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HLA Associations and Risk of Posttransplant Lymphoproliferative Disorder in a Danish Population-Based Cohort

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Background. Posttransplant lymphoproliferative disorder (PTLD) is a feared complication to organ transplantation, associated with substantial morbidity and inferior survival. Risk factors for PTLD include T cell–depleting induction therapy and primary infection or reactivation of Epstein-Barr virus. Possible associations between certain HLA types and the risk of developing PTLD have been reported by other investigators; however, results are conflicting. **Methods.** We conducted a retrospective, population-based study on 4295 Danish solid organ transplant patients from the Scandiatransplant database. Having identified 93 PTLD patients in the cohort, we investigated the association of HLA types with PTLD, Epstein-Barr virus status and time to PTLD onset. The outcomes survival and PTLD were evaluated using Cox regression; mismatching, and the PTLD-specific mortality were evaluated in a competing risk analysis. **Results.** Risk of PTLD was associated with male sex (odds ratio, 1.70; 95% confidence interval, 1.07-2.71), and, in women, HLA-DR13 conferred an increased risk (odds ratio, 3.22; 95% confidence interval, 1.41-7.31). In multivariate analysis, HLA-B45 and HLA-DR13 remained independent predictive factors of PTLD. Mismatching in the B locus was associated with a reduced risk of PTLD ($P < 0.001$). Overall survival was poor after a PTLD diagnosis and was significantly worse than that in the remaining transplant cohort ($P < 0.001$). **Conclusions.** Our data indicate risk-modifying HLA associations, which can be clinically useful after transplantation in personalized monitoring schemes. Given the strong linkage disequilibrium in the HLA region, the associations must be interpreted carefully. The large size, virtually complete ascertainment of cases and no loss to follow-up remain important strengths of the study.

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The HLA genes are highly polymorphic, resulting in a wide variety of specificities in the peptide-binding grooves. The HLA specificities play a crucial role in infectious and inflammatory conditions[1] and have also been associated with susceptibility to certain hematological diseases.[2-4] Post-transplant lymphoproliferative disorder (PTLD) is a feared complication to organ transplantation, associated with substantial morbidity and inferior survival.[5] Confirmed risk factors for PTLD include T cell–depleting induction therapy, and primary infection or reactivation of Epstein-Barr virus (EBV),[6] underlining the importance of T-cell surveillance in antitumor immune responses. Possible associations between certain HLA types and the risk of developing PTLD have been reported by other investigators.[7-10] However, conflicting results and short follow up (3-10 years) have made it difficult to draw firm conclusions with regard to the importance of these associations. Because the incidence of EBV-negative PTLD increases with follow-up, this might be an underreported group, and nonviral causes need also to be considered. With the increasing possibilities for monitoring and preemptive treatment, information regarding risk factors, even before transplantation, could be of considerable importance in the management of these patients.

METHODS

Study Population

This was a retrospective, population-based study including all 4295 heart, kidney, heart-lung or lung solid organ transplantations (SOTs) performed in Denmark during the period January 1 1995, to Feb 19th 2013. Data were recorded in Scandiatransplant,[11] which contains data on all SOTs carried out in Denmark since 1995, including information on HLA-typings of both donors and recipients. All HLA typings were performed in laboratories in Copenhagen and Aarhus, accredited by the "European Federation for Immunogenetics" in accordance with current standards. For MHC class I, HLA-A and -B, and for MHC class II, HLA-DR were recorded. It was possible to determine the killer immunoglobulin-like receptor ligand HLA-Bw4 and HLA-Bw6 status in 3962 and in 3973 patients, respectively, based on http://hla.alleles.org/antigens/bw46.html.

Cases of PTLD were identified in the Danish Scandiatransplant cohort by combined retrieval of data from the Danish Cancer Registry and the Danish Lymphoma Group Registry. This strategy was applied to maximize capture of incident lymphoma cases. The Danish Cancer Registry has recorded incident cancer cases on a nationwide scale since 1943 with accurate and almost complete ascertainment of cases.[12] Lymphoma diagnoses were coded according to the *International Classification of Diseases* (ICD) *for Oncology* (ICD-O-1-3) from 1977 to 2003 and according to ICD-10 since 2004. All cases recorded from 1978 onward have subsequently been converted to ICD-10 codes, including codes for lymphoma subtypes. The PTLDs occurring up to 3 months after graft loss, and subsequent tapering of immunosuppressive therapy, were included. Patient status (alive or deceased) was retrieved from the Danish civil registration registry, including date if deceased. All data sources were linked using the civil registration number, a unique 10-digit personal identifier assigned at birth or immigration to all Danish residents since 1968. The number encodes sex and date of birth. It permits valid and unambiguous linkage between all registries and clinical databases in Denmark and ensures no loss to follow-up.

This initial strategy identified 88 PTLDs in the Danish Scandiatransplant cohort. A further 5 PTLDs in patients transplanted in the period 1990 to 1995, and in which cases, HLA data were available, were identified through the participating pathology departments. Thus, a total of 93 PTLDs were included in the correlation analyses.

