Expression of human leukocyte antigens in diffuse large B cell lymphomas

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Chapter 5

The relationship between HLA class II polymorphisms and somatic deletions in testicular B cell lymphomas of Dutch patients.

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A shorter version of this chapter has been submitted for publication

Abstract

Several risk factors have been established for non-Hodgkin lymphoma (NHL), including immune deficiencies, infections and auto-immune diseases. For diffuse large B cell lymphoma, the most common type of lymphoma, no risk factors have been described, which may be due to the intrinsic heterogeneity of this disorder. Previously, we reported that in contrast to nodal DLBCLs, the majority of testicular DLBCLs showed complete loss of HLA-DR and DQ expression, often associated with homozygous deletions of the corresponding genes. To assess the role of HLA class II polymorphisms and the role of individual class II genes in lymphomas with and without deletions, we applied DNA typing for *HLA-DRB1* and *HLA-DQB1* on 50 Dutch patients with testicular and 48 with nodal DLBCL and compared the frequencies with a cohort of healthy Dutch controls. Both the patients with nodal and those with testicular DLBCL showed a significantly higher frequency of HLA-DRB1*15 compared to the controls ($p < 0.018$, odds ratio 2.09 and $p < 0.013$, odds ratio 2.12 respectively). Moreover, a positive association was seen with *HLA-DRB1*12* ($p = 0.043$, odds ratio 4.17) in the patients with testicular DLBCL, and a negative association was seen with HLA-DRB1*07 ($p = 0.022$, odds ratio 0.13) in the patients with nodal DLBCL. Homozygous deletions of the HLA-DR/DQ region, evaluated by interphase FISH, were seen in 20 of 48 testicular tumours. No preferential loss or retention of a particular HLA-DR or DQ allele was seen, as all alleles were at least once retained or involved in a homozygous deletion.

Introduction

Non Hodgkin's lymphoma (NHL) is a neoplasm showing an upward trend in incidence in adults in the last few decades in both Europe and the USA [1-3]. The aetiology of NHL is unexplained for most of the cases but environmental as well as genetic factors presumably play a role. Some risk factors have been firmly established including immune deficiencies, infections with *Helicobacter pylori, Epstein-Barr virus* or *Hepatitis C virus*, exposure to pesticides and in particular also autoimmune disorders such as Sjögren, Hashimoto and Celiac disease that have all been associated with Human Leukocyte Antigens (HLA) [4-6]. Also a family history of haematological malignancies was shown to increase the life time risk of developing NHL including diffuse large B cell lymphoma (DLBCL) in population-based case-control studies from northern Europe and the United States [2,3 7-10]. Of all NHL, DLBCL is by far the most common type (WHO) [11]. In 40% of the cases, DLBCL primarily presents at extra-nodal sites including the testis [12,13].

Primary lymphoma of the testis is rare, accounting for 1-2% of all NHL [14], but it is the most common testicular tumour in the elderly [14-17]. Predisposing factors for primary lymphoma of the testis in non-immune compromised patients have not been established, although some authors reported associations with trauma, cryptorchism or chronic orchitis [18,19]. Previously, we reported complete loss of HLA-DR expression in the majority of primary testicular and CNS DLBCL. In contrast, few primary nodal DLBCL showed loss of expression [20]. In both the testicular and CNS lymphomas loss of HLA-DR expression was often due to homozygous deletions of the HLA-DR/DQ region on chromosome 6p21.3 [20,21]. Interestingly, both sites are considered as immune sanctuaries. Hypothetically, loss of HLA expression in these lymphomas may reflect a sequential development of (auto-immune) inflammation in which (pre)neoplastic clones develop, that upon infiltration by T cells and disruption of the immune sanctuary, may escape the immune attack by losing HLA class I and II expression.

Associations with certain HLA class II alleles have been reported for several haematological malignancies [22-25]. In the only study addressing HLA-DR/DQ typing in a group of Caucasoid DLBCL patients, the HLA-DR2 allele was reported to be an independent factor for survival [26]. One could speculate that the polymorphism of the HLA class II molecules plays a role early during lymphomagenesis through presentation of tumour- associated antigens (TAA). Malignant B cells express idiotypes (Id) on their cell surface, antigenic determinants localised to the variable (V) regions of immunoglobulin (Ig). Because of the extreme diversity of the V region caused by the initial Ig recombinations and subsequent somatic hypermutation, each neoplastic B cell clone will express highly unique monoclonal Ig that may function as TAA [27,28]. Indeed, in mouse models and in human patients, immunization

