Further investigation of the role of HLA-DPB1 in adult Hodgkin’s disease (HD) suggests an influence on susceptibility to different HD subtypes

GM Taylor¹, DA Gokhale¹, D Crowther², PJ Woll²*, M Harris³, D Ryder⁴, M Ayres¹ and JA Radford²

¹Immunogenetics Laboratory, Department of Medical Genetics, St Mary’s Hospital, Hashterse Road, Manchester M13 0JH, UK; Departments of ²Medical Oncology, ³Pathology and ⁴Medical Statistics, Christie Hospital, Manchester, UK

Summary It has been suggested in a number of studies that susceptibility to adult Hodgkin’s disease (HD) is influenced by the HLA class II region, and specifically by alleles at the HLA-DPB1 locus. Since HD is diagnostically complex, it is not clear whether different HLA-DPB1 alleles confer susceptibility to different HD subtypes. To clarify this we have extended a previous study to type DPB1 alleles in 147 adult HD patients from a single centre. We have analysed patients with nodular sclerosing (NS), mixed cellularity (MC) or lymphocyte predominant (LP) HD, and gender in relation to HLA-DPB1 type, in comparison with 183 adult controls. The results confirmed previously reported associations of DPB1*0301 with HD susceptibility (relative risk (RR) = 1.42; 95% confidence interval (CI) 0.86–2.36) and DPB1*0201 with resistance to HD (RR = 0.49; CI 0.27–0.90). However, analysis by HD subtype and gender showed that *0301-associated susceptibility was confined to females with HD (RR = 2.46; CI 1.02–5.92), and *0201-associated resistance to females with NS-HD (RR = 0.28; CI 0.10–0.79). Susceptibility to NS-HD was also associated in females with *1001 (RR = 11.73; CI 1.32–104.36), and resistance with *1101 (RR = 0.08; CI 0.01–0.65). In contrast, susceptibility to LP-HD was associated in males with *2001 (RR = 32.14; CI 3.17–326.17), and to MC-HD with *3401 (RR = 16.78; CI 2.84–99.17). Comparison of DPB1-encoded polymorphic amino-acid frequencies in patients and controls showed that susceptibility to MC-HD was associated with Leucine at position 35 of DPB1 (RR = 8.85; CI 3.04–25.77), Alanine-55 (RR = 15.17; CI 2.00–115.20) and Valine-84 (RR = 15.94; CI 3.55–71.49). In contrast, Glutamic acid 69 was significantly associated with resistance to MC-HD (RR = 0.14; CI 0.03–0.60). Certain DPB1 alleles and individual DPB1 polymorphic amino acid residues may thus affect susceptibility and resistance to specific HD subtypes. This may be through their influence on the binding of peptides derived from an HD-associated infectious agent, and the consequent effect on immune responses to the agent.

Keywords: Hodgkin’s disease; HLA-DPB1; HVR; susceptibility; resistance; polymorphic amino acid

There have been numerous suggestions that Hodgkins disease (HD) may have an infectious aetiology (Vianna et al, 1971; Viana, 1974; Alexander et al, 1991a; Mauch et al, 1993), though the epidemiological and diagnostic complexity of HD has hindered a complete understanding. Studies suggesting that the peak of HD cases in early adulthood HD resembles that of paralytic poliomyelitis in the pre-vaccine era (Gutensohn and Cole, 1977) support an aetiological pathway in which HD arises as a rare outcome of a common infection (Gutensohn and Cole, 1981). There is now extensive evidence to suggest that the infectious agent, at least in some cases, is Epstein–Barr virus (EBV) (Weiss et al, 1989; Wu et al, 1990; Khan et al, 1992; Jarrett, 1993; Oudejans et al, 1997). However, the ubiquitous distribution of EBV in the normal population, without accompanying disease (Miller, 1990), suggests that other factors, possibly of host origin, contribute to the aetiology of HD.

Immune responses to infectious agent causally associated with HD may be influenced by inter-individual genetic variations. Cell-surface heterodimers encoded by the HLA class II (DR, DQ and DP) loci play a pivotal role in the control of immune responses through the presentation of peptide antigens to T-cells (Brown et al, 1988; Germain, 1994; Hammer, 1995). A number of recent studies have suggested that alleles at the HLA-DPB1 locus, the most centromeric of the three major HLA class II loci (DR, DQ and DP), may increase susceptibility and resistance to HD. Thus, HLA-DPB1*0301 was found to be associated with susceptibility, and *0201 with resistance to HD (Bodmer et al, 1989; Tonks et al, 1992; Cesbron et al, 1993; Pellegris et al, 1993; Klitz et al, 1994; Oza et al, 1994).

