Chapter 6

Summary, discussion and future perspectives
SUMMARY AND DISCUSSION

Based on the composition of the microenvironment, classical Hodgkin lymphoma (cHL) can be classified into four subtypes i.e. nodular sclerosis (NS), mixed cellularity (MC), lymphocyte rich (LR) and lymphocyte depleted (LD). Approximately 30-40% of cHL population in the Western Europe carry Epstein Barr virus (EBV) in the tumor cells. EBV infection is clearly implicated in the etiology of a proportion of cHL with distinct pathology and has been shown to be associated with prognosis (1). Generally EBV+ cHL is associated with the MC subtype, whereas EBV- cHL usually are of the NS subtype. Unlike most other cancers, which show an increased incidence with age, cHL has a characteristic bimodal age incidence. The first prominent peak in young adults of 15-30 years is usually EBV- and associated with NS subtype. The second peak after 50 years usually presents as EBV+ with MC or NS subtype. Moreover, in young children, cHL is predominantly EBV+.

Genetic associations with specific HLA alleles have been reported in both sporadic and familial cHL. Initial HLA association studies in cHL were restricted to cHL overall without taking age, subtype or EBV status into account. Pioneer studies led by us and others stratified cHL based on EBV and found associations of specific HLA-A alleles with EBV+ cHL. The HLA locus at 6p21.31 is the most polymorphic region of the genome, with more than 2,000 different HLA alleles. Each HLA allele is functionally different and has a distinct capacity to present EBV (in case of EBV+ cHL) or aberrant tumor cell derived antigenic peptides to the immune system. These differences in antigen binding capacity may explain the risk or protective effects to develop cHL. The aim of this PhD thesis was to fine screen the HLA region to determine the specific association of HLA alleles with cHL overall and cHL stratified based on EBV, age and HLA class I/II expression at the time of diagnosis. This will allow us to better understand the molecular basis of HLA related susceptibility mechanisms in cHL.

Genetic associations in classical Hodgkin lymphoma

In chapter 2 we summarized current knowledge on genetic polymorphisms associated with cHL and discuss the consistent results associated with cHL susceptibility, prognosis and treatment related secondary malignancies and toxicities in population based targeted gene approach studies as well as in genome wide association studies (GWAS). Targeted gene approach studies focusing on the HLA region showed strong association of HLA class I alleles with EBV+ cHL, whereas HLA class II alleles were found to be associated with cHL overall, EBV- cHL or NS cHL. These associations with the HLA region have been confirmed in multiple GWAS.
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Targeted gene association studies also revealed several associations with non-HLA loci, which were mostly noted in cHL overall, as most of these studies did not perform subgroup specific analysis. Previously associated single nucleotide polymorphisms (SNPs) mapping to the interleukin 4 receptor alpha (IL4RA), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKBIA), signal transducer and activator of transcription 6 (STAT6), tumor protein 63 (TP63) and xeroderma pigmentosum, complementation group C (XPC) gene loci were also observed in a GWAS data set with nominal significant p-values (p<0.05) (2). However, most of the previously reported SNPs were non-significant (p>0.05) in the GWAS indicating that these associations could not be reproduced and hence are most likely false positive results.

An interesting observation is that multiple of the associated genes, i.e. IL13, IL4RA and STAT6, are related to the T helper cell type 2 (Th2) differentiation pathway. These proteins have already been shown to be expressed and essential for growth and survival of HRS cells (3). Studies on genetic polymorphisms affecting prognosis and treatment related secondary toxicities and malignancies were limited. A GWAS identified an association of the PR domain containing 1, with ZNF domain (PRDM1) gene with risk developing any secondary malignancies after radiotherapy for cHL.