The histopathology of all cases of PTLD identified among cohort members were reviewed by expert hematopathologists (coauthors K.B. or S.H.D.) and reclassified according to current World Health Organization (WHO) 2008 criteria,[13] provided a sufficient amount of tumor tissue was available. In cases with inadequate tumor tissue (n = 41), the original pathology reports and chart review unequivocally confirmed the PTLD diagnosis.

Detailed clinical data were obtained from the Danish Lymphoma Group Registry and from medical records. The study was approved by the Central Denmark Region Committees on Health Research Ethics (record no. 41309) and the Danish Data Protection Agency (record no. 1-16-02-247-13) and was conducted in accordance with the Helsinki Declaration.

Immunohistochemistry for Revision of Tumor Classification

Primary diagnostic formalin fixed paraffin-embedded tissue samples containing representative PTLD were available from 52 patients; samples of these were included in a tissue microarray (TMA). Briefly, three 1-mm diameter tissue cores were identified in tumor areas, punched out and re-embedded in recipient blocks using a TMA master-01 (3DHISTECH Ltd., Budapest, Hungary).

Immunohistochemical stains were performed on 4 μm formalin fixed paraffin-embedded tissue sections according to standard antibody-specific protocols, optimized in house for use with the Ventana Benchmark automated staining system (Ventana Medical Systems, Tucson, AZ). For review of the classification of the tumors according to WHO 2008 criteria, a panel of markers was applied consisting of CD3 (polyclonal, Dako, Glostrup, Denmark), CD5 (SP19, Ventana Medical Systems), CD4 (SP35, Ventana), CD8 (SP57, Ventana), CD10 (SP67, Ventana), CD20 (L26, Ventana), CD79a (MRQ-48, Ventana), CD30 (Ber-H2, Dako) and MUM1 (MUM1P, Dako). The EBV antigens were visualized using antibodies to LMP (LMP clones CS.1-4, Dako) and EBNA2 (PE2; Abcam, Cambridge, UK). EBV-RNA (EBER) expression was identified by in-situ hybridization (ISH iView Blue Detection kit; Ventana). In each case, EBV status in the PTLD cell population was evaluated by the authors (S.H.D. and M.V.) and recorded as either positive or negative.

HLA Supertypes

The class I major histocompatibility complex molecule presents intracellularly derived peptides from protein processing. Although these receptors are extremely polymorphic, class I molecules can be clustered into 9 so-called supertypes that bind largely overlapping peptide structures.[14] In addition to carrier frequencies, the HLA class I phenotypes were also categorized according to the deduced supertype.

Statistical Analyses

Associations between HLA typings, deduced HLA supertypes, EBV status and PTLD occurrence were evaluated using

logistic regression, and risk estimates presented as odds ratios (OR). Time to PTLD was examined by linear regression after log-transformation to obtain normal distribution of data. Only HLA types constituting 1% or greater in our cohort were included in the analyses. To adjust for multiple hypothesis testing, the first Benjamini-Yekutieli method[15] was applied to control the false discovery rate within each HLA type and adjusted *P* values (q values) were reported.

Outcomes were evaluated by performing Kaplan-Meier time-to-event analyses and were compared using the log-rank test or Wilcoxon test for nonproportional data. Overall survival (OS) was calculated from time of diagnosis of PTLD to death from any cause, and progression-free survival (PFS) as time from first diagnosis to confirmed relapse, progression or death from any cause. For both OS and PFS, patients were censored at November 24, 2014. The PTLD-associated mortality was evaluated in a competing risk model using a pseudovalues method,[16] and outcome was assessed by the methods described by Pepe and Mori.[17] Similarly, HLA mismatching of donors and recipients and PTLD occurrence were evaluated in a competing risk model with death without PTLD and graft loss without PTLD as competing events; in the analysis for EBV-positive PTLD, EBV-negative PTLD was also considered a competing risk, and vice versa for EBV-negative PTLD. Risk estimates at fixed timepoints were calculated using the pseudovalues approach and expressed as relative risk (RR) with 95% confidence intervals (95% CI).[18] Cox proportional hazards regression analyses were employed to evaluate independent prognostic factors for outcome. Variables with *P* less than 0.10 in univariate analysis were entered into the multivariate analysis as covariates.

Significance was defined as *P* less than 0.05, and all statistical analyses were performed using STATA IC 12 (StataCorp, College Station, TX).

RESULTS

Clinicopathological Features of the Patients

The characteristics of the patients are summarized in Table 1 (the subgroup of patients in the TMA has been described previously[19]). Patients developing PTLD were transplanted at a significantly younger age (mean age 41 years vs 44 years; *P* = 0.018), and the majority (74%) were men. Epstein-Barr virus was present in the tumor cell population in 60 of 77 (78%) cases with known EBV status. Comparing patients with EBV-negative and EBV-positive PTLDs, no significant age differences were found at either the time of transplantation or at the time of PTLD presentation. The median time to PTLD was 2.2 (interquartile range, 0.8-5.9) and 7.2 (interquartile range, 2.4-9.1) years for EBV-positive and EBV-negative PTLD, respectively (*P* = 0.011), and 3.3 years (interquartile range, 1.0-7.7 years) for the combined PTLD cohort. In the whole cohort, the median follow-up from transplantation to censoring was 7.1 years (interquartile range, 3.7-11.8 years) and did not differ between the patients who developed PTLD and those who did not (*P* = 0.116).