The diagnostic complexity of HD and the excess of males with certain HD subtypes (Alexander et al, 1991b; Glaser and Jarrett, 1996) suggests that these two DPB1 alleles (*0301, *0201) may not be the only ones to contribute to HD susceptibility and resistance. This view is supported by the fact that since DPB1 subunits are made up of 2–3 polymorphic amino acid residues at each of six hypervariable regions (HVRs) in exon 2 (Bugawan et al, 1990), some alleles may have identical residues at certain HVRs, but not at others. In a previous study we alluded to a relationship between DPB1 alleles and subtypes of HD (Taylor et al, 1996), but there were insufficient patients for a detailed analysis. To clarify this we have now extended the study to include 147 HD patients classified by subtype, gender and polymorphic amino acid alleles, and compared them with 183 controls.
MATERIALS AND METHODS

Patients and controls

The patients consisted of 147 unrelated adult UK Caucasians with HD, aged between 15 and 75 years, from the North West of England. The series included 118 patients described in a preliminary report (Taylor et al, 1996) together with a further 29 patients. The patients all attended a single treatment centre (The Medical Oncology Clinic, Christie Hospital, Manchester, UK). Further details of the patients are given in Table 1. The control group consisted of 183 anonymous healthy adult blood donors, collected by the National Blood Transfusion Service (NBTS) in Manchester, UK. This compares with 92 adult controls in our previous study (Taylor et al, 1996). They were a randomly collected series consisting of 27.9% males, and 61.2% females. In 10% of cases, the sex of the donor was not specified by the NBTS for reasons of confidentiality. Gender analysis was thus confined to the controls where this information was available. The youngest control subject was 19 years and the oldest was 67 years, the mean and median ages being 41.4 and 42 years respectively.

Histopathology

Review of tissue sections from diagnostic biopsies for all 147 patients was carried out in the Department of Histopathology at the Christie Hospital under the supervision of a cancer pathologist (MH). Assignment of HD subtype was performed in accordance with the Rye modification of the Lukes classification system (Lukes et al, 1966a, 1966b). For the purposes of this study, the HD cases were classified into nodular sclerosing (NS), mixed cellularity (MC), lymphocyte-predominant (LP), lymphocyte-depleted (LD) and unclassified. LP is included as a subtype of HD in this study for completeness and comparative purposes, even though LP is generally accepted to be a separate disease entity (Pan et al, 1996).

DPB1 genomic DNA amplification

Genomic DNA was extracted from the whole blood of patients and controls using established methods, and a 288-bp fragment of HLA-DPB1 exon 2 was amplified using the polymerase chain reaction (PCR) primers DPB1-5′ (GAG AGT GGC GCC TCC GC TCA T) and DPB1-3′ (GCC GGC CCA AAG CCC TCA CTC) using PCR conditions described previously (Taylor et al, 1996).

DPB1 typing with SSO probes

DPB1 typing was carried by the method of Bugawan et al (1990) as described previously (Taylor et al, 1996) using 24 sequence-specific oligonucleotide (SSO) probes. DNA samples that could not be assigned a DPB1 type following SSO hybridization were further analysed by direct DNA sequencing of PCR products.

Allele assignment

DPB1 alleles were assigned from patterns of SSO probe reactivities according to the XIth HLA workshop (Tait et al, 1992) and as detailed in the Report of the HLA Nomenclature Committee (Marsh, 1996). Polymorphic amino acids encoded by six HVRs situated in exon 2 were deduced from allele assignments as previously described (Taylor et al, 1996).

Data analysis

DPB1 allele, phenotype and genotype frequencies in the patients and controls were computed, and results of phenotype frequency analysis compared by χ^2 and Fisher’s exact tests. Phenotype relative risks (RR) were calculated using the method of Sheehe (1966) and 95% confidence intervals (95% CI) of RR calculated by the Mantel and Haentzel (1959) method using the CONTING, 2by2 and ReRi utilities for the IBM-PC provided by Professor Jurgen Ott (Columbia University, NY, USA; see: http://linkage.rocketeer.edu/soft/linkutil). Significance values for the χ^2 distribution and Fisher’s exact test were subjected to the Bonferroni correction by multiplying P-values by the number of comparisons, unless otherwise indicated. Differences in DPB1 phenotype distributions in HD subtype and control groups were determined using the log-likelihood ratio or G-test of Sokal and Rohlf (1981) as described by Klitz et al (1994). The G-statistic was calculated using a resampling computer algorithm (rxc.exe) written and provided by Dr George Carmody (Carleton University, Ottawa, Canada). The algorithm

### Table 1

| Diagnostic subtype          | Males | Females | Total |
|----------------------------|-------|---------|-------|
| Nodular sclerosing (NS)    | 47    | 50.5%   | 43    | 79.6| 90 | 61.2 |
| Lymphocyte predominant (LP)| 19    | 20.4%   | 3     | 5.5 | 22  | 14.9 |
| Mixed cellularity (MC)     | 18    | 19.3%   | 6     | 11.1| 24  | 16.3 |
| Lymphocyte depleted (LD)   | 0     | 0%      | 1     | 1.8 | 1   | 0.6  |
| Unclassified               | 9     | 9.6%    | 1     | 1.8 | 10  | 6.8  |
| Total                      | 93    | 100%    | 54    | 100 | 147 | 100  |

*Number (n) of patients within each diagnostic subgroup. Percentage (% of patients in each subgroup.

