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Pre-transplant donor-specific Interferon-gamma-producing cells and acute rejection of the kidney allograft

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ABSTRACT

Background: Our retrospective study included a cohort of 47 patients who underwent living donor kidney transplantation. The pre-transplant frequencies of donor-specific Interferon-gamma (IFN-γ) producing cells were defined using enzyme-linked immunosorbent spot (ELISpot) assay and correlated with incidence of acute cellular (ACR), antibody-mediated rejection (AMR) and kidney graft survival up to one year after transplantation.
Results: We found a statistically significant correlation between the frequencies of IFN-γ-producing cells and the number of mismatches in HLA antigens between patients and their respective donors – for Class I – A and B ($r = 0.399$, $p < 0.01$) and for Class I and Class II antigens – A, B and DR ($r = 0.409$, $p < 0.01$). No significant relationship was observed between the numbers of IFN-γ-secreting cells and incidence of acute rejection (neither ACR, nor AMR). However, there was a trend of elevated frequencies of IFN-γ-producing cells in patients who developed ACR or AMR in comparison with kidney recipients free of rejection (91 ± 82 and 114 ± 75 vs. $72 \pm 70/5 \times 10^4$ peripheral blood mononuclear cells respectively). Patients with concurrent acute cellular and antibody-mediated rejection had also higher numbers of IFN-γ-producing memory/effector cells compared to patients with cellular rejection only.
Conclusion: Pre-transplant determination of the numbers of IFN-γ-producing donor-specific memory cells using the ELISpot technique may provide clinically relevant results when evaluating the risk of development of acute cellular and antibody-mediated rejection. These frequencies are influenced by the degree of HLA mismatching between patients and their respective kidney donors.

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1. Introduction

Predicting the risk of acute rejection is essential in preventing the development of long-term immunological complications and graft failure after kidney transplantation [1,2]. Besides assays used to define antibodies to HLA antigens, biomarker measurements of immune memory or activation and tests assessing the cellular immune responses are also applied [3]. The ELISpot method is used in the transplantation setting to measure the numbers (frequencies) of cytokine-producing cells, such as IFN-γ-producing memory/effector T cells [4,5]. A number of publications have provided evidence that patients with elevated numbers of pre-transplant donor-specific IFN-γ-secreting cells have increased risk of rejection and worse graft function [4,6,7,8,9,10]. It has been also reported that the frequencies of IFN-γ-producing memory cells may not be influenced by risk factors of rejection such as HLA mismatching or high panel-reactive antibodies [4,7]. However, a recent study has suggested that a correlation may exist between the frequencies of pre-transplant IFN-γ secreting cells and the quantity of HLA Class I mismatches between patients and their respective kidney donors [11]. In addition, the incidence of acute rejection and the numbers of IFN-γ producing cells might not always directly correlate, due to variations in immunosuppressive regimens in different transplantation centers [12], time of occurrence and evaluation of rejection episodes [11] or other unknown factors.

2. Objective

We aimed to assess in a single-center study the clinical significance of the measurement of frequencies of donor-specific Interferon-γ-

Abbreviations: ACR, Acute cellular rejection; AMR, Antibody-mediated rejection; DSA, Donor-specific antibodies; ELISpot, Enzyme-linked immunosorbent spot assay; HLA, Human leukocyte antigens; IFN-γ, Interferon-gamma.

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producing cells in order to predict acute cellular (ACR) and antibody-mediated rejection (AMR) after kidney transplantation from living donors. The numbers of pre-transplant IFN-γ-secreting cells were correlated with the incidence of AMR and ACR, HLA mismatches and with other selected risk factors for rejection.

3. Material and methods

3.1. Patients

47 recipients transplanted with kidneys from living donors during the years 2010–2012 were included. The study was performed according to the regulations of the institute's ethical committee and written informed consent was obtained from all patients. The patients' demographic characteristics are shown in Table 1. 29 transplants were performed between genetic relatives (parent/child). Panel-reactive antibodies (PRA) were assessed using the complement-dependent cytotoxicity (CDC) test on the HLA-defined cells of 50 healthy blood donors. The levels and specificity of HLA antibodies in sensitized patients were further determined using LABScreen Mix and LABScreen Single Antigen Class I and II tests (One Lambda Inc.). Crossmatches for detection of donor-specific antibodies were performed before transplantation using the CDC test and flow cytometry technique (FCXM). All patients were negative before transplantation in the CDC crossmatch test. Positivity in the FCXM crossmatch test on both T and B lymphocytes was considered as a contraindication for transplantation; however, patients who were solely positive on B lymphocytes were transplanted.