HLA associations in classical Hodgkin lymphoma

Previous HLA associations studies in cHL were limited to particular HLA loci or used microsatellite markers mapping to HLA region. Most of these studies were restricted to cHL overall and did not take EBV status or age at diagnosis into account. In **chapters 3** and **chapter 4** we performed a detailed fine screening of the HLA region and performed an association analysis of classical HLA class I and class II allele to cHL overall and cHL subgroups stratified based on EBV status and age at diagnosis. In an ongoing study we stratified cHL patients based on HLA class I and class II expression. In these studies we analyzed the HLA region based on individual sequence specific oligonucleotide probes (SSOPs), which are used to define HLA alleles and on SNPs mapping to HLA region obtained from the GWAS data set (2).

SSOPs analysis revealed that not the individual SSOPs, but a specific combination of them reflecting particular HLA alleles are responsible for the associations (**chapter 3**). Previously it has been reported that, all individuals that carry a five SNP haplotype (rs204999-rs9268528-rs9268542-ra6903608-rs2858870) mapping to the HLA class II region could explained 60% of the decreased risk. Individuals carrying this haplotype also carried the HLA-DRB1*07 allele, a known protective allele (4), supporting the relevance of the HLA allele. Two HLA class I SNPs associated with EBV+ cHL, were strongly linked to previously well-established alleles
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HLA-A*01 and HLA-A*02 respectively (2,4). Although it is difficult to distinguish between SNPs and HLA allele driving susceptibility effects it is likely that the associations of HLA alleles can better explain the susceptibility effects because they represent a functional polymorphisms.

For the HLA alleles, these analyses have resulted in significant associations of multiple HLA alleles to cHL overall and to specific cHL subgroups (Figure 1, Table 1 and Table 2). Some of the associated alleles are unique to a particular cHL subgroup whereas others are associated with multiple cHL subgroups.

Two association were observed in the cHL overall patient group. The allele frequency of HLA-B*51/52 (B5) was significantly increased and the HLA-DRB1*07 allele frequency was significantly decreased in the cHL overall compared to controls. In addition, from our ongoing study of association of HLA alleles in HLA class I and class II expression stratified cHL revealed that the risk allele HLA-B*51/52 (B5) is significantly increased in the HLA class I+ cHL. HLA-DRB1*07 was also associated with EBV- cHL albeit with borderline significance. The protective HLA-DRB1*07 is significantly decreased in HLA class II+ cHL. More than 75% of the HLA class II+ cHL cases are EBV- and majority of cHL cases are EBV+, hence, the association of HLA-DRB1*07 observed in EBV- and HLA class II+ cHL is mostly explained by cHL overall.

For EBV+ cHL multiple association were observed. The allele frequencies of HLA-A*01, HLA-B*37 and HLA-DRB1*10 and the putative haplotype frequency HLA-A*01-B*37-DRB1*10 were significantly increased in the EBV+ cHL compared to controls. Whereas, the HLA-A*02 allele frequency was significantly decreased in the EBV+ cHL compared to controls. The association of HLA-A*01 and HLA-A*02 with EBV+ cHL is consistent with previous studies. The frequency of EBV+ cHL risk alleles HLA-A*01 and HLA-B*37 were increased and the protective allele HLA-A*02 was decreased in HLA class I+ cHL compared to controls. These results are explained by strong association of EBV+ cHL with HLA class I+. The association of HLA class I alleles with EBV+ cHL suggests involvement of antigen presentation in the context of HLA class I alleles in the pathogenesis of EBV+ cHL. Effective presentation of EBV derived antigens by HLA class I alleles can be an important factor to prevent development of EBV+ cHL. The consistent association of HLA-A*01 as a risk allele and HLA-A*02 as a protective allele for developing EBV+ cHL can be explained by their differential affinity for EBV latent peptides to present to T cells. HLA-A*01 has no/low affinity for EBV latent peptides, whereas HLA-A*02 can present EBV LMP1/LMP2 antigens and induce cytotoxic T cells (CTLs) response (5,6).