### Table 2

| Patients | Present study | Total group |
|----------|---------------|-------------|
| Number of patients (n) | 114a | 897b |
| Median age (years) | 28.9 | 30.9 |
| Male:Female ratio | 1.59:1c | 1:73:1 |
| Total survival | 105 (92.1)d | 694 (77.4) |
| Histopathology | | |
| Nodular sclerosing | 76 (66.7)a | 467 (52.1) |
| Lymphocyte predominant | 15 (13.2) | 140 (15.6) |
| Mixed cellularity | 18 (15.8) | 228 (25.4) |
| Lymphocyte depleted | 2 (1.8) | 26 (2.9) |
| Unclassified | 3 (2.6) | 36 (4) |
| Staging | | |
| Stage I | 15 (13.2) | 173 (19.3) |
| Stage II | 47 (41.2) | 335 (37.3) |
| Stage III | 24 (21.1) | 160 (17.8) |
| Stage IV | 28 (24.6) | 229 (25.5) |

*Of 147 DPB1 typed HD patients, 114 with relevant clinical data were compared with 897 from the same centre. *Data from 897 patients with Hodgkins disease patients presenting at the Department of Medical Oncology, Christie Hospital between 1974 and March 1995. *Male:female ratio for total patient series. *Figures in parentheses are percentage values.
was set to simulate 10,000 random distributions in which two columns (patients and controls) were compared in a \(2 \times N\) array, where \(N\) = number of \(DPB1\) phenotypes (rows). \(DPB1\) allelic diversity \((h)\) was calculated according to the method of Nei and Roychoudhury (1974) to provide an estimate of expected heterozygosity. This value was compared with the observed heterozygosity obtained from the frequency of each \(DPB1\) genotype.

### RESULTS

#### Patients

Details of the patients, classified by histological subtype and gender, are shown in Table 1. Of the 147 patients, 90 (61.2%) were NS, 22 (14.9%) were LP, 24 (16.3%) were MC, one (0.68%) patient was LD and ten (6.8%) patients were of unclassifiable histological subtype. By gender, 50.5% of male patients compared with 79.6% of female patients were NS, 20.4% of males and 5.5% females were LP, and 19.3% of males and 11.1% of females were MC. Overall, male exceeded female patients by 37% (male:female ratio (M:F) = 1.72:1). The M:F for NS patients was 1.09, compared with 6.3 and 3.0 for LP and MC patients respectively.

The one LD and ten unclassifiable patients were excluded from the \(DPB1\) analysis of the non-NS patients, but were included in the overall analysis. Of the 22 LP patients, nine (40.9%) were classified as the nodular LP variant, but were not analysed separately from the other LP cases.

The mean and median ages of the total patient group were 33 years and 30 years respectively. The number of cases was highest in the 20- to 30-year age group, with an equal number of males and females in the 20- to 24-year group. Males exceeded females in most of the other age categories. The 19 male LP and 18 male MC cases were spread over the age range 15–49. Although a bimodal age distribution (Glaser and Jarrett, 1996) was not strongly evident, there was an increase in males > 60 years.

#### Patient ascertainment

The patient group was a retrospective, hospital-based series referred for treatment and follow-up to a single centre. Patients were recruited to the HLA study either at presentation (i.e. before treatment) or during follow-up (i.e. at varying times after treatment) between 1990 and 1994. Patients presenting before this period, but lost to follow-up, were not included in the study. The

| Footnote | \(DPB1\) | Statistical comparison | Test | RR | 95% CI | Uncorrected \(P\)-value | Correction factor | Corrected \(P\)-value |
|---|---|---|---|---|---|---|---|---|
| a | *0201 | Total HD vs controls | \(\chi^2\) | 0.49 | 0.27–0.90 | 0.0191 | 21 | >0.05 |
| b | *0201 | Female HD vs female controls | \(\chi^2\) | 0.31 | 0.12–0.80 | 0.0130 | 42 | >0.05 |
| c | *0201 | Female NS-HD vs female controls | \(\chi^2\) | 0.28 | 0.10–0.79 | 0.0133 | 126 | >0.05 |
| d | *0301 | Total HD vs controls | \(\chi^2\) | 1.42 | 0.86–2.36 | 0.0171 | 21 | >0.05 |
| e | *0301 | Female HD vs female controls | \(\chi^2\) | 2.46 | 1.02–5.92 | 0.0423 | 42 | >0.05 |
| f | *1101 | Female NS-HD vs female controls | Fisher’s | 11.73 | 1.32–104.46 | 0.0405 | 252 | >0.05 |
| g | *1101 | NS-HD vs controls | Fisher’s | 0.08 | 0.01–0.65 | 0.0111 | 126 | >0.05 |
| h | *2001 | LP-HD vs controls | Fisher’s | 44.76 | 4.45–449.90 | 0.0111 | 126 | >0.05 |
| i | *2001 | Male LP-HD vs male controls | Fisher’s | 32.14 | 3.17–326.17 | 0.0201 | 252 | >0.05 |
| j | *3401 | Male MC-HD vs male controls | Fisher’s | 16.78 | 2.84–99.17 | 0.0083 | 252 | >0.05 |

Correction factors were applied to \(P\)-values as follows: x21: Number of phenotypes detected; x3: Number of histological subtypes; x2: Gender (male or female); x2: 2-tailed Fisher’s exact test.