Host-Related Risk Factors of PTLD

An overview of all HLA phenotype frequencies in transplanted patients is provided in **Table S1** (**SDC**, http://links.lww.com/TXD/A7). Comparing the PTLD cohort with the rest of the transplanted patients, HLA-B45 was more common in PTLD patients (4.3% vs 1.4%).

The risk of developing PTLD was strongly associated with male sex (age-adjusted $OR_{♀}$, 0.59; 95% CI, 0.37-0.93; $OR_{♂}$, 1.71; 95% CI, 1.07-2.71; *P* = 0.040). This applied to all HLA specificities and deduced supertypes. For women only, HLA-DR6 and HLA-DR8 expression tended to correlate with a higher risk ($OR_{♀}$, 2.28; 95% CI, 1.02-5.14 and

TABLE 1.

Baseline characteristics of all transplantations

	PTLD patients N (%)	Transplantations without PTLD N (%)	*P*
Number	93 (100)	4202 (100)	
Age at transplantation, mean (range), y	41 (1-68)	44 (0.5-83)	0.018
Sex (%)			
Male	68 (73)	2595 (62)	0.026
Female	25 (27)	1607 (38)	
Organ transplant (%)			
Kidney	72 (77)	3175 (75)	0.158
Heart	12 (13)	469 (11)	
Lung	8 (9)	550 (13)	
Heart and lung	1 (1)	8 (1)	
Transplantation per calendar period (per year)			
Before 1995 (not included in rate calculation)	5	0	0.000
1995-1999	33 (6)	1045 (208)	
2000-2004	26 (4.6)	1125 (225)	
2005-2009	21 (4.0)	1160 (232)	
2010-2013[a]	8 (2.5)	872 (277)	
Induction therapy			
Antithymocyte globulin	31 (33)	1367 (33)	0.638
CD25 antibody	39 (42)	1928 (46)	
EBV serostatus			
R–/D+	10 (11)	66 (2)	[b]
R+/D+	3 (3)	566 (13)	
Unknown	80 (86)	3570 (85)	
Previous malignancy			
Nonmelanoma skin cancer	13 (14)	Unknown	
Other[c]	6 (6)	Unknown	
Histological subtype of PTLD			
Early lesions	14 (15)	NA	
Polymorphic lesions	6 (7)		
Monomorphic lesions	73 (78)		
Diffuse large B-cell lymphoma	58 (62)		
Classical Hodgkin lymphoma	5 (5)		
Burkitt lymphoma	3 (3)		
Marginal zone lymphoma	2 (2)		
T-anaplastic large cell lymphoma	2 (2)		
Malignant lymphoma unspecified	2 (2)		
Mantle cell lymphoma	1 (1)		

[a] Cutoff at February 19, 2013.

[b] Due to large amount of missing data statistical analyses were regarded inappropriate.

[c] 1 renal cell carcinoma, 2 prostate cancer, 1 melanoma, 1 carcinoid tumor, 1 ductal carcinoma of the breast.

$OR_{♀}$, 3.20; 95% CI, 1.07-9.55), respectively, adjusted *P* values were not significant). When looking at the splits of HLA-DR6, this higher risk was solely attributable to the HLA-DR13 split ($OR_{♀}$, 3.22; 95% CI, 1.41-7.31; *P* = 0.045). Comparing the EBV-positive PTLD patients versus patients without PTLD, an increased OR for carriers of the split HLA-B45 (OR, 5.83; 95% CI, 2.25-15.10; *P* < 0.001) was found. None of these phenotypes was associated with EBV-negative PTLD. In the latter, only HLA-B57 (OR, 5.78; 95% CI, 1.62-20.54; *P* = 0.072) showed a trend toward an increased risk. All estimates were adjusted for sex.

The relevant ORs are presented in Figure 1, and the sex-specific ORs in **Figure S1 (SDC,** http://links.lww.com/TXD/A7).

Differences in HLA Phenotypes Between EBV-Positive and EBV-Negative PTLD

In the PTLD cohort, the EBV-positive lymphoproliferations developed significantly earlier after transplantation than the EBV-negative ones (see above). However, this effect of EBV was influenced by the patient's HLA type. The presence of HLA-A2 and HLA-B15 were consistently associated with a shorter time to PTLD onset for EBV-positive tumors (*P* = 0.028 and 0.03, respectively); for the HLA types, A10, A11, B14, B18, B21, and B22, the EBV-positive PTLD presented earlier regardless of whether the HLA type was expressed or not (all *P* values, 0.011-0.037). In contrast, for HLA-A1, -A3, -A9, -A19, -A28, -B5, -B7, -B8, -B13, -B16, -B17, -B27, -B35, -B37, -B40, -DR2, -DR3, -DR5, -DR7, -DR8, and -DR9, when present, the time to PTLD was not associated with the EBV status of the tumor (all *P* values between 0.011 and 0.043). When examining the splits, the results were consistent with those of the broad specificities, except for HLA-B45[12], in which EBV-positive PTLD always had a shorter time to onset, no matter the expression status (*P* = 0.015; group 2).

