CHAPTER V

Minor H antigen matches and mismatches are equally distributed among recipients with or without complications after HLA identical sibling renal transplantation

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Abstract

Studies of the effect of minor H antigen mismatching on the outcome of renal transplantation are scarce and concern mainly single center studies. The International Histocompatibility and Immunogenetics Workshops (IHIWS) provide a collaborative platform to execute crucial large studies. In collaboration with 16 laboratories of the IHIWS, the role of 15 autosomal, 10 Y-chromosome encoded minor H antigens and 3 CD31 polymorphisms, was investigated in relation to the incidence of renal graft rejection and graft loss in 444 HLA-identical sibling renal transplantations. Recipient and donor DNA samples were genotyped for the minor H antigens HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, SP110, PANE1, UGT2B17, C19Orf48, LB-ECGF-1, CTSH, LRH-1, LB-ADIR and HY. The correlation between minor H antigen mismatch and the primary outcome graft rejection or graft loss was statistically analyzed. The incidence of rejection was very low and no correlation was observed between one or more minor H antigen mismatch(es) and a rejection episode (n=36), of which only 8 resulted in graft loss. In summary, in our study cohort of 444 renal transplants, mismatching for neither autosomal nor HY minor H antigens correlate with rejection episodes or with graft loss.

Introduction

Short term graft survival of renal transplants has improved significantly over the last years. Long term graft survival however is still poor, as around 35% of cadaveric renal transplants survive for more than ten years. Even with living donors, 10 year graft survival does not exceed 55%[1, 2]. Overall, 60% of graft failures are cellular or humoral immune mediated rejections while 40% of rejections have a non-immunological cause[3]. As expected, rejection is more frequent in HLA partially-matched or fully-mismatched donor-recipient combinations. However, even in HLA-identical sibling transplants, rejection of renal transplants occurs and 10-year survival is calculated at 68-70% in recent multi-center analyses[4, 5]. Furthermore, the vast majority of renal transplant recipients, including HLA-identical sibling transplants, needs lifelong immunosuppression[6]. This prompted us to investigate the role of minor H antigens in HLA-identical renal transplantation.

Minor H antigens are immunogenic peptides derived from polymorphic self proteins presented in the context of HLA[7-9]. The tissue distribution of individual minor H antigens differs; they are either broadly expressed or their expression is restricted to the hematopoietic system[10]. Minor H antigenic disparities between related and unrelated individuals, sharing the relevant MHC class I and II minor H peptide presenting molecules, can induce strong CD8 and CD4 cellular immune responses respectively[11]. T cell mediated rejection has been described in HLA-identical kidney transplantation[12]. Beside cellular also humoral responses against minor H antigens have been reported[13, 14].

More than 30 years ago, the influence of HY minor H antigens was reported in sex-mismatched HLA-A2 positive renal transplants[15, 16]. More recent, the effect of gender mismatch in HLA matched renal transplantation was confirmed[14, 17].

Studies addressing the role of autosomally encoded minor H antigens in related renal transplantation show different results. HA-1 might be involved in chronic rejection[18], but can also lead to transplantation tolerance[19]. These effects were not confirmed in a larger cohort of mainly cadaver transplants[20]. Note that the latter transplants were not HLA-identical, but HLA-A, -B and -DRB1 matched.

Apart from minor H antigens, polymorphisms in the adhesion molecule CD31 (PECAM-1) might influence the outcome of renal transplantation; these polymorphisms have been described to act in a 'minor-like' fashion[21-23]. Until now, the few studies executed on the influence of CD31 polymorphisms do not show any effect. A small study showed no difference of PECAM-1 polymorphisms on acute renal rejection[24]. More recently, a larger study by Heinold et al. did not show any correlation between three different polymorphisms in PECAM-1 and 5 year renal graft survival[25].

In the context of the 15th International Histocompatibily and Immunogenetics Work-

shop (IHIWS) we had the unique opportunity to perform a worldwide multicenter study to analyze the role of autosomal and Y chromosome encoded minor H antigens in a relatively large cohort of recipients of HLA-identical sibling donor transplants. Herein, 581 HLA-identical sibling renal transplantations from the 16 participating centers were enrolled.

Material and Methods

Enrollment of recipient -donor pairs

HLA-identical sibling renal transplantations from 16 participating centers were enrolled from January 2008 until September 2009. Recipient-donor pairs should be positive for one (or more) of the HLA-restriction molecules, HLA-A1, -A2, -A3, -A24, -A29, -A31, -A33, -B7, -B44, -B60, presenting the studied autosomally encoded minor H antigens.