### Table 3

| \(DPB1\) allele | All Hodgkin’s disease (%) | Male HD (%) | Female HD (%) |
|---|---|---|---|
| | Total | NS | LP | MC | Total | NS | LP | MC | Total | NS | LP | MC |
| *0101 | 6.1 | 6.7 | 4.5 | 4.2 | 6.5 | 8.5 | 5.3 | 0 | 6.3 | 5.6 | 4.7 | 0 |
| *0201 | 11.6 | 12.2 | 4.5 | 12.5 | 10.8 | 12.8 | 5.3 | 11.1 | 16.1 | 13.0 | 11.6 | 0 |
| *0202 | 3.4 | 3.3 | 0 | 8.3 | 4.3 | 4.3 | 0 | 11.1 | 1.8 | 1.9 | 2.3 | 0 |
| *0301 | 27.2 | 28.9 | 27.3 | 33.3 | 20.8 | 22.6 | 25.5 | 21.1 | 27.8 | 23.2 | 35.2 | 32.6 | 66.7 | 50 |
| *0401 | 74.1 | 68.9 | 81.8 | 87.5 | 69.9 | 72.0 | 63.8 | 78.9 | 88.9 | 74.1 | 77.8 | 74.4 | 100 | 83.3 | 64.7 |
| *0402 | 15.6 | 16.7 | 22.7 | 8.3 | 22.4 | 17.2 | 17.0 | 26.3 | 11.1 | 24.1 | 13.0 | 16.3 | 0 | 23.5 |
| *0501 | 4.8 | 3.3 | 9.1 | 8.3 | 2.7 | 4.3 | 2.1 | 0 | 5.6 | 6.7 | 7.0 | 0 | 7.8 |
| *0601 | 3.4 | 4.4 | 0 | 4.2 | 3.3 | 2.2 | 2.1 | 0 | 5.6 | 1.8 | 1.8 | 2.3 | 0 | 7.8 |
| *0901 | 1.4 | 2.2 | 0 | 0 | 2.7 | 2.2 | 4.3 | 0 | 5.6 | 2.7 | 2.7 | 0 | 0 | 0 | 2.0 |
| *1001 | 3.4 | 5.6 | 0 | 8.3 | 2.2 | 1.1 | 2.1 | 0 | 0 | 1.8 | 7.4 | 0 | 0 | 7.8 |
| *1101 | 2.0 | 0 | 0 | 0 | 6.0 | 3.2 | 0 | 0 | 6.3 | 0 | 0 | 0 | 0 | 0 | 3.9 |
| *1301 | 4.1 | 5.6 | 0 | 4.2 | 2.2 | 4.3 | 6.4 | 0 | 5.6 | 2.7 | 3.7 | 4.7 | 0 | 0 | 0 |
| *1401 | 4.1 | 3.3 | 4.5 | 0 | 4.9 | 5.4 | 4.3 | 0 | 5.3 | 3.6 | 1.9 | 2.3 | 0 | 5.9 |
| *1501 | 2.0 | 1.1 | 4.5 | 4.2 | 0.5 | 2.2 | 0 | 5.3 | 5.6 | 0.9 | 1.9 | 2.3 | 0 | 2.0 |
| *1601 | 1.4 | 1.1 | 0 | 0 | 2.2 | 2.2 | 2.1 | 0 | 0 | 1.8 | 0 | 0 | 0 | 0 | 2.0 |
| *1701 | 1.4 | 2.2 | 0 | 0 | 2.2 | 2.2 | 4.3 | 0 | 0 | 1.8 | 0 | 0 | 0 | 0 | 2.0 |
| *1901 | 0.7 | 1.1 | 0 | 0 | 0.5 | 1.1 | 2.1 | 0 | 0 | 0.9 | 0 | 0 | 0 | 0 | 0 |
| *2001 | 1.4 | 0 | 9.1 | 0 | 0 | 2.2 | 0 | 10.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *2601 | 0 | 0 | 0 | 0 | 0.5 | 2.2 | 0 | 0 | 0 | 0.9 | 0 | 0 | 0 | 0 | 0 |
| *3401 | 2.0 | 0 | 0 | 4.2 | 0.5 | 3.2 | 0 | 0 | 16.7 | 0.9 | 0 | 0 | 0 | 0 | 0 |

\(n = 147\) 90 22 18 183 93 47 19 18 112 54 43 3 6 51
minimum time interval from diagnosis to blood sampling was 1 week to 13.5 years (median 1.84 years). To assess the extent to which patient selection biased the HLA analysis, an HLA-typed patient group (n = 114) for which there was detailed clinical data was compared with a total evaluable group of patients (n = 897) attending the clinic between 1974 and 1995. The total evaluable group included the DPB1-typed patients.

