Genetic susceptibility to Hodgkin’s lymphoma and to secondary cancer: workshop report

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Although the occurrence of familial Hodgkin’s lymphoma (HL) is a rare event, genetic susceptibility as a cause of HL and its influence on treatment outcome may not be rare. However, results obtained from the analysis of HL families will probably have broad implications with regard to understanding common pathogenic factors leading to the development of the disease. The description of anticipation among the affected offspring of HL patients further strengthens the view that heritable factors contribute to development of HL. Moreover, the finding that particular human leukocyte antigen (HLA) alleles are associated with susceptibility to HL may be regarded as a hint to the presence of an as yet undefined infectious agent, leading to the growth of a malignant lymphoma cell clone in those patients that are more susceptible to this agent due to their HLA genotype. In addition, since an intrinsic genomic instability was observed in a proportion of HL patients, it is plausible that these patients are not only susceptible to the causation of HL, but are also at a higher risk of developing therapy-related (TR) secondary cancers following treatment. Estimation of sister chromatid exchange was established as a tool to identify patients at higher risk of TR cancer. In this context the use of therapeutic agents known to increase genomic instability should be carefully considered prior to determining the best treatment. The future identification of heritable factors contributing to HL will be of importance both with regard to diagnosis as well as treatment of HL patients.

Key words: heritability, Hodgkin’s lymphoma, risk, secondary cancer, susceptibility

Introduction

Hodgkin’s lymphoma (HL) is a heterogeneous hemopoietic malignancy, and little is known about the etiology of this disease. However, an increased risk of developing HL in the siblings and monozygotic twins of patients has been reported [1–3]. In siblings <45 years of age, a seven-fold increased risk of HL was observed [1], but this was absent for older siblings diagnosed after the age of 45 years. Moreover, cytogenetic analysis of Hodgkin Reed–Sternberg (HRS) cells, the malignant cells in the HL tissue, has provided evidence for the constitutive genomic instability of these cells [4, 5]. Chromosomal instability was also observed in peripheral blood lymphocytes obtained from HL patients prior to treatment [6, 7]. Thus it appears that heritable factors may contribute to the risk of developing HL in certain patients as well as of developing secondary cancer following therapy for HL [therapy related (TR) cancer]. These issues were considered at a workshop held at the Fifth International Conference on Hodgkin’s Lymphoma in Cologne in September 2001. The data presented at this workshop are summarized below.

Familial HL and anticipation

In 1998, based on published cases obtained from the literature, it was first suggested that anticipation may play a role in the clinical presentation of familial HL [8]. More recently, Shugart et al. [9] estimated the heritability of HL and tested the hypothesis of genetic anticipation using a high quality cancer database of the Swedish population. Heritability was estimated by employing a threshold-liability model, and this gave a value of ~28%.

To test the hypothesis of anticipation, the t-test procedure [9] was used to test whether there is a difference in age of onset of cancer between parents and offspring who were affected with HL. A randomization test was carried out to test the validity of the P values. Overall, the mean age of onset in the 12 pairs was 38.41 years [standard deviation (SD) 14.61] for parents and 25.58 years (SD 10.91) for children. Based on the birthdate of the parents, data were divided into two groups: pre-1930 and post-1930. In the pre-1930 group, the mean age of onset was 27.2 years for the parents (SD 5.3) and 19 years
(SD 5.2) for the affected children. The mean anticipation for the combined sample is 12.8 years (SD 11.2). The corresponding paired t-test statistic was 3.98 ($P = 0.0011$) and the valid $P$ value obtained for the systematic randomization procedure was 0.0007. Separate estimates for anticipation were 17.5 years (SD 13.2) for the group in which parents were born prior to 1930, and 8.2 years (SD 7) for the group in which the parent was born after 1930 ($P = 0.011$). The associated paired t-test statistics were 3.25 ($P = 0.011$) and 2.86 ($P = 0.018$), respectively. The strength of this study is that Shugart and coworkers. used a population-based cancer registry with 100% coverage and a registry of the whole population. However, several weaknesses need to be pointed out: (i) oversampling of parents with late disease onset, because the HL parents with early age of onset may have limited reproductive capability; and (ii) younger offspring may not be old enough to reproduce at the time this investigation was carried out. These facts may have biased the results. However, to some extent, their choice of study design and statistical approaches have allowed them to reduce some ascertainment bias in the reported estimate for anticipation in familial HL. More recently, a second study was performed which included 56 non-HL (NHL) parent–child pairs [10]. Results show that the anticipation level was even more pronounced among the NHL–NHL pairs than in HL–HL pairs (difference = 1.26 years; $P = 0.0003$). In the near future, this study will be expanded to test for anticipation in individuals affected with different types of hematological malignancy. The familial aggregation of various cancers will also be explored further.