In our cohort a small percentage of EBV+ cHL patients still carried the protective HLA-A*02 allele. Based on a few reports, it might be possible that these patients have been infected with a mutant EBV strain (7–9) that lost known HLA-A*02
restricted antigenic peptides \( (10,11) \). It is also possible that mutations in HLA-A*02 gene itself or in HLA class I associated genes such as beta-2-microglobulin \( (B2M) \), transporter associated with antigen processing 1/2 \( (TAP1/2) \), endoplasmic reticulum aminopeptidase 1 \( (ERAP1) \) etc. in the EBV+ tumor cells might affect its expression and functionality. Our data in an ongoing study indicated that six out 12 EBV+/HLA-A*02+ cases have lost the HLA class I expression. However, sequencing of antigen binding region of HLA-A*02 allele in two cases revealed no mutations.

For EBV- cHL two HLA class II risk alleles have been identified. Allele frequencies of HLA-DRB1*11/12 (DR5) and HLA-DRB1*15/16 (DR2) were both significantly increased in EBV- cHL compared to controls. Association of HLA class II alleles with EBV- cHL suggests that presentation of tumor cell derived antigens by HLA class II alleles is involved in the pathogenesis of EBV- cHL. In addition, HLA-DRB1*15/16 allele frequency is significantly increased in cHL patients of \( \leq 45 \) years of age of diagnosis and a suggestive increase in HLA class II+ cHL compared to controls. These associations partly explained by overrepresentation of EBV- cHL in young patients of \( \leq 45 \) years and in HLA class II+ cHL. Although tumor associated antigens in EBV- cHL are less known, expression of melanoma-associated antigen 4 (MAGE-A4) has been documented in 28% of HRS cells and MAGE-A4 specific CTL therapy has been proven promising for EBV- cHL \( (12,13) \).

In the age of diagnosis stratified analysis the allele frequencies of both HLA-DRB1*15 and HLA-DQB1*06 and the putative haplotype DQB1*06-DRB1*15 were significantly increased in cHL patients with \( \leq 45 \) years of age. Additionally, we found two new HLA allele associations with cHL age stratified analysis. Allele frequencies of HLA-B*08 and HLA-DRB1*03 and the putative haplotype B*08-DRB1*03 were significantly increased in cHL of \( > 45 \) years. Interestingly, the HLA-A*02 allele, a protective allele for EBV+ cHL that forms a common haplotype with DQB1*06-DRB1*15, and the HLA-A*01, a risk allele for EBV+ cHL, that forms a common haplotype with B*08-DRB1*03 did not show associations in age of diagnosis stratified analysis. This indicates that the association of HLA-A*01 and HLA-A*02 with EBV+ cHL is independent of age of diagnosis.
In the HLA class I and II expression stratified analysis we found several alleles associated with HLA expression, some of which have already been mentioned above. In addition to these alleles, we found one extra allele with an increased allele frequency, i.e. HLA-B*07, in HLA class I- cHL compared to controls. Association of HLA alleles with HLA expression implies that HLA expression modulates the risk and

### Figure 1. Summary of HLA allele association with classical Hodgkin lymphoma stratified based on EBV status, age at diagnosis, HLA class I and HLA class II expression status. In each bar graph, allele frequency percentage is plotted for cHL subgroups compared to controls (light grey). Significant associations are shown in color bar with asterisk, suggestive ones are without asterisks (red: increased, green: decreased allele frequency compared to controls), Linked HLA alleles colored similar.
protective effects of HLA alleles. At the time of diagnosis ~70% of HRS cells lack cell surface expression of HLA class I, most often in EBV- cHL. Lack of HLA class II membranous expression is observed in ~40% of EBV+ and EBV- cHL cases (14). We previously proposed that HRS cells may downregulate expression of HLA to escape from antitumor immune responses. Based on our new data, we now postulate that the selective pressure for doing so might be higher if the patient carries a protective allele and lower or even favor retention of HLA expression for risk alleles. This was indeed observed for the cHL risk allele HLA-B*51/52 (B5) and the cHL protective HLA-DRB1*07 allele. A consistent pattern was also observed for the EBV+ cHL associated risk alleles, HLA-A*01 and HLA-B*37. The protective HLA-A*02 allele in EBV+ cHL was also consistent, although this association was only suggestive. However, it should be noted that EBV status and HLA class I loss are not independent of each other. Similarly we also found a consistent pattern for the EBV- cHL risk allele, HLA-DRB1*15/16(DR2). The HLA-B*07 frequency was increased specifically in HLA class I negative cHL compared to controls. Association of HLA-B*07 allele with HLA class I- cHL implicate that this allele drives the downregulation of HLA expression. However, as ~60% of HLA class I- cases are HLA class II+, the association of HLA-B*07 might be driven by its strong linkage with HLA-DRB1*15/16, which is associated with HLA class II+ cHL.

