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Association of high HLA-E expression during acute cellular rejection and numbers of HLA class I leader peptide mismatches with reduced renal allograft survival

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ABSTRACT

Non-classical Human Leukocyte Antigen (HLA)-E preferentially presents leader peptides derived from classical HLA-class I molecules. HLA-E can trigger opposed immune responses by interacting with inhibitory NKG2A or by activating NKG2C receptors on NK and T-cells. We studied the impact of HLA-E on renal allograft survival during acute cellular rejection. HLA-E expression was up-regulated in acute cellular rejection (ACR) biopsies (n = 12) compared to biopsies from 13 renal allografts with no rejection-signs. HLA-E up-regulation was correlated with numbers of HLA-class I leader peptide mismatches ($p = 0.04$). CD8+ and CD56+ infiltrating cells correlated with HLA-E expression ($p < 0.0001$ and $p = 0.0009$, respectively). Activating NKG2C receptor dominated on effector cells in biopsies and peripheral blood during ACR potentially allowing HLA-E-mediated immune activation. Moreover, HLA-E expression correlated with deterioration in renal allograft function ($p < 0.008$) and reduced allograft survival ($p = 0.002$). Our findings provide evidence that during renal allograft rejection HLA-E along with high numbers of mismatched HLA-class I leader peptides might represent additional targets for immune-activating responses.

1. Introduction

Allograft rejection remains the leading problem after renal transplantation. Incompatible classical Human Leukocyte Antigens (HLA) (Susal and Opelz, 2012) class I and II on allograft surface are the primary targets for immunological responses (Susal and Opelz, 2012; Alonso Arias et al., 2012; Mytilineos et al., 1997). Recognition of incompatible classical HLA molecules by the immune system represents the key event during allograft rejection. The importance of the classical HLA-class I (A, B, C) molecules in transplantation rejection processes is well established. However on allograft tissue other molecules do exist, which influence immune responses and have impact on transplant success. One molecule of interest that exhibits a wide-range of functions in innate and adaptive immune system is the non-classical HLA-class I molecule HLA-E. HLA-E has the ability to stimulate either activating or inhibitory signals suggesting that it might be critical in regulating immune responses. Studies in pregnancy, tumor diseases and transgenic xeno-transplantation have attributed immunosuppressive features to HLA-E. However, as for now, little data is available on the immune modulatory efficacy of HLA-E in solid organ transplantation. For stable cell surface expression HLA-E requires a nonameric peptide repertoire comprising mainly peptides derived from leader sequences of classical HLA-class I molecules (Romagnani et al., 2004; Petrie et al., 2008; Braud et al., 1998, 1997; Maier et al.,

Abbreviations: HLA, Human Leukocyte Antigen; LP, Leader peptide; ACR, Acute cellular rejection; IF/TA, Interstitial fibrosis and tubular atrophy; MM, Mismatch.

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2000; Lee et al., 1998; Ulbrecht et al., 1998; O'Callaghan, 2000). The presentation of these leader peptides (LP) by HLA-E allows immune effector cells the indirect monitoring of classical HLA-class I molecule expression (Bland et al., 2003; Hoare et al., 2008). HLA-E/peptide-complex recognition by NK cell and cytotoxic T lymphocyte (CTL) receptors mediates either an inhibitory or a stimulatory signaling (Bland et al., 2003; Gooden et al., 2011; Garcia et al., 2002; Pietra et al., 2009). With regard to these diametrically opposed functions of HLA-E within the immune system, the key questions in allogeneic solid organ transplantation setting are: (i) Is HLA-E expressed during acute cellular rejection in the allograft? (ii) Which factors favor HLA-E up-regulation after transplantation? (iii) Which type of receptor is preferentially expressed in immediate vicinity of HLA-E?

To address these issues we evaluated HLA-E surface expression, numbers and types of infiltrating effector cells in renal allograft biopsies with pathologically confirmed acute cellular rejection (ACR) or with no rejection-signs [interstitial fibrosis and tubular atrophy (IF/TA)] and correlated our findings with clinical and laboratory variables. In addition, presence of HLA-E specific CD94/NKG2A and CD94/NKG2C receptors was studied in biopsies and in peripheral blood. Our results provide substantial evidence that HLA-E along with high numbers of mismatched classical HLA-class I LP may represent additional targets for immune activating responses in ACR.