with idiotype vaccines resulted in tumour specific CTL clones and molecular and clinical remissions in lymphoma patients [29-31]. Several T cell epitopes were identified in each Id and these were presented by different HLA class I and II molecules resulting in polyclonal T cell responses [32]. Apart from Id, testicular DLBCL may express other TAA i.e. testis cancer antigens [33] or antigens derived from aberrant proteins, as these lymphomas showed a high rate of somatic hypermutation [34]. In this respect, similarities to DLBCL of the CNS, another immune privileged site are apparent. In CNS lymphomas a very high burden of somatic hypermutation of the IgH genes, but also BCL-6, PIM-1, MYC, PAX5 and RhoH/TTF, leading to idiotype changes of the Ig proteins as well as amino acid replacements of the involved proteins have been found [35].

The quality of an anti-tumour immune response not only depends on the nature of the antigen presentation by the various class I and II molecules [36,37] but also on the level of cell surface expression [38,39]. Interestingly, expression levels of the individual HLA-DRB1 genes that make up the various haplotypes are highly variable, which is related to the configuration of the individual promoter sites. Moreover, the different haplotypes also differ in the number of expressed HLA-DR genes [40-43]. This further warrants the investigation of HLA class II polymorphisms in these particular lymphomas.

The frequent occurrence of distinct somatically acquired homozygous deletions of HLA-DR and HLA-DQ genes in the tumour cells of primary DLBCL of the testis and CNS suggests the presence of frail sites in this region. In addition, the DNA content of the different haplotypes is variable with the DR3 and DR11-14 haplotypes containing the minimal amount of DNA in the DR-DQ region with DR2 having approximately 30 kb and the DR4, DR7 and DR9 haplotypes having approximately 110 kb more DNA in the DR subregion [44-46]. The difference in DNA content between the haplotypes may be the result of meiotic recombination between recombination hotspots. Indeed previous investigations in mice showed that recombination frequencies differed between haplotypes and that high sequence homology was present at recombination hotspots [47,48]. By extrapolation, certain haplotypes or genotypes may therefore also be relatively vulnerable to somatic deletion in tumour cells.

In the present study we compared HLA-DRB1 and HLA-DQB1 allele frequencies in 50 patients with testicular DLBCL, 48 with nodal DLBCL and a cohort of healthy Dutch controls [49]. In addition to HLA-DR/DQ typing, we investigated in the testicular DLBCLs whether the HLA class II genotype of the patients was of influence for genomic deletions of this region in the tumour cells.

Materials and Methods

Patients and controls

In total 98 lymphomas matching the criteria for diffuse large B cell Lymphoma (DLBCL) (WHO classification) were collected [11]. In all cases, a B cell origin was confirmed by immunohistochemical staining for CD20 or CD79a on formalin- fixed paraffin embedded tissue blocks. Fifty DLBCL were primarily derived from the testis and 48 from lymph nodes. DLBCL that secondarily presented at these sites, or DLBCL of immune compromised patients were excluded from this study.

Lymphomas were retrieved from the tissue banks of the pathology departments of the Leiden University Medical Center (Leiden, The Netherlands), VU Medical Center (Amsterdam, The Netherlands), University Medical Center Groningen (Groningen, the Netherlands), Haga Hospital (The Hague, The Netherlands), or were obtained from the NHL Registry of the Comprehensive Cancer Center West in The Netherlands between 1981 and 1989 [50]. Tissue blocks of 19 testicular lymphomas were collected by L. Looijenga from the Josephine Nefkens Institute (Rotterdam, The Netherlands). All testicular DLBCL and many of the nodal DLBCLs have been described in previous reports [20,21,51].

A panel of randomly chosen HLA-DR, -DQ typed healthy Dutch Caucasoid blood donors (N = 2400) served as a control population [49]

The *Code for Proper Use of Human Tissue* from the Federation of Medical Societies, published in 2002 and approved by Institutional Review Boards of the University Medical Centers in the Netherlands, was used.

Genomic HLA typing

HLA-DR and HLA-DQ typing of the patients were performed on genomic DNA isolated from either tumour tissue or whole blood. For generic HLA-DRB1 and DQB1, in-house designed biotin labelled sequence-specific oligonucleotide (SSO) probes were hybridized with genomic DNA and amplified with polymerase chain reaction (PCR) as has been described previously [52]. In case of typing on tumour tissue (often containing homozygous and hemizygous deletions in the lymphoma cells), we were always able to obtain a full genotype, due to the presence of large numbers of reactive T cells, dendritic cells, endothelial cells and residual Leydig and Sertoli cells within the tumour tissue. In one case with a homozygous deletion and trisomy 6 we found only one HLA-DR and one HLA-DQ allele. As we had only tumour tissue were not certain that the patient was homozygous for both alleles, we excluded this case from further analysis.