**HLA associations in classical Hodgkin lymphoma of a Brazil population**

One of the important factors to consider in HLA associations in cHL is ethnic background, as common and well-documented allele frequencies show marked differences between populations. In Western Europe HLA-A*01 is a risk allele, whereas HLA-A*02 is a protective allele for EBV+ cHL. In the Chinese EBV+ cHL population, we showed that the risk pattern is different from that of Caucasians. The HLA-A*02:07 sub-allele is a risk allele for EBV+ cHL and a protective allele for EBV- cHL (15). Interestingly the difference between HLA-A*02:01 and HLA-A*02:07 is a single amino acid (Y99C) at a key position in the peptide binding cleft. It has been demonstrated that HLA-A*02:07 specific CTLs are less responsive to LMP2 peptides as compared to HLA-A*02:01, HLA-A*02:06 or HLA-A*02:09 (16). The driving mechanisms of the protective effect of HLA-A*02:07 of EBV- cHL is less clear. In theory, it is expected HLA-A*02:07 can induce strong anti-tumor immune responses against tumor specific antigenic peptides in EBV- cHL. In chapter 5 we explored the HLA-A associations in a Brazilian cHL cohort. Similar to Western Europe, the HLA-A*01 allele carrier frequency was significantly increased, whereas the HLA-A*02 allele carrier frequency was significantly reduced in EBV+ cHL compared to controls. Similar to the European and Chinese population HLA class I expression was significantly more often retained in EBV+ cHL as compared to EBV- cHL (17). An
An unexpected finding in this study was the low percentage of EBV+ cHL (34%) as compared to other studies from South America that usually show EBV+ percentage up to 67% (18). The low percentage of EBV+ cHL cases raises some concerns with respect to the ethnicity of our Brazil cHL population, which may in fact be a mix of Hispanics and Caucasians.

**Table 1: HLA class I allele frequencies with significant and suggestive differences between HLA I+, HLA I- cHL and controls.**

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>HLA I+ (n=140)</th>
<th>HLA I- (n=336)</th>
<th>Controls (n=15,114)</th>
<th>HLA I+ vs. HLA I- *</th>
<th>HLA I+ vs. Controls *</th>
<th>HLA I- vs. Controls *</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*01</td>
<td>39 (27.9)</td>
<td>55 (16.5)</td>
<td>2669 (17.7)</td>
<td>ns</td>
<td>0.0016</td>
<td>ns</td>
</tr>
<tr>
<td>A*02</td>
<td>27 (19.3)</td>
<td>115 (34.4)</td>
<td>4830 (32.0)</td>
<td>0.0010</td>
<td>0.0013</td>
<td>ns</td>
</tr>
<tr>
<td>B*07</td>
<td>19 (13.6)</td>
<td>76 (23.0)</td>
<td>2451 (16.2)</td>
<td>ns</td>
<td>ns</td>
<td>0.00093</td>
</tr>
<tr>
<td>B*37</td>
<td>9 (6.4)</td>
<td>5 (1.5)</td>
<td>277 (1.8)</td>
<td>ns</td>
<td>0.00006</td>
<td>ns</td>
</tr>
<tr>
<td>B*51/52</td>
<td>19 (13.6)</td>
<td>22 (6.7)</td>
<td>798 (5.3)</td>
<td>ns</td>
<td>0.00001</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Chi-square test, with significant (p<0.001) are shown in bold, suggestive ones are in italic (p<0.003). ns: not significant.