HLA Mismatching and Risk of PTLD

The implications of HLA mismatching on PTLD development are depicted in Figure 2. At the HLA-B locus, zero mismatches were associated with a significantly higher incidence of PTLD (*P* < 0.001) compared with 1 to 2 mismatches; this corresponded to an RR for 1 to 2 mismatches at 5 and 10 years of 0.42 (95% CI, 0.23-0.75; *P* = 0.003) and 0.52 (95% CI, 0.31-0.86; *P* = 0.010), respectively. At 5 years after transplantation, HLA-DR mismatching was associated with a small, but statistically significant, risk reduction for EBV-negative PTLD compared with no mismatches (RR, 0.07; 95% CI, 0.008-0.57; *P* = 0.013). The HLA-A mismatching had no impact on PTLD incidence. The number of mismatches for both HLA-A and -B was significantly lower in living donor transplantations (*P* < 0.001 for both). However, donor type itself (i.e., living versus deceased) was not associated with PTLD occurrence (*P* = 0.525).

The risk of PTLD, as illustrated by the incidence rate, differed according to time after transplantation and to the EBV status of the tumor (**Table S2, SDC,** http://links.lww.com/TXD/A7). The previously reported bimodal pattern[20] is in our data attributable to the variation in EBV expression (Figure 3), with PTLD cases from the first incidence peak developing soon after transplantation being largely EBV positive, followed over 15 to 20 years after transplantation by a rise in EBV-negative PTLD.

Results of the univariate and multivariate analyses for risk of developing PTLD are listed in Table 2. The individual variables were chosen based on the results of the regression analyses and predefined variables of interest. The multivariate analysis was adjusted for sex, because of the observed sex-related differences. Expression of HLA-B45–, HLA-DR13–, and the HLA-A2–associated supertypes remained independent predictive factors for the risk of developing PTLD (Table 2).

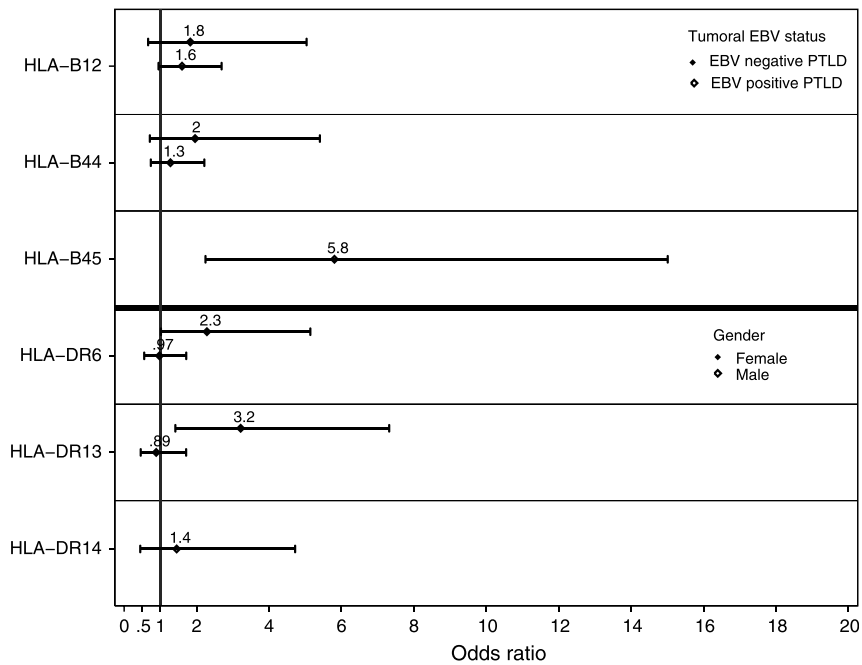

FIGURE 1. Sex- and EBV-specific odds ratios for significant split specificities with regard to development of posttransplant lymphoproliferative disorder.

