HLA-associated susceptibility to childhood B-cell precursor ALL: definition and role of HLA-DPB1 supertypes

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Childhood B-cell precursor (BCP) ALL is thought to be caused by a delayed immune response to an unidentified postnatal infection. An association between BCP ALL and HLA class II (DR, DQ, DP) alleles could provide further clues to the identity of the infection, since HLA molecules exhibit alleloype-restricted binding of infection-derived antigenic peptides. We clustered >30 HLA-DPB1 alleles into six predicted peptide-binding supertypes (DP1, 2, 3, 4, 6, and 8), based on amino acid di-morphisms at positions 11 (G/L), 69 (E/K), and 84 (G/D) of the DPβ1 domain. We found that the DPβ11-69-84 supertype GEG (DP2), was 70% more frequent in BCP ALL (n = 687; P < 10^{-4}), and 98% more frequent in cases diagnosed between 3 and 6 years (P < 10^{-6}), but not < 3 or > 6 years, than in controls. Only one of 21 DPB1 supergenotypes, GEG/GKG (DP2/DP4), was significantly more frequent in BCP ALL (P = 0.00004) than controls. These results suggest that susceptibility to BCP ALL is associated with the DP2 supertype, which is predicted to bind peptides with positively charged, nonpolar aromatic residues at the P4 position, and hydrophobic residues at the P1 and P6 positions. Studies of peptide binding by DP2 alleles could help to identify infection(s) carrying these peptides.

Keywords: HLA-DPB1; supertypes; BCP ALL; case–control comparison; allele frequency; peptide-binding pockets

Acute lymphoblastic leukaemia (ALL) is the most common childhood malignancy in developed countries, where it constitutes over 30% of childhood cancers (Stiller et al, 1998; Smith et al, 1999). The striking age-incidence peak between 2 and 5 years of age consists mainly of common, B-cell precursor (BCP) ALL (Greaves et al, 1993, 1998, 2002). The highly polymorphic HLA DR, DQ, and DP loci are encoded by genes in the human major histocompatibility complex (MHC), and are responsible for the binding and presentation of infection-derived peptides to CD4+ T cells, leading to adaptive immune responses to infections (Cooke and Hill, 2001). The affinity of different HLA class II alleles for infection-derived peptides is influenced by a series of discrete peptide-binding pockets (PBP) embedded in the antigen-binding groove of the HLA class II αβ heterodimer (Hammer et al, 1997).

Since T-cell responses to infection in the presymptomatic phase of BCP ALL are not readily accessible to functional analysis, the primary contribution of alleles at these loci makes it difficult to distinguish the primary contribution of alleles at these loci. Contrasting patterns of DR-DQ allelic linkage disequilibrium (LD) in different ethnic groups (Oksenberg et al, 2004) could resolve this problem, but such studies have yet to be reported in childhood leukaemia. Since the HLA-DR locus is only weakly linked to DR-DQ (Begovich et al, 1992; Cullen et al, 2002), analysis of DP alleles in BCP ALL should identify associations independent of DR-DQ. We and others have previously reported associations between DP alleles and human leukaemia (Pawelec et al, 1988; Taylor et al, 1995, 2002). Furthermore, DP alleles are known to be associated with, or to act as restriction elements for a number of parasitic (Meyer et al, 1994; May et al, 1998), microbial and viral diseases, including hepatitis B and rabies (Celis and Karr, 1989; Celis et al, 1990), herpes simplex...
Peptide binding by HLA class II alleles, including DP, is the outcome of interactions between the amino acid side chains of the peptide and four major peptide-binding pockets (1, 4, 6, and 9; Hammer et al., 1997). Since different alleles can have overlapping peptide-binding properties, depending on the number of PBP that they share (Southwood et al., 1998), this has permitted DR alleles with the same amino acid polymorphisms lining specific peptide-binding pockets to be clustered into supertypes (Sette and Sidney, 1998; Southwood et al., 1998; Doytchinova and Flower, 2005). Using a similar approach, Castelli et al. (2002) defined three DP supertype clusters with shared amino acid residues in the P1 (β84) and P6 (β11) PBP. However, the P4 peptide-binding pocket, at position β69, also makes an important contribution to antibody and peptide-binding (Arroyo et al., 1995; Chicz et al., 1997), T-cell responses (Berretta et al., 2003; Diaz et al., 2003) and disease susceptibility (Potolucchio et al., 1999; Wang et al., 1999). For this reason we clustered > 30 DPB1 alleles into six supertypes based on polymorphisms in three PBP, at positions 11, 69, and 84 of the β1 domain (i.e., pockets 6, 4, and 1). We compared their frequencies in childhood BCP ALL, non-BCP leukaemia and solid tumours recruited as part of the UK Childhood Cancer Study (2000) with newborn controls. We discuss the implications of our findings in relation to an infectious aetiology for BCP ALL.