**Table 2: HLA class II allele frequencies with significant and suggestive differences between HLA II+, HLA II- cHL and controls.**

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>HLA II+ (n=288)</th>
<th>HLA II- (n=190)</th>
<th>Controls (n=15,114)</th>
<th>HLA II+ vs. HLA II- *</th>
<th>HLA II+ vs. Controls *</th>
<th>HLA II- vs. Controls *</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*07</td>
<td>10 (3.5)</td>
<td>14 (7.4)</td>
<td>1320 (10.1)</td>
<td>ns</td>
<td>0.00026</td>
<td>ns</td>
</tr>
<tr>
<td>DRB1*15/16</td>
<td>66 (23.2)</td>
<td>32 (16.8)</td>
<td>2154 (16.4)</td>
<td>ns</td>
<td>0.0022</td>
<td>ns</td>
</tr>
</tbody>
</table>

*Chi-square test, with significant (p<0.001) are shown in bold, suggestive ones are in italic (p<0.003). ns: not significant.
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In summary
In this thesis we determined the associations between classical HLA class I and class II alleles and susceptibility to cHL overall and specific cHL subgroups. Association of risk or protective alleles with retention and downregulation of HLA expression supports the concept that HLA associations in cHL are driven by specific HLA alleles, possibly based on allele specific antigen presentation. These findings support the relevance of HLA alleles rather than individual SSOPs/SNPs in the pathogenesis of cHL. As HLA alleles are the main components of immune system, the HLA associations in cHL are consistent with the immunological nature of the disease. The findings contributed significantly to a better understanding of etiology and underlying immunopathogenetic mechanisms in cHL.

The most important findings of this thesis are:

1. HLA alleles not SSOPs or SNPs are responsible for the HLA susceptibility.
2. HLA-B*51/52 (B5) is a risk allele and HLA-DRB1*07 is a protective allele for cHL overall.
3. The alleles of the relatively common haplotype in Caucasians HLA-A*01-B*37-DRB1*10 are risk for developing EBV+ cHL whereas the HLA-A*02 is a protective allele.
4. Two HLA class II alleles, HLA-DRB1*11/12 (DR5) and HLA-DRB1*15/16 (DR2) are risk for developing EBV- cHL.
5. HLA alleles of the common haplotype in the Caucasians i.e. DQB1*06-DRB1*15 are associated with risk of cHL in individuals below 45 years of age, whereas the alleles of the haplotype HLA-B*08-DRB1*03 are associated with cHL risk in individuals above 45 years age.
6. In the west European Caucasians, the association of HLA-A*01 and HLA-A*02 with EBV+ cHL is independent of age.
7. HLA-A analysis in a cohort from Brazil revealed the HLA-A*01 is a risk allele and HLA-A*02 as a protective allele for developing EBV+ cHL similar to Western Europe.
FUTURE PERSPECTIVES

The studies described in this thesis have provided important clues to the immune related susceptibility mechanisms of cHL. Nevertheless, (new) challenges and questions remain to be addressed in future studies.