The clinical state of patients was followed for one year. Acute cellular (ACR) and antibody-mediated (AMR) rejection were diagnosed in kidney graft biopsy samples according to the updated Banff classification valid in the period 2010–2012 [13,14]. Six patients received induction immunosuppression (antithymocyte globulin and steroids). During the 1-year follow-up after transplantation, 21 patients (45%) developed acute cellular rejection, 4 (19%) of whom developed AMR simultaneously. One patient (2%) experienced AMR without concurrent ACR. ACR was identified in 12 (58%) patients within the first month after transplantation. In seven (33%) patients, ACR was diagnosed up to 3 months, and in two (9%) within one year after transplantation. Out of the six retransplanted patients, four developed ACR and two developed AMR simultaneously. Two patients died for reasons indirectly related to transplantation (heart failure, pneumonia) and two grafts failed — one due to recurrence of the original disease and the other due to acute rejection.

3.2. Mixed lymphocyte cultures (MLC)

Peripheral heparinized blood (20 ml) was obtained from patients and their respective donors (0–310 days before transplantation); mononuclear cells (PBMC) were isolated after centrifugation using Ficoll gradient (900 g, 20 min) and cryopreserved in liquid nitrogen until further testing. Kidney recipient mononuclear cells were stimulated in one-way mixed lymphocyte cultures with donor lymphocytes or autologous cells. DNA replication in donor (or autologous) cells was blocked by Mitomycin C, 250 μg/2 × 10^6 cells/ml for 30 min at 37 °C; cells were then washed twice (10 min, 400 g). Lymphocytes were counted (cell analyzer Cedex XS, Roche, Switzerland), adjusted to a concentration of 1 × 10^6/ml, pipetted into 96-well U-shaped 250 μl cultivation plates and incubated for 24 h (37 °C, 5% CO_2). Kidney recipient cells (5 × 10^4 PBMC/well, 50 μl) were stimulated with 5 × 10^4 PBMC/well of allogeneic donor or autologous control cells and with pooled PBMC from 10 different HLA-typed individuals (positive control). The capacity of cells to produce IFN-γ was also verified by stimulation with an anti-CD3 monoclonal antibody (positive control). Pooled cells incubated with Mitomycin C were used as a control of inhibition of IFN-γ production by Mitomycin C. All cell combinations were pipetted in quadruplicate.

3.3. Enzyme-linked immunosorbent spot (ELISpot) assay

ELISpot kits for detection of human IFN-γ were obtained from Mabtech (cat. no. 3420-2AST-2, Stockholm, Sweden). Strips coated with an anti-IFN-γ antibody were washed with sterile PBS and then blocked for 30 min with 10% fetal calf serum (FCS) in RPMI 1640 medium. After removing the blocking medium, stimulated cells (in MLC, see above) were pipetted into the strips. Anti-CD3 monoclonal antibody was added to nonstimulated cells in positive control wells. Cells were then incubated for 20 h (5% CO_2, 37 °C). After removal of cells by flicking, strips were washed with PBS and detection alkaline-phosphatase labeled antibody 7-B6-ALP was added to each well. After 2 h incubation, strips were washed and substrate solution BCIP/NBT was added to each well. Development of spots was stopped after 10 min by washing.

Table 1
Demographic characteristics of kidney recipients and their donors in relation to the incidence of acute cellular rejection (ACR).

Demographic characteristics		No rejection n = 25	No rejection Min–Max/(%)	ACR[a] n = 21	ACR[a] Min–Max/(%)	p value
Age	Recipient	41 ± 14	(19–70)	38 ± 12	(19–65)	NS
	Donor	54 ± 11	(31–68)	51 ± 10	(32–67)	NS
Recipient gender	Male (n = 32)	17	53%	15	47%	NS
	Female (n = 14)	8	57%	6	43%	
Number of transplantations	=1 (n = 40)	23	58%	17	42%	NS
	>1 (n = 6)	2	33%	4	67%	
AB0 compatibility	Yes (n = 41)	21	51%	20	49%	NS
	No (n = 5)	4	80%	1	20%	
Cold ischemia (min)	(n = 43)	44 ± 26	(12–115)	47 ± 20	(18–90)	NS
Serum creatinine (μmol/l) after transplantation	3 months	147 ± 31	(82–195)	148 ± 31	(106–196)	NS
	6 months	150 ± 53	(77–340)	127 ± 27	(66–181)	NS
	12 months	148 ± 64	(84–408)	131 ± 22	(93–175)	NS
Induction immunosuppression	Yes (n = 6)	3	50%	3	50%	NS
(Antithymocyte globulin)	No (n = 40)	22	55%	18	44%	
Maintenance immunosuppression	Tacrolimus, prednisone (n = 3)	1	4%	2	9%	NS
	Tacrolimus, prednisone, MMF (n = 39)	22	88%	17	81%	
	Cyclosporine A, prednisone (n = 1)	1	4%	0	0%	
	Cyclosporine A, prednisone, MMF (n = 2)	1	4%	1	5%	
	Other (n = 1)	0	0%	1	5%	

ACR — acute cellular rejection, MMF — mycophenolate mofetil and NS — not significant.
[a] Patients with ACR (n = 17) and concurrent AMR (n = 4) were included.

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