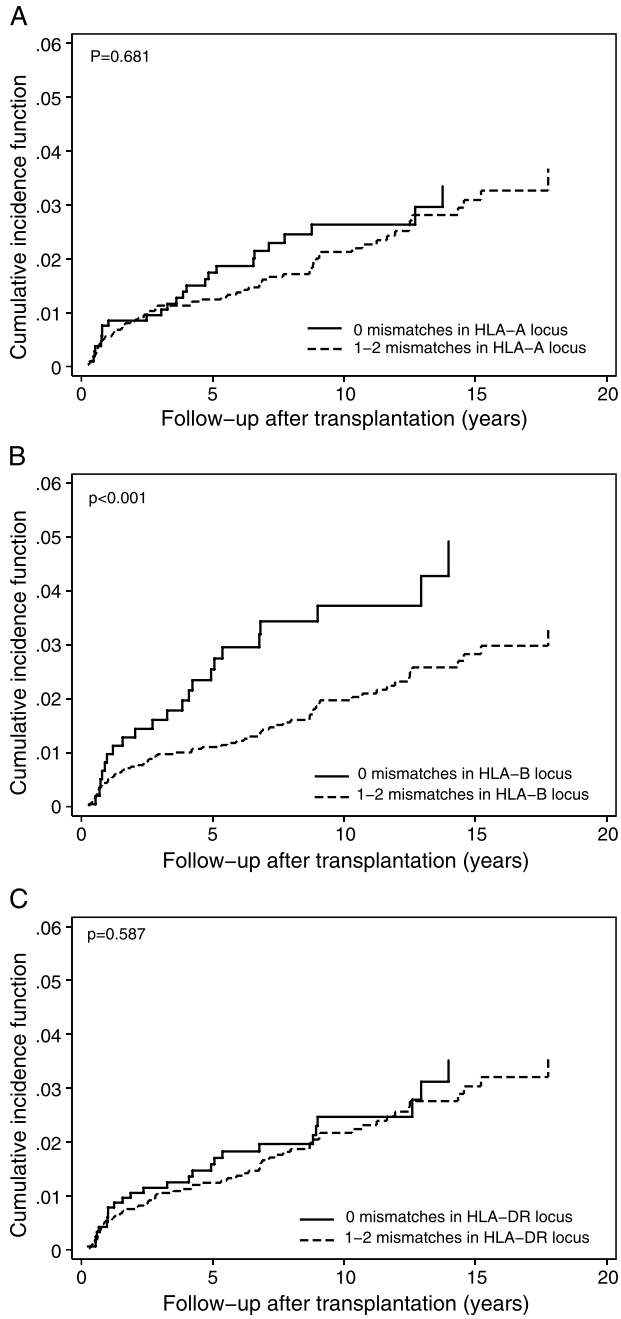

FIGURE 2. Donor-recipient HLA mismatches in PTLD development. Graphs show the PTLD cumulative incidence function of HLA-A mismatches (A), HLA-B mismatches (B), and HLA-DR mismatches (C).

Survival Analyses

With a mean follow-up of 4 years (range, 0-18 years) from onset of PTLD, the 5-year OS and PFS, for all PTLD patients taken as 1 group, were 58% (95% CI, 47-68%) and 51% (95% CI, 38-58%), respectively (Figure 4). As expected, patients with monomorphic disease did considerably worse than patients with early/polymorphic lesions (log-rank $P = 0.022$). Also, mismatching at the DR locus was associated with poorer survival (5-year OS, 70% (45-85%), 62% (47-75%), and 29% (11-49%) for 0, 1, and 2 mismatches, respectively; log-rank $P = 0.013$).

The variables assessed at univariate level are listed in Table 3. In the multivariate Cox regression analysis, we included HLA-DR mismatches, rituximab treatment, WHO performance status, nodal versus extranodal disease, and Ann Arbor stage. All except HLA-DR mismatches retained independent prognostic value in the multivariate analysis (Table 3).

Next, we evaluated PTLD-specific mortality (Figure 4) in the total cohort of transplanted patients. The PTLD patients had a significantly higher mortality compared with the remaining cohort population, with a risk difference of 24% (95% CI, 15-33%) at 5 years and 49% (95% CI, 36-62%) at 10 years after transplantation ($P < 0.001$ for both risk estimates).

DISCUSSION

In this large population-based cohort of Danish SOT patients, we identified several host-related risk factors for the development of PTLD and performed outcome analyses in various patient subsets.

We identified HLA types associated with PTLD according to: time to PTLD onset, tumoral EBV status, sex and survival. So far, the data on HLA typing related to the development of PTLD is scarce and discordant.[7-10] Three studies also included a variety of organ transplantations from both living and deceased donors in mainly white populations; Reshef et al[9] identified HLA-A26 as the main PTLD risk locus, Subklewe et al[7] reported HLA-B18 and -B21 as main risk factors, whereas Lustberg et al[10] pointed to HLA-B40. In our cohort, HLA-A26 was only found in 5% of the transplanted patients and in 8% of those who developed PTLD. Corresponding values for HLA-B21 were 4% and 2% in the 2 groups, respectively, whereas HLA-B18 was found in 6% of patients in both groups. The HLA-B40 was also almost equally expressed in control and PTLD patients. The fourth study by Pourfarziani et al[8] is markedly smaller, included only renal transplantations, and reported data from an Iranian patient cohort with an expectedly different immunological background to our patients. The primary risk loci in our study (HLA-B45 and -DR13) have never been reported before and remained independent predictive factors for the risk of developing PTLD in multivariate analysis. They were both identified in patient subgroups, and we did not identify any risk alleles in the combined cohort. These differences probably reflect the heterogeneity of the analyzed patient cohorts and emphasize the need for a detailed