**RESULTS**

**Case and control characteristics**

The UKCCS is an epidemiological case–control study designed to test the role of environmental factors in the aetiology of childhood cancer and leukaemia (UKCCS, 2000). As part of the UKCCS, we obtained HLA-DPB1 types for 982 cases of childhood leukaemia (Taylor et al., 2002). Ninety-one percent of the leukaemia cases were classified as white, based on parental information, the remainder being Asian (3.8%), Black (1%), mixed ethnicity (1.9%), other ethnic groups (0.5%) or unknown. Of 875 cases of ALL, 559 were identified as BCP ALL, and a further 228 ALL cases were unclassified (Taylor et al., 2002). Subsequent diagnostic information for the unclassified ALL cases enabled us to identify 128 additional BCP ALL, seven Pro-B ALL, and six T ALL cases. These were included in the present study, which therefore comprises 895 DP-type cases of childhood leukaemia with a confirmed diagnosis, of which 687 were BCP ALL and 208 were non-BCP leukaemia cases (Table 1). A mixed diagnostic series of childhood solid tumour cases (n = 409), not including childhood lymphoma (Taylor et al., 2002) is included for comparison. Of these, 405 cases had informative ethnic data, being classified as white in 91% of cases. Cord blood samples from a cross-sectional series of normal white UK term newborns (n = 864) were used as controls.

**Table 1**  
Number of cases and controls in this study.

| Study group* | Total | Male | Female | Male/female ratio |
|--------------|-------|------|--------|------------------|
| Leukaemia    | 895   | 492  | 403    | 1.22             |
| BCP ALL      | 687   | 373  | 314    | 1.19             |
| Non-BCP leukaemia | 208 | 119  | 89     | 1.33             |
| Pro-B ALL    | 26    | 7    | 19     | 0.37             |
| T ALL        | 75    | 51   | 24     | 2.12             |
| AML          | 107   | 61   | 46     | 1.33             |
| Solid tumour | 409   | 218  | 191    | 1.14             |
| Newborn controls | 864 | 436  | 428    | 1.01             |

*Leukaemias are classified as B cell precursor ALL (BCP ALL) and non-BCP acute leukaemia including Pro-B ALL, T ALL and AML.

For further details, see Table 2 and Results. Statistical analysis. As discussed previously (Taylor et al., 2002) ethical constraints precluded the collection of samples from case–matched control children, so we used local white UK newborns as controls. DPB1 alleles with a cumulative frequency of <5% that did not fall within the supertype clustering system were excluded from the analysis. Only sequence variation in the three peptide-binding pockets (positions 11, 69, and 84; pockets 6, 4, and 1, respectively) used for supertype clustering was included in the analysis. Global case–control supertype frequencies were compared using the CLUMP programme of Sham and Curtis (1995), a Monte Carlo method that computes a Pearson χ² statistic (T1) from a series of simulated case–control tables. In univariate analysis, cross-product odds ratios (ORs), and 95% confidence intervals were calculated from case–control supertype and genotype frequencies by the RERI program in the Linkage Utility Package, LINKUTIL, using the Sheehe method. The 2by2 programme in LINKUTIL was used to determine 2-sided P-values for case–control supertype and genotype differences using Fisher’s Exact test. Six supertypes require an uncorrected P-value <0.008, and 21 supergenotypes an uncorrected value <0.002 to achieve significance (P = 0.05). No correction for the total number of classical DP alleles was applied. POPGENE version 1.31 was used to test for two-locus linkage disequilibrium between DPB1 and DQA1, or DQB1 alleles.