Fluorescence in situ hybridization

In total 48 testicular DLBCL were analyzed by interphase FISH for the HLA-DR/DQ region using PAC 93N13 covering *HLA-DRB1* and *HLA-DQA1* as previously described [21,51]. Nuclei were isolated from snap-frozen tumour tissue in 21 cases and from formalin-fixed paraffin embedded tumour tissue in the other 27 cases. The α-satellite centromeric 6-probe (D6Z1, Oncor, Gaitersburg, MD) was labelled with biotin-16-deoxyuridine triphosphate (dUTP; Roche Diagnostics, Mannheim, Germany). The PAC probe was labelled with digoxigenin-12-dUTP (Roche Diagnostics) by standard nick translation. Hybridization and immunodetection were performed as previously described [21]. Homozygous deletions were defined as the complete absence of PAC signals in cells with one or more preserved centromere 6 signals. Hemizygous deletions were defined by the presence of a lower number of locus-specific PAC signals relative to the number of centromere 6 signals. The cut off level for homozygous deletions was fixed at 6% of the cells and for hemizygous deletions at 21% of the cells [21].

Statistical Analysis

Haldane's modification of Woolf's method was used to calculate the odds ratio (OR). The statistical significance that the OR values differed from unity was established by the Fisher's exact test (two-sided) [53,54]. When indicated, P-values were corrected for multiple comparisons using the comparison $P_c = 1 - (1 - P_u)^n$, where P_u is the uncorrected and P_c the corrected P-value and n the number of comparisons (n = 20) [55].

Results

The association of HLA with diffuse large B cell lymphoma was analysed by comparing the frequencies of the different HLA-DRB1 and HLA-DQB1 alleles between 50 Dutch patients with testicular DLBCL, 48 Dutch patients with nodal DLBCL and a large cohort of healthy Dutch controls (see table 1). Our previous results suggested an important role for loss of HLA class II expression in the development of testicular but much less in nodal DLBCL [20,21,51,56] and we therefore analysed both groups separately. The frequency of *HLA-DRB1*15* was 42% in both patient groups compared to 26% in the control group ($p < 0.018$, odds ratio 2.09 and $p < 0.013$, odds ratio 2.12 respectively).

Compared to the controls, nodal DLBCL showed a significant negative association with *HLA-DRB1*07* (OR = 0.13, $p_c < 0.02$) and the testicular DLBCL a positive association with *HLA-DRB1*12* (OR = 4.17, $p_c < 0.043$). Subsequently, we directly compared the allele frequencies between the testicular and the nodal lymphomas. The testicular DLBCL showed

a lower frequency of *HLA-DRB1*03* compared to the nodal group (respectively 16% vs. 35%; OR 0.36, p_u 0.037/ p_c n.s.) but no significance was reached after correction. Interestingly, *HLA-DRB1*08* and its associated *DQB1*04* allele were absent in the testicular group but over represented in the nodal group (15% vs. 5%).

We previously reported homozygous and hemizygous deletions of the HLA-DR/DQ region in a group of 14 testicular DLBCLs [21,51]. In the current study, we investigated 34 additional tumours of patients with a primary DLBCL of the testis by interphase FISH using a probe 93N13 covering *HLA-DRB1* and *HLA-DQA1* in combination with a chromosome 6 specific centromeric probe. Twenty tumours showed homozygous and 14 tumours showed hemizygous deletions of this small class II region thereby confirming our previous notion that deletions of HLA-DR and -DQ genes are a prominent feature of testicular DLBCLs. The remaining 14 cases showed no deletions of this region. To check whether loss of class II genes was associated with the patients genotype, and in particular whether certain alleles were preferentially involved in deletions in the tumour cells, we compared the allele frequencies in the cases with respectively homozygous and hemizygous deletions with the other cases (see Table 2). None of the HLA alleles was explicitly involved in loss, since all alleles showed retention in at least one case. To check if specific HLA alleles precluded the occurrence of deletions in the lymphoma cells, we compared the tumours without deletions with all other tumours. Absolute retention of one particular HLA-DRB1 allele was also not seen, as all HLA-DRB1 alleles were affected by a homozygous deletion at least once. However, some DR alleles showed interesting features. Only one the 20 patients (5%) with a homozygous deletion but 6 of the 14 patients (43%) with a hemizygous deletion carried the *HLA-DRB1*11* ($p < 0.03$/ n.s. after correction). Hemizygous deletions were also frequent in the tumours of *HLA-DRB1*03* positive patients (36%, $p < 0.04$/ n.s. after correction) while again only one patient showed a homozygous deletion in the tumour.