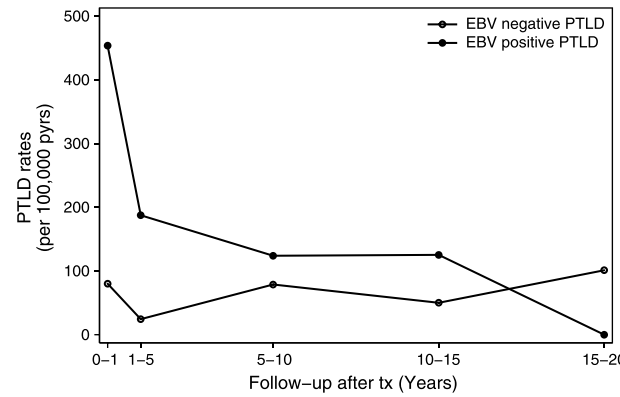

FIGURE 3. Incidence rates for PTLD according to EBV status of the tumor.

TABLE 2.

Cox regression analyses for PTLD development

Variables	PTLD patients N (%)	Univariate HR (95% CI)	Univariate P	Multivariate[a] HR (95% CI)	Multivariate[a] P
Sex					
Male	68 (73)	ref	0.055		
Female	25 (27)	0.64 (0.40-1.01)			
Time covariates					
Age at transplantation, mean(range)	47 (2-77)	0.99 (0.98-1.00)	0.240		
HLA mismatches					
A					
0	25 (27)	ref			
1-2	68 (73)	0.94 (0.60-1.49)	0.801		
B					
0	23 (25)	ref		ref	
1-2	70 (75)	0.57 (0.35-0.91)	0.018	0.61 (0.37-1.01)	0.055
DR					
0	26 (28)	ref			
1-2	67 (72)	0.93 (0.59-1.46)	0.757		
HLA carrier status					
HLA-A2	41 (48)	0.71 (0.46-1.08)	0.108		
HLA-B44	26 (30)	1.34 (0.85-2.12)	0.217		
HLA-B45	4 (5)	3.62 (1.33-9.88)	0.012	3.52 (1.28-9.67)	0.015
HLA-DR13	20 (23)	1.57 (0.96-2.58)	0.072	1.70 (1.03-2.79)	0.037
Supertype A2	41 (48)	0.63 (0.41-0.96)	0.033	0.62 (0.40-0.95)	0.027
Donor type					
Living	24 (26)	ref			
Deceased	66 (71)	1.18 (0.74-1.88)	0.489		
Unknown	3 (3)				
Organ transplanted[b]					
Kidney	72 (77)	ref			
Heart	12 (13)	1.46 (0.76-2.79)	0.255		
Lung	8 (9)	1.23 (0.55-2.73)	0.620		
Heart-lung	1 (1)	6.32 (0.86-46.51)	0.070	4.87 (0.66-36.02)	0.212
Induction therapy					
Antithymocyte globulin	31 (33)	ref			
CD25 antibody	39 (42)	1.26 (0.74-2.17)	0.396		
Unknown[c]	23 (25)				

[a] Adjusted for sex.

[b] Adjusted for transplant center.

[c] The group is formed of kidney transplantation, who all received induction.

characterization of donor, recipient and tumor biology to facilitate cohort comparisons.

One of the strongest associations revealed by our study was the significantly higher risk of PTLD in male patients. The present report is one of the few to identify this association, and is the first to link a sex-related risk to a specific HLA phenotype. An increased risk related to male sex was previously reported by Dharnidharka et al,[21] Morton et al,[22] and Lustberg et al[10] and is consistent with the general male preponderance also seen in lymphomas occurring in non-overtly immunosuppressed individuals. The male preponderance was found throughout all HLA phenotypes and supertypes. Furthermore, our findings indicate a difference in PTLD-associated HLA types in men and women, as is also the case in chronic lymphocytic leukemia.[2] Interestingly, 2 of the disease-associated HLA types identified in this study (HLA-B45 and HLA-DR13) play similar roles in the context of EBV infection because they both present immediate-early proteins (BRLF1 and BZLF1) expressed in the lytic cycle.[23] These same proteins are also presented by HLA-B18, which in a large cohort of diffuse large B-cell lymphomas correlated significantly with outcome, and was associated with PTLD.[3,7] Moreover, in humans, viral peptide specific CD4+ T-cells play a crucial role in controlling both primary and recurrent infections. It has been shown, that sex-specific differences in memory T-cell responses exist,[24-26] although available data are limited, and an increased risk in men might be attributable to factors other than the HLA system. The influence of EBV on PTLD development is unquestionable; however, the rate of EBV-negative PTLD increases with follow-up. In our series, the latest observed case of PTLD occurred 17 years after transplantation; the long follow-up without loss of cases is one of the main strengths of this study. It is unclear whether these "late occurring" EBV-negative