**MATERIALS AND METHODS**

**Cases and controls**

Childhood leukaemia cases were recruited between 1992 and 98 as part of the UK Childhood Cancer Study (UKCCS, 2000). Leukaemias were classified as BCP ALL (CD10 + , CD19 + ; n = 687) or non-BCP acute leukaemia. The non-BCP leukaemias were the sum of Pro-B ALL (CD10−, CD19+), T-ALL (CD2/CD7+, CD19+, DR−), and AML (n = 208). Diagnostic immuno-phenotyping was carried out according to the protocol for UK Medical Research Council leukaemia trials (UKCCS, 2000). Childhood solid tumour cases (n = 409) were also recruited as part of the UKCCS (UKCCS, 2000). Umbilical cord blood samples from a cross-sectional series of normal white UK newborns (n = 864) born in Manchester UK between 1991 and 1997 were used as controls (Taylor et al., 2002). Blood sample collection and HLA molecular typing were carried out with national and local ethical consent. UKCCS patient data (diagnoses, gender, ages, ethnic background) were validated by the UKCCS data centre at the Epidemiology and Genetics Unit, University of York.

**HLA-DPB1 molecular typing**

HLA-DPB1 molecular typing was carried out as previously described in detail (Taylor et al., 2002) by amplifying a 327 bp exon 2 product in each case and control genomic DNA sample using a single pair of generic DPB1 PCR primers, spotting aliquots of each PCR product onto 384 sample nylon filters, and hybridising replicate filters with a panel of 28 32P-labelled sequence specific oligonucleotide probes. Probe hybridisation was detected using real-time autoradiography, and alleles assigned from published DPB1 ideograms.

**Data analysis**

DPB1 alleles in cases and controls were grouped into the six supertype clusters defined in this study (see Table 2 and Results for further details). Supertype allele and genotype frequencies were compared in cases and controls using global and univariate statistical analysis. As discussed previously (Taylor et al., 2002)
Male–female ratios were slightly higher in the leukaemia cases (1.22) than the solid tumours (1.14) and controls (1.01).

**HLA-DPB1 supertypes**

The majority (90%) of >30 DPB1 alleles in the cases and controls could be clustered into six supertypes (Table 2), consisting of three pairs, each pair differing at position 69 for a glutamic acid (E) or lysine (K) in pocket 4, but having the same residues at positions 11 (G or L; pocket 6) and 84 (G or D; pocket 1). We designated the six supertypes by their position 11-69-84 residues as GEG, GKG, LED, LKD, GKD, and GED, corresponding to dimorphisms in the P6-P4-P1 peptide-binding pockets. Using a modification of the hierarchical supertype clustering system for DP alleles developed by Doytchinova and Flower (2005), we have provisionally called these supertypes DP1 (GKD), DP2 (GED), DP3 (LKD), DP4 (GKG), DP6 (LED), and DP8 (GED).

**HLA-DPB1 supertype frequency in childhood leukaemia**

In the total leukaemia case series (n = 895) and the newborn controls (n = 864), we identified 14 DPB1/69K alleles, of which four are DP2 (GEG), seven are DP6 (LED), and three are DP8 (GED). Of 15 DPB1/69K alleles, six are DP4 (GKG), six are DP3 (LKD), and three are DP1 (GKD). In global $\chi^2$ analysis, the supertype frequency in the total leukaemia series was significantly different (P < 10$^{-10}$) from the controls (Table 3), but there was only a marginal difference between the solid tumour cases and controls (P = 0.04). In univariate analysis, DP2 (GEG) (OR, 95% confidence interval (CI): 1.6, 95%, CI, 1.2–2.0; 2 sided P = 0.0002) and DP8 (GED) (OR, CI: 2.9, 1.4–6.3; P = 0.006) were significantly more frequent in leukaemia cases than controls. DP6 (LED) (OR, CI: 1.3, 1.0–1.8; P = 0.04) was only marginally significant without correction for six supertypes, while DP2 and DP8 were significant after correction.

Stratification of the leukaemias into BCP ALL (n = 687) and non-BCP acute leukaemia (n = 208) revealed that DP supertypes in BCP ALL differed significantly from the newborn controls (P < 10$^{-4}$), but non-BCP leukaemia was only marginally significant (P = 0.04) (Table 4). In univariate analysis, DP2 (GED) (OR, CI: 1.7, 1.3–2.1; P < 10$^{-3}$) and DP8 (GED) (OR, CI: 3.2, 1.5–7.0; P = 0.004) were significantly more frequent, after correction for six supertypes, than controls. DP6 (LED) was not significant in BCP ALL, but was significant in non-BCP leukaemia (OR, CI: 1.8, 1.2–2.7; P = 0.007). DP1 (GKD) was significantly less frequent, after correction, than controls in BCP ALL (OR, CI: 0.5, 0.4–0.7; P < 10$^{-3}$), but not in non-BCP leukaemia.