2. Materials and methods

2.1. Study population

25 patients were enrolled in this single center study. Selection criteria included regular follow-ups after renal transplantation, evaluation of renal transplant biopsies by one of the institutional expert renal-pathologist, sufficient available renal biopsy material, biopsies collection prior to anti-rejection treatment, standard triple immunosuppressive regime (low-dose prednisolone, calcineurin inhibitor and mycophenolate-mofetil), patients over 18 years of age, no evidence for malignancy, infection or pregnancy. All patients gave written informed consent. Patients underwent renal transplantation between January 2008 and March 2011 at the University Hospital Essen, Germany, and had a renal allograft biopsy for cause (i.e. evidence for graft dysfunction) presenting histopathological findings of an ACR or IF/TA. Based on histopathological findings patients were stratified into the group of patients with ACR (n = 9 patients with Banff grade IA/IB; n = 3 patients with Banff grade IIA; mean age 50 ± 3.7 years; six men) or with IF/TA (n = 13, mean age 54 ± 3.2 years; seven men). The follow-up period lasted up to a maximum of 60 months. With respect to transplant relevant characteristics there were no significant differences between the two groups. Recipients of both patient groups were comparable regarding age, sex, type of organ donation and time spent on renal transplant waiting list. Moreover, donor-characteristics were similar in both patient groups. Baseline demographic and transplant-related data of the two groups as well as of the corresponding donors, respectively, are shown in Table 1. According to molecular typing results for HLA-A, −B, −C, −E and HLA-DR of donor-recipient pairs there were no significant differences regarding number of re-transplantations, number of HLA-A, −B, −C and HLA-DR as well as HLA-E mismatches between the two patient groups. Recipient-donor pairs were typed for HLA-E using sequence-specific primer-polymerase chain reaction (SSP-PCR) method as described previously (Grimsley et al., 2002). Although median graft survival was reduced in patients with ACR, this did not reach statistical significance (Table 1). In the ACR group the long-term graft-loss was significantly higher compared to the patient group with IF/TA (4 vs. 0; p = 0.039). To investigate the distribution of the NKG2A and NKG2C expression on peripheral blood lymphocytes, blood samples of 15 renal transplant recipients suffering from ACR (n = 6) or having a stable renal transplant function (n = 9) were collected in addition. The study was designed in accordance with the guidelines of the Declaration of Helsinki and approved by the institutional ethics committee.

Table 1
Baseline characteristics, clinical and transplant related data of the patient group with acute cellular allograft rejection and the patient group with interstitial fibrosis and tubular atrophy for recipients and donors, respectively. All values are represented in mean ± standard deviation. §Comparison between the two groups is done by using chi-square test, Mann-Whitney-U test or *t*-test. Abbr.: ns indicates not significant; ACR, acute cellular rejection; IF/TA, interstitial fibrosis and tubular atrophy; HD, haemodialysis; PD, peritoneal dialysis; vs., versus; GN, glomerulonephritis; PKD, polycystic kidney; disease CMV, Cytomegalovirus, HLA, human leukocyte antigen; PRA, panel-reactive antibody; RT, renal transplantation; ΔGFR1, deterioration in renal graft function from baseline till biopsy; ΔGFR2, deterioration in renal graft function from baseline till last follow up, HPF; high power field.

Characteristics	ACR n = 12 (%)	IF/TA n = 13 (%)	p-value§
Recipient related characteristics			
Male/Female (%)	6/6 (50/50)	7/6 (54/46)	*ns*
Age (years, mean ±SD)	50 ±3·7	54 ± 3·2	*ns*
Cause of end-stage renal disease			
Diabetes mellitus	/	1	
Hypertension	/	1	
Chronic GN	4	6	
PKD	2	1	
Others or unknown	6	4	
Time on dialysis (days) ±SD	1214 ± 780	1061 ± 768	*ns*
HD vs. PD vs. none	6 vs. 3 vs. 3	11 vs. 2 vs. 0	
CMV			
CMV-seropositive recipient (%)	8 (66)	7 (54)	*ns*
CMV-seronegative recipient and CMV-seropositive donor (%)	1 (8)	3 (23)	*ns*
Donor related characteristics			
Male/Female (%)	5/7 (42/58)	5/8 (38/62)	*ns*
Age (years, mean ±SD)	51 ± 21	62 ± 8	*ns*
Type of donor			
Living organ donation (%)	7 (58)	4 (31)	*ns*
Related donation (%)	3 (25)	4 (31)	*ns*
Donor-recipient sex mismatch (%)	8 (66)	6 (46)	*ns*
Total cold ischemia time (hours, mean ±SD)	7 ± 7	11 ± 7	*ns*
CMV-seropositive donor (%)	6 (50)	7 (54)	*ns*

2.2. Clinical outcome parameters

Clinical outcome parameters were graft-loss and deterioration in renal allograft function. Graft-loss was defined as return to dialysis. Renal allograft-function was evaluated by estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease formula. Baseline renal allograft-function was defined as highest eGFR within 2 months before biopsy. Deterioration in renal allograft-functions until time point of biopsy (ΔGFR_1) was defined as difference between eGFR at baseline and biopsy. The loss in renal allograft function from baseline till last follow-up (ΔGFR_2) was assessed with the same method. Regarding decline in renal allograft function, the deterioration was steeper in the patient group with ACR than in the patient group with IF/TA for ΔGFR_1 and for ΔGFR_2 (p = 0.006, p = 0.016, see Table 1).

2.3. Immunohistochemical studies

Renal allograft sections were obtained from needle core biopsies (n = 25). All biopsy material was routinely formalin-fixed and processed for paraffin embedding and sectioned at 4 μm. Following