1. Functional studies on the associated HLA alleles

The main function of HLA alleles is to present antigenic peptides of self or foreign origin to T cells. This will invoke specific cytotoxic immune responses against the peptide presenting cell. In cHL it remains unknown how the associated HLA alleles can provide protective or risk effects to develop the disease and which specific antigens might be involved. A main question to be answered is which antigenic peptides are presented by the HRS cells, and what type of immune response is subsequently triggered by the immune system. There are several approaches to identify these antigenic peptides. Peptides that are likely to be presented, such as EBV or MAGE-A4 derived peptides, can be predicted in-silico using bio-informatics prediction applications such as Bimas, Syfpeithi (19,20). These predicted peptides should be tested for their functionality in in-vitro T cell response assays such as enzyme linked immunospot (ELISPOT). Another approach is to elute the naturally presented peptides using immuno-affinity chromatography from EBV transformed B cell lines (LCLs) homozygous for disease EBV+ cHL associated HLA alleles and identification of peptides using liquid chromatography and mass spectrometry (LC-MS) (21). In a similar way, cHL cell lines can be used to elute and identify candidate tumor associated antigenic peptides. Identification of antigenic epitopes presented by HLA alleles may help to generate epitope specific cytotoxic T lymphocyte (CTL) clones for T cell based immunotherapy of cHL (22).

2. Functional studies on associated non-HLA genes

Recent GWAS and targeted gene approach studies have indicated several non-HLA genes for the susceptibility of cHL. To follow up on these findings an expression quantitative trait locus (eQTLs) analysis could be done for the SNPs at the associated gene loci. We showed marked differences in the expression levels of TCF3 in SNP allele stratified LCLs (23). Similar analysis could be done for the other associated gene variants such as IL13, IL4R and STAT6 in order to support their functional relevance in cHL susceptibility. These genes belong to Th2 differentiation pathway and are especially important in cHL biology as they modulate cHL microenvironment in to tumor supportive Th2 type. In addition IL13 has been shown to be an autocrine growth factors for HRS cells (24). The IL13 SNP is a missense SNP that results in change in amino acid at codon 110. Functional studies have reported that the risk
associated 110Q IL13 variant is more effective in enhancing STAT6 phosphorylation and is neutralized less efficiently by an IL13RA2 decoy than the common R110 IL13 variant (25,26). Our preliminary ongoing experiments suggests that cHL cell lines treated with IL13 110Q risk variant had an increased cell growth compared to treatment with wild type IL13. Hence, future functional studies including these candidate genes will contribute to the understanding of the predisposing mechanisms.


Sixteen percent of the EBV+ cHL patients still carry the protective HLA-A*02 allele. In these cases, either the patients might be infected with “HLA-A*02 epitope loss” EBV variants or HLA-A*02 is no longer able to present the EBV epitopes to CTLs. HLA-A*02 epitope loss variants have been documented in nasopharyngeal carcinoma, which has a similar EBV infection pattern as cHL (10). Infection with these strains may thus alter the effectiveness of the immune response mediated by HLA-A*02 (5). Future studies analyzing the sequence variations present in the EBV genome to find a putative loss of HLA-A*02 epitopes should be performed. Especially in EBV+ cHL patients that carry the HLA-A*02 allele and retain expression of the HLA-A*02 allele, since it is striking that these HRS cells survive from effective anti-tumor immune responses. Mutations in the B2M, TAP1/2 and ERAP1 might affect HLA class I expression and provide immune escape for the tumor cells. Mutations in peptide-binding region of the HLA-A*02 gene in the tumor cells of EBV+ and HLA class I+ cHL might lead to loss of antigen presenting capabilities of the HLA-A*02 allele. Future studies aiming at sequencing and screening for point mutations in the HLA-A*02 gene in laser microdissected HRS cells and checking for HLA-A*02 expression in the tumor cells will help identifying possible tumor immune escape mechanisms.

4. Ethnic differences in HLA associations.

HLA associations in EBV+ cHL show ethnic variations. We showed that HLA-A*01 is a risk allele and HLA*02 is a protective allele in the Dutch cHL population, whereas in a Chinese cHL population HLA-A*02:07 is risk allele for developing EBV+ cHL and protective for EBV- cHL (15). In a similar attempt in a Brazilian cHL population, we found a risk pattern consistent with the Dutch or Western Europe populations. Based on EBV percentage, we are concerned about the ethnicity of these patients. Future studies should be performed on a well-defined Hispanic cHL population to study the ethnical differences in the HLA risk pattern. It is also worthwhile to study the genetic differences in other ethnic groups. These studies help us to unravel ethnic group
specific susceptibility mechanisms. Moreover, GWAS in cHL have been restricted to Caucasians populations. Further, GWAS in other ethnic cHL populations will enable us to screen and identify novel susceptibility loci specific for these populations.