The association of BCP ALL with DP2 and DP8 raised the possibility of a chance finding. To test this, supertype frequencies in four BCP ALL case series were compared with controls: (1) cases included in our previous study (n = 559; Taylor et al, 2002); (2) half of the cases in the present study (n = 344); (3) half of the cases in the previous study combined with the ‘new’ cases (n = 343); (4) the ‘new’ cases (n = 128) alone. DP2 and DP8 were significant in all four case series, though only DP2 remained significant after correction (Table 5).

To determine the relationship between the age at diagnosis of BCP ALL and DP supertype, we compared the frequencies in cases diagnosed <3 years of age, >3–6 years, and >6 years, with controls. Figure 1 shows that the risk of BCP ALL was increased by 98% in DP2+ cases diagnosed at >3–6 years (OR, CI: 1.9, 1.4–2.6; P = 10$^{-4}$), but was not significant in BCP ALL diagnosed <3 or >6 years. DP4 was significantly increased in BCP ALL diagnosed <3 years, though not after correction. DP8 was not significant after correction, while DP1 protected from BCP ALL in all age groups.

### Table 2 DPB1 supertypes of DPB1 alleles

| DPB1 supertype | Peptide-binding motif* | DPB1 alleles with this supertype |
|----------------|------------------------|----------------------------------|
| DP2           | GEG                    | 0021, 0022, 3301, 4801            |
| DP4           | GKG                    | 0401, 0402, 2301, 2401, 4901, 5101|
| DP6           | LED                    | 0601, 0901, 1001, 1301, 1701, 2101, 3001 |
| DP3           | LKD                    | 0301, 1401, 2001, 2501, 2601, 3501 |
| DP8           | GED                    | 0801, 1601, 1901                  |
| DP1           | GKD                    | 0101, 0501, 5001                  |

*DPB1 supertypes assigned from di-allelic amino acids at positions β1,1 (GL); β1,9 (EK) and β1,69 (GD).

### Table 3 DPB1 supertype frequency in childhood leukaemia and solid tumours compared with controls

| DPB1 supertype | Leukaemia | Solid tumour | Newborn controls |
|----------------|-----------|--------------|------------------|
| DP2 (GEG)     | 10.3 1.6  1.2–2.0 0.0002* | 7.1 1.1 0.8–1.5 0.6 | 6.8 |
| DP4 (GKG)     | 59.6 1.1 0.9–1.2 0.38 | 60.3 1.1 0.9–1.3 0.3 | 58.0 |
| DP6 (LED)     | 6.8 1.3 1.0–1.8 0.04 | 6.5 1.3 0.9–1.8 0.2 | 5.2 |
| DP3 (LKD)     | 12.0 0.9 0.7–1.1 0.29 | 12.8 0.9 0.8–1.2 0.8 | 13.2 |
| DP8 (GED)     | 1.4 2.9 1.4–6.3 0.006* | 0.6 1.4 0.3–3.9 0.8 | 0.5 |
| DP1 (GKD)     | 6.9 0.6 0.5–0.7 <10$^{-6}$ | 8.6 0.8 0.6–1.1 0.09 | 11.1 |

*Significant (P<0.05) after correction for six supertypes. **Significant (P<0.05) in global $\chi^2$ (CLUMP) analysis.