**Association of HL with particular HLA class II genes**

Epidemiological evidence has long suggested that HL may arise as a result of the rare outcome of late exposure to a specific infectious agent [11]. The possibility that this agent may be Epstein–Barr virus (EBV) is supported by evidence of EBV in the involved lymph nodes in ~50% of patients with HL, depending on age, gender and subtype [12]. However, the widespread distribution of asymptomatic EBV infection in the normal adult population suggests that this virus may not be the only causative factor, implying that gene-environment interactions may also be involved. The key role played by the human leukocyte antigen (HLA) genes in the control of immune responses to viruses suggests that HLA genes might be important in the etiology of HL. Evidence showing that the polymorphic peptide-binding pockets of the HLA class II antigen-binding groove exhibit specificity for different immunogenic peptides suggests that the causation of HL could be defined in terms of HLA class II alleles, or by peptide pocket profiles. This ‘reverse immunogenetic’ footprint of sequences binding to HL-associated HLA class II alleles may enable predictions to be made about the role of EBV in HL.

Some years ago Bodmer et al. [13] reported on the observation of an association of DPB1*0201 with resistance to HL, and DPB1*0301 with susceptibility to HL. Later, an overall association between the nodular sclerosis subtype of HL and HLA class II loci was reported [14], whereby susceptibility seemed to be influenced by more than one haplotype. More recently, Taylor et al. [15] performed a detailed molecular analysis of a hospital-based series of 147 adult HL patients and 183 controls from Manchester, UK. Their results confirmed the previously documented associations with DPB1*0301 [odds ratio (OR) 1.43, 95% confidence interval (CI) 0.86–2.36] and 0201 (OR 0.48, 95% CI 0.27–0.90). However, further analysis revealed both gender and subtype differences in associations with these alleles. Thus, the association of DPB1*0201 with resistance was only significant in females with nodular sclerosing (NS) HL (OR 0.15, CI 0.03–0.67), there being no significant association in males with NS or non-NS, or in each gender with non-NS. In contrast, the association with *0301 was significant in females with non-NS (OR 4.67, CI 1.11–19.57), but absent in females with NS and in males with either subtype. Comparison of the polymorphic amino acids lining the peptide binding groove of *0201 and *0301 revealed that they differ at all positions (11, 57, 69, 76 and 87) except position 36, suggesting that valine at this position is not a key determinant of susceptibility to HL. Support for susceptibility due to lysine at position 69 derives from results showing increased susceptibility in patients heterozygous for *0301/*0401 (OR 13.2, CI 1.60–109.93); these alleles share only a lysine at position 69, suggesting that this may exert a role in the combined susceptibility of these heterozygotes.

One potential explanation for the association of DPB1 alleles with susceptibility and resistance to adult HL is that they are in linkage disequilibrium (LD) with more strongly associated DRB1 alleles. To address this question, Taylor and coworkers DRB1-typed their adult HL series. They found that DRB1*1501 was associated with susceptibility (OR 1.93, CI 1.21–3.05) and 0101 with resistance (OR 0.38, CI 0.21–0.72) to HL. However, unlike DPB1 alleles, there was no major difference in DRB1 susceptibility/resistance with respect to NS and non-NS subtypes. Next, the two DPB1 alleles (*0201, *0301) were tested for LD with the two DRB1 alleles, and no significant evidence of preferential associations was found between the pairs of alleles at the two loci ($P > 0.05$). Although Taylor and colleagues have not yet been able to examine the relationship between the presence of EBV and HLA-DPB1 genotype in detail, their preliminary results in a different case series obtained in collaboration with R. Jarrett, F. Alexander and colleagues [16] indicate that patients typing DPB1*0101/*0301 ‘are more likely to be EBV+’, whilst patients typing DPB1*0101/*0301 ‘are more likely to be EBV−’. The main difference in these two alleles is in the amino acids at positions 11, 36 and 57, but further work is needed to clarify this in a larger series of patients.
The tentative conclusion from this work is that different HLA-DPB1 isotypes bind peptides derived from an infectious agent that is capable of causing HL in the context of the gender of the individual, and that this leads to a T cell response to the agent, which influences the HL subtype that subsequently develops. Although there is preliminary evidence of differential association with EBV, it is not yet clear whether this is a causative association.