	Nodal (N=48)		Testis (N=50)		Controls (N=2400)	Nodal v.s. Controls		Testis v.s. Controls		Testis v.s. Nodal	
HLA-	Positive	%	Positive	%	%	OR (95% c.i.)	Pu/Pc	OR (95% c.i.)	Pu/Pc	OR (95% c.i.)	Pu/Pc
DRB1*01	10	**21**	7	**14**	**20**		n.s.		n.s.		n.s.
DRB1*03	17	**35**	8	**16**	**25**		n.s.		n.s.	0.36 (0.14 - 0.92)	0.037/n.s.
DRB1*04	15	**31**	13	**26**	**28**		n.s.		n.s.		n.s.
DRB1*07	1	**2**	5	**10**	**19**	0.13 (0.03 - 0.68)	0.001/0.022		n.s.		n.s.
DRB1*08	7	**15**	0	**0**	**5**	3.18 (1.43 - 7.07)	0.015/n.s.		n.s.	0.06 (0.00 - 0.99)	0.005/n.s.
DRB1*09	0	**0**	1	**2**	**2**		n.s.		n.s.		n.s.
DRB1*10	1	**2**	4	**8**	**4**		n.s.		n.s.		n.s.
DRB1*11	6	**13**	10	**20**	**14**		n.s.		n.s.		n.s.
DRB1*12	4	**8**	8	**16**	**5**		n.s.	4.17 (1.95 - 8.93)	0.002/0.043		n.s.
DRB1*13	8	**17**	12	**24**	**28**		n.s.		n.s.		n.s.
DRB1*14	3	**6**	8	**16**	**5**		n.s.	3.47 (1.62 - 7.40)	0.006/n.s.		n.s.
DRB1*15	20	**42**	21	**42**	**26**	2.09 (1.18-3.74)	0.018/n.s.	2.12 (1.21 - 3.75)	0.013/n.s.		n.s.
DRB1*16	0	**0**	1	**2**	**2**		n.s.		n.s.		n.s.
DQB1*02	18	**38**	11	**22**	**37**		n.s.	0.49 (0.25 - 0.95)	0.026/n.s.		n.s.
DQB1*0301	13	**27**	19	**38**	**28**		n.s.		n.s.		n.s.
DQB1*0302	9	**19**	9	**18**	**20**		n.s.		n.s.		n.s.
DQB1*0303	0	**0**	1	**2**	**8**	0.12 (0.01-2.00)	0.043/n.s.		n.s.		n.s.
DQB1*04	5	**10**	0	**0**	**3**	3.80 (1.45 - 9.92)	0.023/0.37		n.s.	0.08 (0.00 - 1.46)	0.025/n.s.
DQB1*05	16	**33**	18	**36**	**35**		n.s.		n.s.		n.s.
DQB1*06	25	**52**	30	**60**	**50**		n.s.		n.s.		n.s.

Table 1: Distribution of allelic frequencies of HLA-DRB1 and HLA-DQB1 in 50 patients with testicular DLBCL, 48 patients with nodal DLBCL and 2400 healthy Dutch controls.

Legend: DLBCL = diffuse large B cell lymphoma; OR = odds ratio; P_u = uncorrected P value; P_c = P value corrected for 20 informative comparisons; n.s. = not significant