### Table 4 DPB1 supertype frequency in BCP ALL and non-BCP leukaemia compared with controls

| DPB1 supertype | BCP ALL | Non-BCP leukaemia |
|----------------|---------|-------------------|
| DP2 (GEG)     | 10.8 1.7 1.3–2.1 <10$^{-4}$ | 8.4 1.3 0.9–1.9 0.3 |
| DP4 (GKG)     | 60.0 1.1 0.9–1.2 0.27 | 579 0.9 0.8–1.2 1.0 |
| DP6 (LED)     | 6.2 1.2 0.9–1.6 0.24 | 89 1.8 1.2–2.7 0.007* |
| DP3 (LKD)     | 11.6 0.9 0.7–1.1 0.21 | 130 0.9 0.7–1.3 0.9 |
| DP8 (GED)     | 1.5 3.2 1.5–7.0 0.004* | 1.0 2.2 0.7–6.6 0.4 |
| DP1 (GKD)     | 6.4 0.5 0.4–0.7 <10$^{-6}$ | 8.4 0.7 0.5–1.1 0.1 |

*Significant after correction for six supertypes. **Significant (P<0.05) in global $\chi^2$ (CLUMP) analysis.
Table 5  HLA-DPB1 supertype-associated risk of BCP ALL in replicate series of cases compared with controls

| DPB1 supertype | Series 1* | Series 2 | Series 3 | Series 4 |
|----------------|-----------|----------|----------|----------|
|                | OR        | CI       | P        | OR       | CI       | P        | OR       | CI       | P        |
| DP2            | 1.6       | 1.2–2.1  | 0.001*   | 1.8      | 1.3–2.4  | 0.003*   | 1.6      | 1.1–2.1  | 0.007*   |
| DP4            | 1.1       | 0.9–1.3  | 0.19     | 1.1      | 0.9–1.3  | 0.23     | 1.05     | 0.9–1.3  | 0.59     |
| DP6            | 1.3       | 0.9–1.7  | 0.17     | 1.2      | 0.8–1.7  | 0.48     | 1.3      | 0.9–1.8  | 0.26     |
| DP3            | 0.9       | 0.7–1.1  | 0.33     | 0.9      | 0.7–1.1  | 0.33     | 0.9      | 0.7–1.1  | 0.34     |
| DP8            | 3.0       | 1.3–6.8  | 0.01     | 3.1      | 1.3–7.6  | 0.02     | 3.4      | 1.4–8.2  | 0.01     |
| DP1            | 0.5       | 0.4–0.7  | <10^-5*  | 0.5      | 0.3–0.7  | <10^-3*  | 0.6      | 0.5–0.9  | 0.008*   |

Number =

559 344 343 128

*Series 1: see Taylor et al (2002); series 2: 50% of series 1; series 3: 215 cases from series 1 + 128 cases from series 4; series 4: new cases in this study. *Significant (P<0.05) after correction for six supertypes.

**DISCUSSION**

Selective peptide binding by HLA allotypes is a prerequisite for the recognition of antigens by T cells leading to adaptive immunity (Madden, 1995). Such a mechanism may underpin the immune-mediated progression of pre-ALL to overt leukaemia following delayed postnatal infection (Greaves, 2006). In our previous study, we suggested that the presence in pocket 4 of a glutamic acid (E) residue at position 69 of the DPB1 domain was associated with BCP ALL (Taylor et al, 2002). However, HLA class II allotype-associated peptide binding is not the property of a single PBP; rather, it is the sum of a series of key PBPs forming a DP allotype-associated peptide-binding motif or ‘footprint’. Polymorphisms in PBPs accommodating the P1, 4, 6, and 9 amino acid anchors appear primarily to influence the DP allotype footprint (Hammer et al, 1997; Diaz et al, 2003, 2005). Since pocket 9 is composed of polymorphisms in residues 35, 36, 55, and 56 (Diaz et al, 2003), we excluded this level of complexity. Furthermore, grouping amino acid polymorphisms at positions 36, 56, and 76 failed to define recognised supertypes, and were not associated with leukaemia (data not shown). Clustering of DP alleles into six supertypes based on amino acid dimorphisms at positions 84 (P1 pocket), 69 (P4 pocket), and 11 (P6 pocket) represents an expanded version of the scheme proposed by Castelli et al (2002) based on peptide binding, and a slightly modified version of the hierarchical clustering scheme proposed by Doytchinova and Flower (2005). We have provisionally denoted the six supertypes DP1 (GKD), DP2 (EGE), DP3 (LKD), DP4 (GKG), DP6 (LED), and DP8 (GEG) since they broadly resemble those defined in the primed lymphocyte test (PLT) as DPw specificities (De Koster et al, 1991). Furthermore, HLA-DPBw2 defined by PLT was previously reported to be associated with ALL (Pawelec et al, 1988).