The two groups are compared in Table 2, which shows that the distribution of HD subtypes was similar, although the frequency of NS was slightly greater and MC slightly less in the HLA-typed group compared with the total group. The M:F ratio of DPB1-typed patients was less than in the total group, reflecting a deficit of MC patients. The age and staging of the two groups were not remarkably different. The overall survival rate of the HLA-typed patients was 92.1% compared with 77.4% in the total evaluable group. Comparison of death rates in the total and HLA-typed groups by Cox regression analysis showed that the typed patients had a slightly lower death rate than the total group, but this was not significant (P = 0.23). Given the favourable prognosis of HD in this centre (Radford et al, 1995), we conclude that any selection bias of the HLA-typed patients for survival was small.

**DPB1 allele and phenotype frequency analysis**

HLA-DPB1 typing revealed 19 alleles in the patient series and 20 alleles in the controls. One allele present in the patients was absent, but this was not significant (P = 0.23). Given the favourable prognosis of HD in this centre, we conclude that any selection bias of the HLA-typed patients for survival was small.
from the controls (*2001), whilst two alleles (*2601, *2901) were absent from the patients but present in the controls. Analysis of allelic diversity (h) showed no significant difference in heterozygosity in the total HD group and the controls (data not shown).

The frequency of DPB1 phenotypes in the total patient group and by subtype was compared with the controls using the $2 \times N$ log-likelihood ratio method to compute the G-statistic. No correction for the number of DPB1 alleles is required with this method (Klitz et al, 1994). No significant difference was found in the total patient series or in each subtype alone (NS, LP and MC) compared with the controls (data not shown). However, the combined non-NS group (LP+MC+LD) showed a significant difference from the controls (G-statistic: 40.3, $P = 0.0053$), indicating a difference in the frequency distribution of DPB1 phenotypes due to non-NS-HD.

To identify whether specific DPB1 alleles were associated with susceptibility and resistance to HD, phenotype relative risks and 95% confidence intervals were determined allele-by-allele in 2 tests for each subtype and gender. The complete results, together with the statistical analysis, are shown in Table 3. They can be summarized thus: an excess of females of all HD subtypes typed for DPB1*0301 (RR = 2.46; 95% CI 1.02–5.92); a deficit of all patients typed for DPB1*0201 (RR = 0.49; 95% CI 0.27–0.90) and this was greater in females with NS-HD (RR = 0.28; 95% CI 0.10–0.79); in males, neither *0301 nor *0201 were associated with susceptibility or resistance respectively. In females, DPB1*0101 was associated with resistance to NS (RR = 11.73; 95% CI 1.32–104.46), and *1101 with resistance to HD (RR = 0.08; 95% CI 0.01–0.65). In males, *2001 was associated with susceptibility to LP (RR = 32.14; CI 326.17), and *3401 was associated with susceptibility to MC (RR = 16.78; 95% CI 2.84–99.17).

**Analysis of polymorphic amino acids**

DPB1 alleles are composed of combinations of polymorphic DNA sequences in six HRVs in exon 2 which encode 2–3 polymorphic amino acids at each position. The identity of these amino acids can be predicted from the patterns of SSO hybridization used to assign classical DPB1 alleles. Since none of the HRV sequences is allele-specific, the same sequence can occur in more than one classical DPB1 allele. By comparing the frequency of polymorphic amino acids in patients and controls, corrections need only involve the number of amino acid alleles at that position. We therefore carried out a complete analysis in patients and controls of all polymorphic amino acids encoded at the following positions of DPB1: 8 (Leu/Val), 9 (Phe/His/Tyr), 11 (Gly/Leu), 35 (Phe/Leu/Tyr), 36 (Ala/Val), 55 (Ala/Asp/Glu), 56 (Ala/Glu), 57 (Asp/Glu), 65 (Ile/Leu), 69 (Glu/Lys/Arg), 76 (Ile/Met/Val), 84 (Asp/Gly/Val) and 85–87 (Glu-Ala-Val/Gly-Pro-Met).