**Figure 2. Immunomodulatory role of soluble HLA (sHLA).** The tumor cells produce elevated levels of sHLA class I molecules bearing tumor derived peptide antigens. The sHLA can then bind to the T cell receptor (TCR) of anti-tumor cytotoxic T cells (CTLs) and induce either apoptosis or anergy. SHLA can also bind to the C-type lectin inhibitory receptor (CLIR) or killer Ig-like receptor (KIR) of natural killer (NK) cells inducing NK cell apoptosis.

**5. Soluble HLA as immune modulators.**

Soluble HLA (sHLA) molecules can be found in the circulation of healthy individuals. It has been reported that HLA-A9, HLA-A29, HLA-A33 alleles were associated with increased sHLA class I serum levels in healthy individuals serotyped for HLA-A, HLA-B and HLA-DR loci (27,28). SHLA class I molecules are able to bind to CTLs and induce apoptosis in alloreactive CTLs and EBV specific CTLs (29,30). In addition, it has been reported that sHLA class I engagement with either the C-type lectin inhibitory receptor (CLIR) or killer Ig-like receptor (KIR) on natural killer (NK) cells induced NK cell apoptosis (31). Based on these data, it might be anticipated that tumor cells secrete sHLA class I molecules loaded with tumor-generated peptides as a mechanism to escape from anti-tumor immune responses (Figure 2). To test this hypothesis plasma levels of sHLA should be correlated with HLA type in cHL patients.
and healthy individuals with a specific focus on known risk and protective alleles. Some studies have shown a relation between increased levels of sHLA and adverse prognosis in lymphoma including Hodgkin lymphoma (32,33). Therefore, it might be worthwhile to study allele specific sHLA in serum samples of HLA typed cHL patients as a potential prognostic factor and it can be tested by an allele specific enzyme linked immunosorbent assay (ELISA).

6. HLA based mouse model

The minority of tumor cells in an abundant background of inflammatory cells precluded the development of animal model for cHL. Such a model would, however, be crucial to decipher susceptibility, immune escape mechanisms and design of new therapies. The key question about cHL tumor development concerns the mechanisms responsible for malignant transformation and the contribution of the microenvironment. Studies aiming to propagate cHL cell line L540 in immune-deficient SCID mice had little success, indicating the importance of bystander cells in cHL pathogenesis (34). A chimeric HLA-DR4-H2-E (DR4) homozygous transgenic mouse line spontaneously developed several lymphomas, including Hodgkin-like lymphomas presenting features that are characteristic of HRS cells (35). As HLA alleles are strongly associated with cHL development, an HLA based animal model over expressing cHL related susceptible gene(s) might provide an opportunity to generate a mouse model and learn more about HLA mediated susceptible mechanisms. HLA allele specific transgenic mice have already proven to be versatile models to study pre-clinical HLA restricted CTL responses (36,37). A humanized NOG mice (NOD/Shi-scid/IL-2Rγnull) inoculated with EBV+ hematopoietic stem cells can recapitulate key aspects of EBV infection and remarkably shown the features of humans B cell lymphoproliferative disorder (38). It has also been established that latent EBV infection and stable episomal maintenance in murine B cell lines transfected with hCD21 and HLA-DR, might provide a suitable animal model of EBV infection (39). Thus, HLA-A*01 and HLA-A*02 based EBV infected transgenic mice could be a potential model to study the risk or protective mechanisms associated with HLA-A*01 and HLA-A*02 respectively in EBV+ cHL. Of note, the HLA-A*02:01 transgenic mice have also been proven to be versatile animal models for preclinical evaluation of peptide-based cancer immunotherapy (40).
REFERENCES


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