The DPB1 locus is the second most polymorphic HLA class II locus after DRB1, with at least 120 alleles identified to date (http://anthonynolan.org.uk/HIG/lists/class2list.html). In a rare disease such as BCP ALL in which there are likely to be multiple aetiological factors, weak HLA associations potentially require hundreds of cases and controls to allow for correction for multiple testing. Supertype analysis, in which alleles are clustered according to common functional (i.e., peptide binding) properties, overcomes this problem. DPB1 alleles comprise combinatorial series of six variable regions (A-F) encoded by exon 2 (Bugawan et al, 1988), in which alleles with the same variable region polymorphisms have the same peptide-binding pockets. DP alleles with the same polymorphisms at position 11 in variable region A, position for DPB1*1601, a frequency not significantly greater than in the controls, indicating that the DP-supertype results cannot be explained by LD between DP and DQ alleles.

**Table 5**  HLA-DPB1 supertype-associated risk of BCP ALL in replicate series of cases compared with controls

| DPB1 supertype | Series 1* | Series 2 | Series 3 | Series 4 |
|----------------|-----------|----------|----------|----------|
|                | OR        | CI       | P        | OR       | CI       | P        | OR       | CI       | P        |
| DP2            | 1.6       | 1.2–2.1  | 0.001*   | 1.8      | 1.3–2.4  | 0.003*   | 1.6      | 1.1–2.1  | 0.007*   |
| DP4            | 1.1       | 0.9–1.3  | 0.19     | 1.1      | 0.9–1.3  | 0.23     | 1.05     | 0.9–1.3  | 0.59     |
| DP6            | 1.3       | 0.9–1.7  | 0.17     | 1.2      | 0.8–1.7  | 0.48     | 1.3      | 0.9–1.8  | 0.26     |
| DP3            | 0.9       | 0.7–1.1  | 0.33     | 0.9      | 0.7–1.1  | 0.33     | 0.9      | 0.7–1.1  | 0.34     |
| DP8            | 3.0       | 1.3–6.8  | 0.01     | 3.1      | 1.3–7.6  | 0.02     | 3.4      | 1.4–8.2  | 0.01     |
| DP1            | 0.5       | 0.4–0.7  | <10^-5*  | 0.5      | 0.3–0.7  | <10^-3*  | 0.6      | 0.5–0.9  | 0.008*   |

Number =

559 344 343 128

*Series 1: see Taylor et al (2002); series 2: 50% of series 1; series 3: 215 cases from series 1 + 128 cases from series 4; series 4: new cases in this study. *Significant (P<0.05) after correction for six supertypes.
**Table 6** Risk of BCP ALL, non-BCP leukaemia and paediatric solid tumours associated with DPB1 supergenotypes, compared with controls