	HOZ (N = 20)	%	HEZ (N = 14)	%	RET (N = 14)	%	HOZ v.s. RET OR (95% c.i.)	Pu/Pc	HOZ v.s. OTHERS OR (95% c.i.)	Pu/Pc	HEZ v.s. OTHERS OR (95% c.i.)	Pu/Pc
DRB1*01	1	**5**	1	**7**	4	**29**		n.s.		n.s.		n.s.
DRB1*03	1	**5**	5	**36**	2	**14**		n.s.		n.s.	5.21 (1.14-23.91)	0.037/n.s.
DRB1*04	7	**35**	2	**14**	2	**14**		n.s.		n.s.		n.s.
DRB1*07	2	**10**	1	**7**	2	**14**		n.s.		n.s.		n.s.
DRB1*08	0	**0**	0	**0**	0	**0**		n.s.		n.s.		n.s.
DRB1*09	1	**5**	0	**0**	0	**0**		n.s.		n.s.		n.s.
DRB1*10	1	**5**	2	**14**	1	**7**		n.s.		n.s.		n.s.
DRB1*11	1	**5**	6	**43**	3	**21**		n.s.	0.15 (0.03-0.99)	0.031/n.s.	5.18 (1.25-21.49)	0.045/n.s.
DRB1*12	5	**25**	2	**14**	1	**7**		n.s.		n.s.		n.s.
DRB1*13	6	**30**	2	**14**	4	**29**		n.s.		n.s.		n.s.
DRB1*14	3	**15**	3	**21**	2	**14**		n.s.		n.s.		n.s.
DRB1*15	10	**50**	4	**29**	6	**43**		n.s.		n.s.		n.s.
DRB1*16	1	**5**	0	**0**	0	**0**		n.s.		n.s.		n.s.
DQB1*02	3	**15**	5	**36**	3	**21**		n.s.		n.s.		n.s.
DQB1*0301	7	**35**	6	**43**	7	**50**		n.s.		n.s.		n.s.
DQB1*0302	5	**25**	2	**14**	1	**7**		n.s.		n.s.		n.s.
DQB1*0303	1	**5**	0	**0**	0	**0**		n.s.		n.s.		n.s.
DQB1*04	0	**0**	0	**0**	0	**0**		n.s.		n.s.		n.s.
DQB1*05	5	**25**	6	**43**	6	**43**		n.s.		n.s.		n.s.
DQB1*06	15	**75**	6	**43**	8	**57**		n.s.		n.s.		n.s.

Table 2: Comparisons of percentages of the different HLA-DRB1 and HLA-DQB1 alleles involved in homozygous or hemizygous deletions or retention in testicular DLBCL.

Legend: HOZ = homozygous deletion; HEZ = hemizygous deletion; RET = retention (no deletion); N = number of testicular DLBCL

Discussion

In a cellular immune response, B-cells can initially function as antigen presenting cells via their HLA class II molecules. During the germinal centre cell reaction, B cells undergo immunoglobulin class switching and somatic hypermutation. Both processes are associated with DNA breaks and illegitimate recombinations with oncogenes [57] which may ultimately result in lymphoma cells with autonomous growth. Neoplastic B cell clones expressing tumour specific antigens may be eliminated by cytotoxic T cells (CTLs) or may undergo HLA class II mediated apoptosis [58-61]. We previously found extensive loss of HLA-DR and DQ expression in lymphomas presenting at the central nervous system and the testis [20] often due to homozygous deletions of the corresponding genes [21,51]. By loss of HLA expression these DLBCLs probably escape from immune surveillance. To investigate whether certain HLA class II alleles are associated with DLBCL and with the previously observed homozygous deletions, we performed DNA-typing of *HLA-DRB1* and *HLA-DQB1* alleles in 50 patients with testicular and 48 patients with nodal DLBCL and compared these with a large cohort of healthy Dutch controls.

Associations of HLA class II alleles with lymphoid malignancies have been described before. Familial Hodgkin's lymphoma was associated with the *HLA-DRB1*1501 - DQB1*0602* haplotype and with *HLA-DRB1*1104* [22,23]. Childhood acute lymphoblastic leukaemia showed a moderate positive association with *HLA-DRB1*04* and a strong positive association with homozygosity for *HLA-DRB4*01* in male patients [25]. So far, few reports have addressed the distribution of HLA class II alleles in NHL, probably due to the large number of NHL entities in combination with the rapidly changing classification systems over the past few decades. In a comparative study in Caucasoid, Mexican-American and Negroid B-cell NHL patients, no associations were found with specific HLA-DR alleles. However, in this study, DLBCLs were not analyzed separately [62]. In Thai NHL patients, a higher frequency of *HLA-DRB1*1502* has been reported [63]. However, these lymphomas were not classified according to the REAL or WHO classification [11], making comparisons with our results difficult. One study concerning Caucasoid DLBCL patients has been published [26]. Juszczynski et al. reported a significant and independent association of the HLA-*DRB1*02* allele with adverse outcome. The DR2 frequency however, was not increased in the patients group. The HLA-DR2 allele consists of the *HLA-DRB1*15* and *DRB1*16* alleles of which the former is the most frequent in Caucasoids and we can assume that the DR2 positive patients were in fact *HLA-DRB1*15* carriers. In contrast to the French patients, both our patient groups showed a significant increase of *HLA-DRB1*15* , which suggests an increased risk for DLBCL. *HLA-DRB1*15* has previously been associated with several autoimmune diseases [64-66] as well as

certain malignancies [67,68]. In contrast to *HLA-DRB1*15*, the frequencies of nearly all other HLA-DR and –DQ alleles differed between the nodal and testicular DLBCL patients, but significance was reached for only a few alleles after correction when compared to the control group. A significant positive association with *DRB1*12* was found for the testicular DLBCL patients and a significant negative association with *DRB1*07* for the nodal DLBCL patients.