The results are shown in Table 4 in relation to HD subtype and gender. After correction for the number of comparisons, four amino acid residues (leucine 35, alanine 55, alanine 56 and valine 84) were significantly associated with susceptibility to HD, and one (glutamic acid 69) was associated with resistance. Leuc 35, Ala 55 and Val 84 were associated with susceptibility in males to MC (Leu 35: RR = 8.52, 95% CI 2.58–28.16; Ala 35: RR = 8.69, 95% CI 1.10–68.49; Val 84: RR = 13.72, 95% CI 2.97–63.32). Glutamic acid 69 was associated with resistance to LP (RR = 0.14; 95% CI 0.03–0.60) in males. In contrast to the classical DPB1 phenotype analysis, none of the amino acid comparisons revealed significant associations with HD in females.

**DISCUSSION**

A role for the HLA class II region and specifically for alleles at the HLA-DPB1 locus in the aetiology of Hodgkin’s disease has been proposed in a number of studies (Bodmer et al, 1989; Tonks et al, 1991, 1992; Klitz et al, 1994; Oza et al, 1994; Taylor et al, 1996). What has emerged from these studies is that DPB1*0301 appears to confer susceptibility, and DPB1*0201 resistance to HD. However, relative risks associated with these alleles were small, implying either that DPB1 is only a minor contributor to HD susceptibility, or that they are masked by the diagnostic heterogeneity of the disease.

The present study extends our previous work (Taylor et al, 1996) by the addition of 29 patients and 91 adult controls, using a more detailed analytical approach. Our results, obtained with patients from a specific geographical region treated at a single specialist centre, confirm previous findings of an increase in DPB1*0301 as an indicator of susceptibility (Tonks et al, 1992) and a decrease in *0201, indicative of resistance (Bodmer et al, 1989) in HD. They also show that subclassification of HD by histological subtype and gender reveal additional associations which were not previously reported. Our results do not rule out a contribution from other HLA class II loci (Klitz et al, 1994) and, bearing in mind the greater incidence of non-NS-HD in males, a role for HLA-associated, X-linked susceptibility genes.

We found that the increase in DPB1*0301 was greatest in females with non-NS, and the decrease in DPB1*0201 was greatest in females with NS. Neither allele appeared to contribute to susceptibility or resistance to HD in males. Interestingly, susceptibility in males was associated with two rare alleles *2001 and *3401, and involved LP and MC-HD respectively. We have previously reported a family with HD in two sisters, both of whom typed for DPB1*2001 (Gokhale et al, 1995). In females, susceptibility to NS-HD was also associated with DPB1*1001. At first sight, these results appear to suggest that the HD subtype and the patient’s gender might influence the DPB1 association. However, the more likely explanation is that it is a person’s gender and DPB1 type influencing the HD subtype that develops following an aetiological event such as infection with a virus. If this is the case, it raises the possibility that certain DPB1 alleles may do this by their influence on the presentation of infection-derived peptides to T-cells. Precisely how an ensuing immune response to the infectious agent affects HD pathology remains to be determined. One possibility is that the magnitude or type of T-cell response elicits the proliferation of a lineage-specific premalignant clone.

The present study was carried out on a hospital-based patient series which consisted of retrospectively and prospectively ascertained HD patients. We sought to minimize sources of bias by limiting the study to a single specialist treatment centre with diagnostic review by the same cancer pathology department. The excellent overall survival rate of the Manchester patients (Radford et al, 1995) suggests that there was only minimal selection bias favouring survivors. This was confirmed by comparing the survival of the DPB1-typed patients with a total evaluable patient group from the same centre. There was no major difference in overall survival despite a small deficit of MC patients in the DPB1-typed group. Previous studies of HLA class II alleles in HD patients (Tonks et al, 1992; Klitz et al, 1994) have also involved hospital-based patient series.

Although we tested and confirmed a prior hypothesis that DPB1*0301 was associated with susceptibility and DPB1*0201...
with resistance to HD, the significance of these and other allele associations was lost when the null hypothesis of no association was corrected for the number of tests (DPB1 alleles, HD subtype and gender). This emphasizes one of the problems encountered in studying the contribution of a highly polymorphic genetic system to the aetiology of a rare, diagnostically heterogeneous disease, especially where the genetic contribution may be indirect and of low penetrance. Even though uncorrected allele associations may be chance findings, they are of value in calculating the size of the patient and control groups required to verify such observations.

Since DPB1 peptide diversity is determined by polymorphic amino acids coded by each of the six DPB1 HVRs (Bugawan et al, 1990) an alternative approach was to analyse their frequency, instead of analysing classical DPB1 alleles (i.e. *0101*, *0201* etc). Each HVR shows only limited polymorphism, so the magnitude of the Bonferroni correction at each position is much less than required when correcting for the number of classical DPB1 alleles. Furthermore, since the polymorphic amino acids encoded by each HVR are themselves probably involved in determining antigenic peptide-binding and disease susceptibility, each polymorphic position in exon 2 is arguably at least, if not more, important than classical alleles.