Table 1. Minor H antigens in the IHIWS 15th workshop minor typing kit

Minor H antigen	HLA restriction molecule	Tissue distribution
HA-1/A2	HLA-A2/HLA-B60	Restricted: hematopoietic/solid tumor cells
HA-2	HLA-A2	Restricted: hematopoietic cells
HA-3	HLA-A1	Broad
HA-8	HLA-A2	Broad
HB-1	HLA-B44	Restricted: hematopoietic cells
ACC-1	HLA-A24	Restricted: hematopoietic/solid tumor cells
ACC-2	HLA-B44	Restricted: hematopoietic/solid tumor cells
UGT2B17/A29	HLA-A29/HLA-B44	Restricted: hematopoietic cells
LRH-1	HLA-B7	Restricted: hematopoietic/solid tumor cells
SP110/ HwA9	HLA-A3	Restricted: hematopoietic cells
PANE1/ HwA10	HLA-A3	Restricted: hematopoietic cells
C19Orf48/ HwA11	HLA-A2	Restricted: solid tumor cells
LB-ECGF-1	HLA-B7	Restricted: solid tumor cells
CTSH/A31	HLA-A31/HLA-A33	Restricted: hematopoietic cells
LB-ADIR	HLA-A2	Restricted: hematopoietic/solid tumor cells
CD31 exon 3	Not applicable	Broad
CD31 exon 8	Not applicable	Broad
CD31 exon 12	Not applicable	Broad
HY	HLA-A1/A2/A33/B7/B8/B52/ B60/DR15/DQ5/DRB3.0301	Broad

Minor H antigen typing

DNA was isolated using standardized protocols. Genotyping of 15 autosomally encoded minor H antigens, HY and 3 CD31 polymorphism (table 1) was performed using the PCR-SSP technique (Invitrogen, http://www.invitrogen.com). Hereto, a modified minor H antigen typing kit was developed in collaboration with Invitrogen. This kit is an extended version of the kit developed for the purpose of the first minor H antigen workshop, carried out under the auspices of the 14th IHIWS[26]. All collaborating centers used the same batch of the Dynal Allset Minor Histocompatibility Antigen (mHA) Typing Kit, kindly provided by Invitrogen (Bromborough, United Kingdom). The typing was performed according to the manufacturer as described before[26]. Briefly; PCR mixes contained 85.5 ul (50 ng/l) of genomic DNA, 3.6 ul (5U/l) AmpliTaq DNA polymerase and 360 ul of DynaMix Plus. 10 ul of this mix was added in each well of the typing tray. The PCR program started with 2 minutes of 96 C, followed by 10 cycles of 15 seconds at 96 C, and 60 seconds at 65 C. Subsequently, 20 cycles were run using the following conditions: 10 seconds at 96 C, 50 seconds at 61 C, and 30 seconds at 72 C. IHW1077 and IHW1166 were used as standardized controls by all centers.

Table 2. Patient characteristics

	Tx with rejection episode N= 36	**Tx without rejection episode N=408**	**Tx without rejection, follow-up ≥ 10 years N=44**
Recipient age (years)	41,4 (23-64)	40,5 (12-71)	36,8 (19-54)
Cause of ESRD			
Glomerulonephritis	3	68	6
Diabetes	4	36	9
Hypertension	5	27	0
Chronic renal failure	10	131	3
Other	12	125	20
Unknown/missing	2	21	6
Initial Immunosuppressive drugs regimen			
Azathiprine	26	280	23
Corticosteroids	32	329	28
Cyclosporin A	27	290	20
Other/unknown	4	52	5
None	0	27	11

Table 3. Frequency of minor H antigen mismatches in the study cohort

	Tx with rejection episode (%)# N= 36	Tx without rejection (%) N=408	Tx without rejection, follow-up ≥ 10 years (%) N=44
Gender mismatch*, N=	5 (14)	75 (18)	12 (27)
HY mismatch, N=			
0	33 (92)	348 (85)	36 (82)
1	1 (3)	29 (7)	4 (9)
≥ 2	2 (6)	31 (8)	4 (9)
Autosomal mismatch, N=			
0	25 (69)	267 (65)	27 (61)
1	11 (31)	109 (27)	14 (32)
≥ 2	0	32 (8)	3 (7)
CD31 exon3 mismatch, N=	13 (36)	121 (30)	15 (34)
CD31 exon8 mismatch, N=	13 (36)	137 (34)	14 (32)
CD31 exon12 mismatch, N=	11 (31)	136 (33)	15 (34)

* female recipient with male donor
# percentages of the number in the respective column

Statistical analyses

Minor H antigen mismatches were analyzed in the direction of recipient against donor. CD31 polymorphisms were analyzed being matched or mismatched, since until now it is not known which polymorphism is immunogenic. For statistical analysis of the data IBM SPSS Statistics 20 was used. Two-sided Pearson Chi-square test in combination with logistic regression was used since the numbers were too small to perform multi-variate analyses. Statistical significance was defined by p-value < 0.05.