In this study, we confirm in a large group of 48 testicular DLBCL, our previous report on deletions of the HLA-DR and DQ genes [21]. In 40% of the tumours, the HLA-DR/DQ region was homozygously lost and in another 30% hemizygously lost. The PAC probe we used contains the *HLA-DRB1* and *HLA-DQA1* genes but not the *HLA-DRA*, *DRB3*, *DRB4*, *DRB5* and *HLA-DQB1* genes. Loss of DQA1 will however, result in complete lack of HLA-DQ expression as no stable dimer with DQB1 can be formed. In the DR1, DR8 and DR10 positive tumours, carrying only one functional DRB gene, loss of *HLA-DRB1* will result in complete lack of DR expression [69]. The other haplotypes may still express HLA-DR although at lower levels, except for the tumours carrying the highly expressed *DRB3 gene* (the DR3, DR11-14 haplotypes) [40]. However, previously nearly all homozygous deletions also included the DRA and other DRB genes [21] and therefore, most likely the currently observed homozygous deletions resulted in complete loss of HLA-DR expression. None of the alleles were exclusively involved in loss or in retention but the DRB1*12 positive patients showed frequent homozygous and the DRB1*11 positive patients frequent hemizygous deletions in the tumours.

The frequency of HLA-DRB1*03 was very low in the testicular DLBCL patients and just one HLA-DRB1*03 carrier showed a homozygous deletion in the tumour. In five HLA-DRB1*03 positive patients a hemizygous deletion was seen. In two of these, we previously established through mutation analysis that *HLA-DRB1*03* was retained while the other alleles were deleted [56]. In the tumours with a hemizygous deletion we attempted to establish which of the two alleles was retained by DNA typing on flow cytometric purified tumour cells [70]. Unfortunately, we did not succeed in this. Nevertheless, it is tempting to speculate that genomic loss of *HLA-DRB1*03* would be less profitable for a testicular lymphoma. Most Caucasoids positive for HLA-B8 and HLA-DR3 carry the 8.1 (A1-B8-DRB1*03) highly conserved ancestral haplotype (AH), associated with a wide range of autoimmune diseases [71]. Healthy individuals with this AH show a repressed natural killer cell activity [72] and a prevalent Th-2 cytokine profile predominantly enhancing humoral immune responses. The latter might be due to higher spontaneous serum levels of TNF-α [73] which is also associated with a high level of apoptosis in Th-1 cells [74] thereby affecting the anti-tumour cytotoxic T cell response [75].

Also *HLA-DRB1*11* was only once involved in a homozygous deletion. The explanation for this low frequency, may be found at the molecular level as both *DRB1*03* and *DRB1*11* belong to haplotypes that contain approximately 110 kb less DNA in the HLA-DR region than the *DRB1*04*, *DRB1*07* and *DRB1*09* containing haplotypes and 30 kb less than the *DRB1*02* haplotype [44-46]. The molecular organisation of the shorter DR3 and DR11 segments might render them less susceptible to recombination and subsequent deletions. However, the DR12 haplotype which is positively associated with testicular DLBCL is equally short while the majority of the carriers did show homozygous deletions of the DR-DQ region in the tumours. In addition, differences were seen in deletion frequencies between combinations of haplotypes with equal, shorter or longer DNA lengths.

Thus far, the aetiology and pathogenesis of testicular lymphomas are not known and one can only speculate about the exact role of HLA class II in the initiation of lymphomagenesis and lymphoma progression. The antigen specificity of the different HLA class II molecules might be important during a putative episode of inflammation preceding lymphoma development. Later on, loss of certain HLA alleles involved in presentation of specific yet unknown peptides, may help the tumour cells to escape from a CTL response. However, one could also argue that for testicular DLBCL, not the polymorphism of HLA class II molecules is crucial but the loss of expression of particularly all HLA-DR molecules, as interaction with HLA-DR molecules may directly mediate apoptosis of B cells [58-61].

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