The results of this analysis showed that even after correction for the number of comparisons (amino acid, subtype and gender), there were significant associations between specific amino acids and histological subtypes. Four amino acid residues were found to be involved in susceptibility to MC-HD, two of which (leucine 35 and valine 84) were significantly increased by virtue of their presence in rare DPB1 alleles. The increased frequency of leucine 35 in males with MC can be related to its presence in male controls. Glutamic acid at position 69 seems to protect males against LP, due to its occurrence in MC-HD. The allele *0201* was corrected for the number of classical **DPB1** alleles. The increased frequency of leucine 35, alanine 55 and valine 84 in MC could indicate a direct role in interactions with viral peptides. The gender difference in the amino acid association could mean that the response to an aetiological agent is modified by X-linked genes. It is worth noting that the gene for X-linked lymphoproliferative disease, a disease associated with an inability to combat EBV infection, maps to Xq25 (Skare et al, 1989). A variant of the XLP gene, together with HLA-DPB1 polymorphic amino acids, could perhaps contribute to susceptibility to MC-HD.

**ACKNOWLEDGEMENTS**

We are grateful for the dedicated assistance of Diane Meynell and Amanda Watson during this project. The work was supported by grants from the Leukaemia Research Fund and Kay Kendall Leukaemia Fund to GM Taylor, and the Cancer Research Campaign to D Crowther.

**REFERENCES**

Alexander FE, Ricketts TJ, McKinney PA and Cartwright RA (1991a) Community lifestyle characteristics and incidence of Hodgkin's disease in young people. *Int J Cancer* 48: 10–14

Alexander FE, McKinney PA, Williams J, Ricketts TJ and Cartwright RA (1991b) Epidemiological evidence for the 'two-disease hypothesis' in Hodgkin's disease. *Int J Epidemiol* 20: 354–361

Bodmer JG, Tonks S, Oza AM, Lister TA and Bodmer WF (1989) HLA-DP-based resistance to Hodgkin's disease. *Lancet* i: 1455–1456

Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL and Wiley DC (1993) Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 364: 33–39

Bugawan TL, Begovich AB and Erlich HA (1990) Rapid HLA-DPB typing using enzymatically amplified DNA and nonradioactive sequence-specific oligonucleotide probes. *Immunogenetics* 32: 231–241

Cesbron A, Moreau P, Rapp MJ, Chenneau ML, Herry P, Bonneville F, Muller JY, Harrouesse JL and Bigmon JD (1993) HLA-DPB and susceptibility to Hodgkin's disease. *Hum Immunol* 36: 51

Fu XT, Bono CP, Wouffe SL, Sweatengin C, Summers NL, Sinagaglia F, Sette A, Schwartz BD and Karr RW (1995) Pocket 4 of the HLA-DR (αβ) *0401* molecule is a major determinant of T cell recognition of peptide. *J Exp Med* 181: 915–926

Germain RN (1994) MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 76: 287–299

Glaser SL and Jarrett RF (1996) The epidemiology of Hodgkin's disease. *Ballière's Clin Haematol* 9: 401–416

Gokhale DA, Evans DG, Crowther D, Woll P, Watson CJ, Dearden SP, Fergusson WD, Stevens RF and Taylor GM (1995) Molecular genetic analysis of a family with a history of Hodgkin's disease and dyschondrosteosis. *Leukemia* 9: 826–833

Gutensohn N and Cole P (1977) Epidemiology of Hodgkin's disease in the young. *Int J Cancer* 19: 595–604

Germain RN and Cole P (1977) Epidemiology of Hodgkin's disease in the young. *Int J Cancer* 19: 595–604
Gutensohn N and Cole P (1981) Childhood social environment and Hodgkin’s disease. *N Engl J Med* **304**: 135–140

Hammer J (1995) New methods to predict MHC-binding sequences within protein antigens. *Curr Opin Immunol* **7**: 263–269

Hammer J, Gallazzi F, Bono E, Karr RW, Guenot J, Valsasnini P, Nagy ZA and Smigaglia F (1995) Peptide binding specificity of HLA-DR4 molecules: correlation with rheumatoid arthritis association. *J Exp Med* **181**: 1847–1855

Jarrett RF (1993) Viruses and Hodgkin’s disease. *Leukemia* **7**: S78–S82

Khan G, Coates PJ, Gupta RK, Kangro HO and Slavin G (1992) Presence of Epstein-Barr virus in Hodgkin’s disease is not exclusive to Reed–Sternberg cells. *Am J Pathol* **140**: 757–762

Klitz W, Aldrich CL, Filides N, Horning SJ and Begovich AB (1994) Localization of predisposition to Hodgkin’s disease in the HLA class II region. *Am J Hum Genet* **54**: 497–505

Krupp RJ, Butler JH and Hicks EB (1966) Is Hodgkin’s disease infectious? *Tumori* **52**: 269–272

Lukes RJ, Craver LF, Hall TC, Rappaport H and Ruben P (1966) Genetic distance. *J Natl Cancer Inst* **22**: 733–736

Lukes RJ, Butler JI and Hicks EB (1966) Combination of log relative risk in retrospective studies of disease. *J Natl Cancer Inst* **22**: 719–748

