]. High frequencies of donor-reactive IL-10 pc determined by Elispot assay were found in PBMC from recipients who did not develop GVHD, while recipients who developed GVHD had low numbers of IL-10 pc [14]. Petersen et al. showed high levels of donor-directed IL-10 mRNA in PBMC from recipients who did not develop GVHD, while low IL-10 mRNA levels were found in recipients who developed GVHD [13]. In the HLA-identical LR kidney transplant setting, VanBuskirk et al. showed, using a trans-vivo delayed-type hypersensitivity (DTH) analysis, a possible role for Tr1 cells by secretion of IL-10 and TGF-β [30]. Moreover, Rodriguez et al. demonstrated, also using a trans-vivo DTH-analysis, that matching for HLA molecules resulted in enhanced immune regulation in kidney transplant recipients [31]. In HLA-identical LR kidney transplant recipients, the latter group also found reduced T cell responses against non-HLA antigens by Tr1 cells through the secretion of IL-10. Therefore, we propose that HLA-identical LR kidney transplant recipients are developing peripheral tolerance against non-HLA antigens by Tr1 cells through the secretion of IL-10.

It has been suggested that the presence of PRA reactivity was associated with long-term graft loss in kidney transplants from HLA-identical sibling donors [4], which could reflect immune reactivity directed to non-HLA antigens or mHAgs. In the present study, we found no correlation between PRA before HLA-identical LR kidney transplantation and the number of IFN-γ pc, IL-10 pc and GrB pc. Also, in the HLA-mismatched group, no relation was found between PRA before kidney transplantation and the number of cytokine producing cells.

After HLA-mismatched kidney transplantation, high donor-reactive IL-10 pc was associated with stable graft function, and low IL-10 was related to rejection [15]. In our study, three HLA-mismatched LR kidney transplant recipients with excellent renal function had high numbers of IL-10 pc in combination with low numbers of IFN-γ pc. Because we assume that donor-reactive IL-10 pc could be derived from Tr1 cells, we suggest to reduce the immunosuppressive load in patients with stable graft function and high numbers of donor-reactive IL-10 pc in combination with low numbers of IFN-γ pc. Whether the immunosuppressive medication influences the balance between IFN-γ and IL-10 should be investigated by e.g. in vitro addition of immunosuppressive drugs, or by discontinuation of the immunosuppressive medication in those transplant recipients. Furthermore, the autologous response was comparable between healthy individuals and transplant recipients (data not shown), suggesting that immunosuppression has no influence on the autologous response.

Studies reported that CD4+CD25bright regulatory T-cells might play a beneficial role in mediating peripheral tolerance after kidney transplantation [33,34]. Unfortunately, not enough cells were available to determine the percentage CD4+CD25bright regulatory T-cells by flow cytometry.
Cytokine profiles after HLA-identical living-related kidney transplantation

In conclusion, high numbers of donor-reactive IL-10 pc in combination with low reactivity in autologous control, low numbers of donor-reactive IFN-γ pc, IL-13 pc and GrB pc, after HLA-identical LR kidney transplantation may reflect active downregulation of reactivity against non-HLA molecules.

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Conflict of interest statement. None declared.

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