Sister chromatic exchanges as a predictor for the development of secondary malignancies in HL patients

HL survivors face an increased risk of developing second cancers. Strom et al. [17, 18] evaluated sister chromatid exchange (SCE) in peripheral blood lymphocytes as predictors of second cancer risk in a cohort of 105 newly diagnosed adult patients seen at M. D. Anderson Cancer Center, Houston, TX, USA. They analyzed whether having a high frequency of pre-treatment SCEs was associated with an increased risk of developing a second cancer. During the follow-up time (average 7 years, range 1–13 years), 14 new cancers occurred. Multivariate Cox regression analysis revealed that high levels of SCEs [relative risk (RR) 3.9, \( P = 0.03 \), 95% CI 1.1–13.7] and age as a continuous variable (RR 1.1, \( P = 0.03 \), CI 1–1.1) predicted second cancer risk. Histology, stage and treatment (with alkylating agents versus others) were not associated with elevated risk. These data suggest that baseline SCE may be a useful marker to identify patients at increased risk of developing second cancers. Likewise, as described by Kelly and Peraentesis [19], the detection and characterization of polymorphisms of drug metabolizing enzymes may be a good tool for identifying HL patients at risk of treatment-related complications. HL patients at particular risk of developing secondary leukemia can be identified using fluorescence in situ hybridization (FISH) methods in order to detect leukemia-specific chromosomal aberrations in the developing leukemia cell clone prior to clinical manifestation. This method is described in detail by Lillington et al. [20].

Granulocyte colony stimulating factor influences allelic replication in HL patients

Relapse is the major obstacle for successful autologous stem cell transplantation (autoSCT) in HL. Event-free survival of HL patients transplanted during the second achieved complete remission status or later is 30%. Moreover, survival and disease-free survival of HL patients with refractory disease or with resistant relapse is extremely low. Allogeneic SCT (allo-SCT) might offer a graft-versus-tumor effect, which may be of therapeutic potential [21, 22]. However, alloSCT applicability has been usually limited by conditioning-related toxicity. Fludarabine-based low-intensity conditioning (LIC) or non-myeloablative conditioning (NST) is a realistic option, since it can be administered with very low organ toxicity [23]. Most LIC transplants are being carried out with granulocyte colony stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSC). As secondary leukemias are an emerging problem for HL patients post-autoSCT [24], and as most of the donors in an alloSCT setting are first-degree relatives of the patients, Nagler et al. [25] evaluated the possible effect of G-CSF on genomic stability and changes in the temporal order of allelic replication in lymphoma patients, as well as in normal HLA-matched sibling donors of G-CSF-mobilized PBSC. They used the FISH replication assay, which is based on in situ hybridization with a fluorescently labeled probe that identifies either the TP53 or the D21S55 loci, which are highly associated with hematological malignancies [26]. In this method, the confirmation of the signal indicates the replication status, thus alleles that replicate synchronously characterize concomitantly expressed genes in the common bi-allelic mode. In patients with hematological malignancies including HL and NHL, pre-alloSCT significantly high level of mono-allelic replication of both loci was observed after evaluation of bone marrow (BM) and peripheral blood (PB) samples. Similarly high levels of mono-allelic replication were observed in normal donors that received 5 days of subcutaneous injections of G-CSF (10 \( \mu \text{g/kg} \)) for mobilization. Moreover, the effect of G-CSF could be mimicked in vivo by adding the growth factor to PB cells in cultures. No increase in mono-allelic replication could be observed in the control cultures. It was therefore concluded that the frequency of asynchronous replication of the TP53 and D21S55 loci is low in PB and BM samples of normal donors, and high in patients with hematological malignancies including HL. Furthermore, in normal donors treated with G-CSF in vivo and in vitro, the frequency increases to a level indicative of mono-allelic replication. This effect is reversible. Long-term follow-up of the stem-cell donors may, therefore, be required. The significance of these preliminary findings has yet to be elucidated.

Concluding remarks

Although it is now well established that HRS cells in the majority of cases are clonally derived from germinal center B lymphocytes, the factors contributing to the development of an HL cell clone remain to be elucidated. Epidemiological studies suggest that heritable factors are important in terms of susceptibility. Further investigations will reveal whether a person can be identified as being at high risk of developing HL prior to the onset of disease, or at least prior to the clinical manifestation of the lymphoma clone. Moreover the identification of HL patients that are at high risk for developing of secondary cancer following chemotherapy may help in determining the best available treatment as well as the manner of aftercare following treatment. Further identification of intrinsic host factors contributing to the development of HL, as well as TR secondary cancers in HL patients, will thus have
broad implications in the future with regard to early diagnosis or even prevention of the disease, as well as in terms of management of the disease.

References