Manel N and Haenszel W (1959) Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* **22**: 719–748

Mach P, Kalish LA, Kadin M, Colemain CN, Osteen R and Hellman S (1993) Patterns of presentation of Hodgkin disease. *Cancer* **71**: 2062–2071

Miller G (1990) Epstein–Barr virus: biology, pathogenesis and medical aspects. *In: Field’s Virology*, pp. 1921–1958. Raven Press: New York

Nair M and Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. *Genetics* **76**: 379–390

Oudejans JJ, Jiwa NM and Meijer CJLM (1997) Epstein–Barr virus in Hodgkin’s disease: more than just an innocent bystander. *J Pathol* **181**: S78–S82

Pan LX, Diss TC, Peng HZ, Norton A and Isaacs PG (1996) Nodular lymphocyte predominance Hodgkin’s disease: a monoclonal or polyclonal B-cell disorder? *Blood* **87**: 2428–2434

Pellegrin G, Lombardo C, Cantoni A, Devizzi L and Balzarotti M. (1993) Study of the HLA-DRB1 locus by the polymerase chain reaction technique in patients with Hodgkin’s disease. *Tumori* **79**: 133–136

Potolicchio I, Mosconi G, Forni A, Nemetery B, Seghizzi P and Sorrentino R (1997) Susceptibility to hard metal lung disease is strongly associated with the presence of glutamate 69 in HLA-DR beta chain. *Eur J Immunol* **27**: 2741–2743

Radford JA, Crowther D, Rohatiner AZS, Ryder WDJ, Gupta RK, Oz a, Deakin DP, Arnott S, Wilkinson PM, James RD, Johnston RJ and Lister TA (1995) Results of a randomized trial comparing MVPP chemotherapy with a hybrid regimen, CHVP/IE, in the initial treatment of Hodgkin’s disease. *J Clin Oncol* **13**: 2379–2385

Sheireh PR (1986) Combination of log relative risk in retrospective studies of disease. *Am J Pub Health Nations Health* **56**: 1745–1750

Skare JC, Grierson HL, Sullivan JL, Nussbaum RL, Putito DT, Sylla BS, Lenoir GM, Reilly DS, White BN and Milansky A (1989) Linkage analysis of seven kindreds with the X-linked lymphoproliferative syndrome (XLP) confirms that the XLP locus is near DXS42 and DXS37. *Human Genet* **82**: 354–358

Sokal RR and Rohlf FJ (1995) *Biometry*, 3rd edn. WH Freeman: New York

Tait BD, Bodmer JG, Erlach HA, Ferrara GB, Albert E, Begovich A, Kimura A, Varney MD and Klitz W (1991) DNA typing: DPA and DPB analysis. In: *HLA 1991: Proceedings of the Eleventh International Histocompatibility Workshop and Conference*, Vol. 1. Tsuji K, Aizawa M and Sasazuki T (eds), pp. 485–496. Oxford Science: Oxford

Taylor GM, Gokhale DA, Crowther D, Woll P, Harris M, Alexander F, Jarrett R and Cartwright RA (1996) Increased frequency of HLA-DRB1*0301 in Hodgkin’s disease suggests that susceptibility is HVR-sequence and subtype associated. *Leukemia* **10**: 854–859

Tonks S, Oz a AM, Lister TA, Bodmer JG and Collaborating Centres (1994) An international study of the association between HLA-DR and Hodgkin’s disease. *Leukemia* **8**: 190–195

V arney MD and Klitz W (1991) DNA typing: DPA and DPB analysis. In: *HLA 1991: Proceedings of the Eleventh International Histocompatibility Workshop and Conference*, Vol. 1. Tsuji K, Aizawa M and Sasazuki T (eds), pp. 539–544. Oxford Science: Oxford

Tonks S, Oz a AM, Lister TA and Bodmer JG (1992) Association of HLA-DR with Hodgkin’s disease. *Lancet* k. 340: 968–969

Vianna NJ, Greenwald P and Davies JNP (1971) Nature of Hodgkin’s disease agent. *Brit J Cancer* (1999) **80**(9), 1405–1411

Wei S, Movahed LA, Warnke RA and Sklar J (1989) Detection of Epstein–Barr viral genomes in Reed-Sternberg cells of Hodgkin’s disease. *N Engl J Med* **320**: 502–506

Wu T-C, Mann RB, Charache P, Hayward SD, Staal S, Lambe BC and Ambinder RF (1990) Detection of EBV gene expression in Reed-Sternberg cells of Hodgkin’s disease. *Int J Cancer* **46**: 801–804

Zerva L, Cizman B, Mehra NK, Alahari SK, Murali R, Zmijewski CM, Kamoun M and Monos DS (1996) Arginine at positions 13 or 70–71 in pocket 4 of HLA-DRB1 alleles is associated with susceptibility to tuberculoid leprosy. *J Exp Med* **183**: 829–836