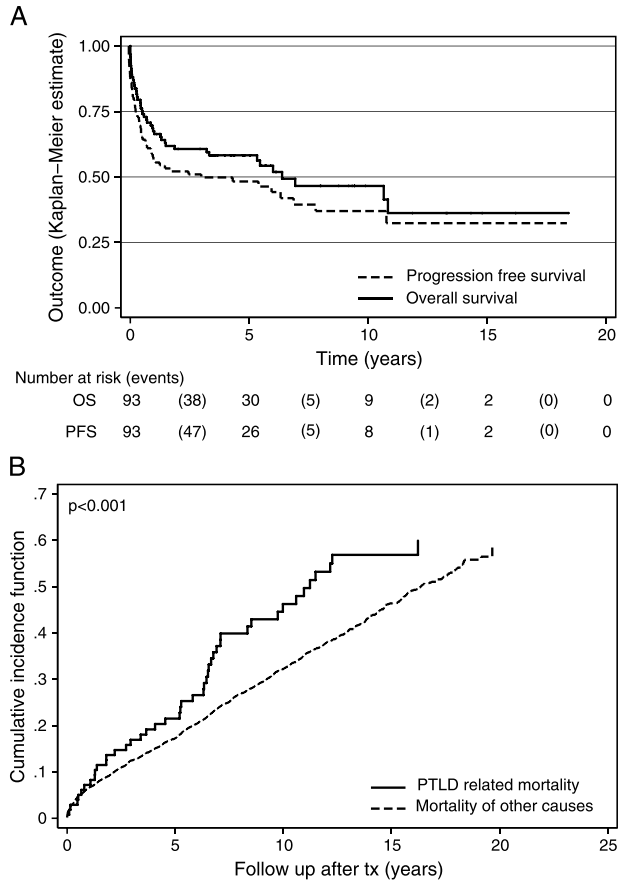

FIGURE 4. Outcome. Progression-free survival, overall survival and PTLD-related mortality. A, All PTLD patients. B, The PTLD-related mortality compared with mortality in the remaining cohort of transplant patients.

lymphomas represent sporadic disease, result from "hit and run" EBV infection, or evolve after many years as the result of the accumulated effect of various possible transplantation-associated factors, such as chronic antigen stimulation by the graft or long-term immunosuppressive medication. We identified 1 HLA-allele (HLA-B45) associated with EBV-positive lymphoproliferations, in line with the findings from 2 gene expression profiling studies, that suggest that PTLDs segregate according to EBV status[27,28] rather than histological subtype. The differences between EBV-negative and EBV-positive PTLDs based on tumoral EBV status were investigated in 2 studies[7,9]; these identified differently associated HLA types, although these did not overlap our results. A recent study was not able to confirm these reported findings,[29] despite using the largest EBV-positive cohort to date. Differences in EBV latency programs and varying tumor histology (B-cell vs T-cell, indolent vs aggressive, polyclonal vs monoclonal) may explain this, as well as the inconsistent results.

In line with previous reports, we found the HLA-A2 supertype to be correlated with a lower occurrence of lymphoproliferation.[4,30] HLA-A2 is capable of inducing effective cytotoxic T-cell responses against EBV latent antigen peptides in many individuals,[23] thereby probably protecting against reactivation. Interestingly, HLA-A2 presents various minor H antigens (mHags).[31] In allogeneic hematopoietic stem cell transplantation, mHag disparities increase the frequency and strength of graft-versus-host and graft-versus-leukemia effects. In a multicenter study, a significantly lower relapse rate in patient/donor pairs mismatched for hematopoietic mHags was demonstrated,[32] and it is plausible that the cytotoxic T-cell response elicited by mHag mismatching translates into a more effective tumor control.

Our findings indicate a protective effect of mismatches in the B-loci overall, and of DR-mismatches in EBV-negative PTLD. Because the mechanisms of how HLA types are associated to PTLD development are largely unknown, possible explanations for the protective effect of HLA-B-locus mismatching against PTLD development remain speculative. Roughly a quarter of our patients received a graft from a living donor, some of which were related and thus possibly matched for unknown antigens, which might have been of greater importance in determining susceptibility to PTLD than the possible impact of HLA type matching. Wong et al[33] found a similar association, although in lung transplant patients, and attributed this observation to impaired tumor surveillance secondary to decreased alloreactivity in matched patients. Also in this field, published results are conflicting. Thus, 2 large studies by Caillard et al[34] and Faull et al,[20] which also included both deceased and living donors, found no associations between HLA mismatching and risk of PTLD. In contrast 3 other large studies[35-37] reported increased risks of PTLD with, respectively, increased HLA-DR-, HLA-B- and overall number of mismatches. However, several factors make direct comparisons with our work difficult. The study by Opelz et al[35] included only deceased-donor transplantations and cases of posttransplant non-Hodgkin lymphomas. In addition, their follow-up was 3 years after transplantation, thus excluding most EBV-negative and a substantial proportion of the EBV-positive PTLDs from their analysis. We also used a different methodology (a competing risk model) as the present competing events would bias the Kaplan-Meier estimate.[38] Similarly, the estimates in the study by Bakker et al[36] are not easy to interpret, as the authors included several variables in a Cox regression model which counted 20 events.[39] Caillard et al[34] later reported no association with HLA mismatches. Although no definitive causal explanations can be given, we find it likely that mismatching results in a more vigorous immune response against donor-derived peptides, thereby creating a less permissive tumor microenvironment.