Results

Study cohort

In total 581 transplantations were enrolled in our standardized database. Transplantations were excluded for the following reasons: recipient and donor were: not HLA-identical (n=3), non-living related donor transplantation (n=1), monozygotic twins (n=1), recipient received a bone marrow transplant before renal transplantation from the same donor (n=1), recipients receiving a second transplant (n=2), incomplete HLA-typing (n=2), no follow-up (n=8) and incomplete minor H antigen typing (n=119). The remaining 444 renal transplant recipient-donor pairs were included in our analyses. Recipients' characteristics are described in table 2.

Table 4. Number of minor H antigen mismatches in the study cohort

Minor H antigen mismatch	Tx with rejection episode,N= 36	Tx without rejection, follow-up ≥ 10 years, N=44	p-value*	OR (95% CI)**
HA-1	2	2	0.84	0.81 (0.11-6.1)
HA-2	1	1	0.87	0.81 (0.05-13.5)
HA-8	1	3	0.42	2.56 (0.26-25.7)
HB-1	0	2	0.99	1384694938 (0.0-∞)
ACC-1	1	0	1.0	2030882689 (0.0-∞)
ACC-2	0	3	0.99	0.0 (0.0-∞)
SP110/ HwA9	1	0	1.0	2030882689 (0.0-∞)
C19Orf48/ HwA11	3	4	0.91	0.91 (0.19-4.4)
LB-ADIR	2	4	0.55	0.59 (0.10-3.4)
HY	3	8	0.21	2.44 (0.60-10.0)

Minor H antigens without mismatches in the studied cohort are not included.
* Two-sided Pearson Chi-square test
** OR=odds ratio by logistic regression (95% confidential interval)

No significant influence of minor H antigens on graft rejection and graft loss

The incidence of one or more rejection episodes and rejection with graft loss was very low in our cohort with n=36 (8,1%) and n=8 (1,8%) respectively. Renal graft rejection was defined by decreased renal function and confirmed by biopsy in almost all patients (34 out of 36 patients).The follow-up time of the recipients varied from a few days to 23 years, with a median time of follow up of 3,6 years. We therefore decided to compare patients who had one or more rejection episode(s) with (n=8) or without (n=28) graft loss, with patients with a minimum follow-up of ten years without complications (n=44). There was no significant difference between these two groups in respect to overall gender mismatch, HLA-restricted HY mismatch, 1 or more autosomally encoded minor H antigen mismatch and CD31 mismatches (table 3). The numbers of the individual minors were too small to analyze their association with a rejection episode separately (table 4).

Discussion

Although our study is unique in its quantity of HLA-identical sibling renal transplantations, no significant influence was observed of neither autosomally nor Y chromosome encoded minor H antigen in relation to graft rejection. In our cohort the incidence of a rejection episode and especially graft loss was very low. This can partly be explained

by the short follow-up time (median 3.6 years) of most of these transplantations. It is likely that a proportion of the studied transplantations will have a rejection episode in the coming years since the number of rejections increases in time[1, 2]. Another plausible explanation is the use of immune suppressive drugs in most but not all of the studied patients. Note that all patients were on immunosuppressive drugs during a transplant rejection episode with or without graft loss. Lately, mainly in HLA-identical renal transplantations, immunosuppressive drugs are tapered and sometimes completely stopped which is successful in a number of cases[27-29]. Although today new immunosuppressive drugs, with less side effects, are introduced, lifelong immune suppression still leads to significant comorbidity[30].

Evidently, the ultimate goal in allografting is transplantation tolerance. Indeed, long term renal transplant tolerance in a HLA identical sibling transplant recipient was shown to be associated with minor HA-1 antigen mismatch[19]. Related follow up studies are currently performed in order to identify patients with natural transplantation tolerance prior to organ transplantation[31]. In our current study, we cannot identify minor H antigens associated with graft survival as marker of tolerance since the minor H mismatches are equally distributed amongst the few recipients with rejection episodes and amongst those with long term graft survival.

With the current rapid identification of new minor H antigens, kidney tissue specific antigens might be identified as well. It seems reasonable that especially these antigens might play a role in HLA-identical renal transplantation.

In conclusion, this study, executed under the auspices of the IHIWS with the participation of 16 laboratories, enabled statistical analyses of 15 autosomally and 10 Y-chromosomally encoded minor H antigens in 444 HLA identical renal transplantations. Taken into account that minor H antigen mismatching may affect different outcomes in HLA matched renal transplantation, a much larger study cohort combined with a case-control study is required to give insight into minor H antigen immune responses in transplantation tolerance and rejection. Above all, long term in vitro monitoring of the phenotype of minor H antigen specific immune responses in recipients of HLA identical grafts, with or without immunosuppression, is crucial.

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