Patient survival was poor after a diagnosis of PTLD, although the introduction of treatment with the CD20 antibody rituximab has improved survival and also remained an independent prognostic factor. Poor performance status and widespread disease were also independently associated with a dismal outcome, reflecting both aggressive disease and probably also the frailty of the patients, which does not allow for too aggressive treatment strategies. The impact of HLA mismatching on OS is in line with previous reports.[40-42]

A major limitation of our study is the lack of information on the immunosuppressive treatment used in the total cohort after transplantation, because different regimens are associated with risk of PTLD.[43] We did, however, adjust for transplantation site in the Cox analysis to account for different strategies at the various transplantation centers, and this did not show any association with PTLD development. Also, we had only scarce information on donor/recipient EBV seromismatches at time of transplant, which might explain some of the varying HLA associations in the literature, and our results should be interpreted in the light of this. The large

TABLE 3.

Patient and PTLD characteristics with cox regression univariate and multivariate analyses for mortality

			Univariate Analyses		Multivariate Analysis	
Variables		**Patients N (%)**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Sex						
Male		68 (73)	ref			
Female		25 (27)	0.54 (0.24-1.12)	0.104		
Time covariates						
Time to PTLD, mean (range)		5 (0.25-17)	0.96 (0.90-1.03)	0.297		
Year of transplantation (5-y intervals)		93 (100)	0.91 (0.69-1.20)	0.498		
HLA mismatches						
A	0	19 (22)	ref			
	1	52 (60)	1.59 (0.70-3.62)	0.219		
	2	16 (18)	1.85 (0.67-5.12)	0.233		
B	0	17 (20)	ref			
	1	40 (46)	0.76 (0.32-1.77)	0.523		
	2	30 (34)	1.47 (0.66-3.28)	0.348		
DR	0	20 (23)	ref			
	1	47 (54)	1.01 (0.44-2.27)	0.994		
	2	20 (23)	2.57 (1.07-6.18)	0.035	2.40 (0.86-6.69)	0.094
HLA carrier status						
HLA-A2		40 (47)	0.87 (0.46-1.65)	0.675		
HLA-B44		26 (30)	1.25 (0.64-2.43)	0.513		
HLA-B45		4 (5)	1.54e-15 (0)	1.0		
HLA-DR13		20 (23)	0.82 (0.38-1.78)	0.614		
Supertype A2		41 (48)	0.86 (0.46-1.64)	0.655		
Supertype B44		45 (48)	0.79 (0.44-1.43)	0.438		
Immunosuppressive treatment						
Cyclosporin		69 (79)	1.78 (0.69-4.56)	0.230		
Tacrolimus		35 (41)	0.59 (0.29-1.18)	0.136		
MMF		65 (75)	0.72 (0.37-1.43)	0.351		
Azathioprine		20 (24)	1.90 (0.96-3.79)	0.067		
Induction therapy[a]						
Antithymocyte globulin		31 (33)	ref			
CD25 antibody		39 (42)	0.63 (0.27-1.50)	0.297		
Presentation						
Early (<1 y)		23 (25)	ref			
Late (>1 y)		70 (75)	1.11 (0.56-2.20)	0.762		
Stage						
I-II		54 (59)	ref			
III-IV		38 (41)	2.29 (1.26-4.16)	0.007	2.22 (1.31-4.36)	0.020
Primary localization						
Nodal		43 (46)	ref			
Extranodal		50 (54)	2.23 (1.20-4.15)	0.012	2.57 (1.18-5.57)	0.017
WHO performance score						
0-2		73 (81)	ref			
3-4		17 (19)	5.73 (2.96-11.07)	0.000	6.32 (3.00-13.35)	0.000
EBV status of tumor						
Positive		60 (78)	ref			
Negative		17 (22)	0.88 (0.38-2.01)	0.753		
Graft PTLD						
Present		8 (9)	1.61 (0.68-3.84)	0.280		
Absent		85 (91)	ref			
Type PTLD						
Early/polymorphic lesion		20 (22)	ref			
Monomorphic PTLD		64 (78)	1.61 (1.05-2.47)	0.029		
PTLD treatment						
Rituximab		45 (49)	2.05 (1.09-3.85)	0.025	2.96 (1.48-5.93)	0.002

[a] Adjusted for organ transplanted.

size, inclusion of both early and monomorphic lesions, virtually complete ascertainment of cases, and no loss to follow-up remain important strengths of the study.

In conclusion, we report several risk-modifying HLA associations, which can be clinically useful in personalized monitoring schemes after transplantation.

Given the strong linkage disequilibrium in the gene-rich HLA region, each of the HLA associations has to be interpreted carefully, as other strongly linked alleles might actually represent the true causative variant, and other non-HLA genes in the region might also be involved. Our present data should therefore be supplemented and confirmed by more extensive genomic analyses, such as whole genomic sequencing.

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