| Super-genotype     | OR  | 95% CI  | P       | OR  | 95% CI  | P       | OR  | 95% CI  | P       |
|--------------------|-----|---------|---------|-----|---------|---------|-----|---------|---------|
| DP2/DP2 (GEG/GEG)  | 0.9 | 0.4–1.9 | 0.99    | 0.9 | 0.3–2.8 | 0.99    | 1.02| 0.4–2.4 | 0.99    |
| DP2/DP4 (GEG/GKD)  | 2.1 | 1.5–2.9 | 0.00004*| 1.6 | 0.9–2.7 | 0.14    | 1.1 | 0.7–1.8 | 0.7     |
| DP2/DP6 (GEG/LED)  | 2.5 | 1.0–5.8 | 0.06    | 2.5 | 0.8–7.7 | 0.3     | 1.5 | 0.5–4.5 | 0.6     |
| DP2/DP3 (GEG/LKD)  | 1.5 | 0.7–3.1 | 0.42    | 0.8 | 0.3–3.6 | 0.99    | 1.1 | 0.4–2.8 | 0.99    |
| DP2/DP8 (GEG/GED)  | 3.8 | 0.3–4.1 | 0.88    | 12.5| 1.1–138.5| 0.38   | —  | —       | —       |
| DP2/DP1 (GEG/GKD)  | 1.1 | 0.5–2.7 | 0.96    | 0.6 | 0.1–1.7 | 0.99    | 0.9 | 0.3–2.6 | 0.99    |
| DP3/DP4 (GKG/GKD)  | 0.9 | 0.7–1.1 | 0.40    | 0.8 | 0.6–1.1 | 0.17    | 1.1 | 0.9–1.4 | 0.3     |
| DP3/DP6 (GKG/LKD)  | 1.9 | 1.2–3.0 | 0.006   | 2.7 | 1.6–4.9 | 0.002*  | 1.7 | 1.0–2.9 | 0.06    |
| DP3/DP3 (GKG/LKD)  | 1.1 | 0.8–1.4 | 0.80    | 1.2 | 1.2–1.4 | 0.1     | 1.1 | 0.7–1.5 | 0.8     |
| DP3/DP8 (GKG/GED)  | 2.7 | 0.9–7.1 | 0.08    | 1.9 | 0.1–15.3| 0.99    | 0.6 | 0.1–2.9 | 0.7     |
| DP4/DP1 (GKG/GKD)  | 0.8 | 0.6–1.2 | 0.40    | 0.9 | 0.6–2.0 | 0.8     | 0.6 | 0.4–0.9 | 0.04    |
| DP4/DP6 (LED/LED)  | 0.6 | 0.2–1.7 | 0.48    | 1.1 | 0.3–4.0 | 0.99    | 1.7 | 0.7–4.3 | 0.4     |
| DP4/DP3 (LED/LKD)  | 0.5 | 0.2–1.1 | 0.09    | 1.1 | 0.04–3.1| 0.67    | 0.2 | 0.07–0.81| 0.02    |
| DP4/DP8 (LED/GED)  | —   | —       | —       | —   | —       | —       | 6.3 | 0.6–70.2| 0.16    |
| DP6/DP1 (LED/LKD)  | 0.8 | 0.3–2.3 | 0.89    | 2.2 | 0.7–6.7 | 0.37    | 0.9 | 0.3–2.8 | 0.99    |
| DP6/DP3 (LKD/LKD)  | 0.7 | 0.4–1.2 | 0.23    | 0.8 | 0.4–1.8 | 0.76    | 1.1 | 0.6–1.9 | 0.9     |
| DP6/DP8 (LKD/GED)  | 3.3 | 0.8–12.8| 0.16    | 2.5 | 0.4–15.0| 0.95    | 2.1 | 0.4–10.5| 0.7     |
| DP6/DP3 (LKD/GKD)  | 0.5 | 0.2–1.2 | 0.15    | 1.6 | 0.5–3.9 | 0.85    | 1.2 | 0.5–2.7 | 0.8     |
| DP8/DP1 (GKG/GKD)  | 3.8 | 0.3–4.1 | 0.88    | —   | —       | —       | —   | —       | —       |
| DP8/DP1 (GKG/GKD)  | 0.2 | 0.1–0.5 | 0.00004*| 0.4 | 0.1–1.06| 0.06    | 0.8 | 0.4–1.5 | 0.6     |

*Significant (P<0.05) after correction for 21 supergenotypes.

Influenced by low frequency alleles. Nevertheless, our results require confirmation with independent case–control series. Although associations between childhood ALL and DR, DQ and DP alleles have been reported in previous studies (Dorak et al, 1995, 1999; Taylor et al, 1995, 2002), there has been no test of the effect of LD between alleles at the different loci. We found no evidence that the association of BCP ALL with DP2 could be explained by LD with DQ alleles, suggesting that DP has a primary role in susceptibility to BCP ALL.

It is unlikely that the association of BCP ALL with DP2 is due to a defect in the immune response to an oncogenic virus (immun evasion). There is no evidence that childhood BCP ALL is caused by an oncogenic virus (MacKenzie et al, 2006), and the positive association with DP2 suggests that binding of specific peptide(s) and T-cell activation are involved in causation, which is inconsistent with immune evasion by an oncogenic virus. The negative association of DP1 with BCP ALL may be due to the binding and recognition of TEL-AML1 peptide(s) in children with pre-ALL with this super-type, as discussed elsewhere (Taylor et al, 2008), since a TEL-AML1 junctional peptide has been shown to elicit a DPB1*0501-restricted (DP1) CD4+ T cell response (Yun et al, 1999).

The delayed response to infection hypothesis for BCP ALL (Greaves et al, 2008) proposes that a child carrying an in utero-initiated preleukaemic clone is vulnerable to the development of leukaemia if it is insulated from infection during the early postnatal period, but exposed at a later age. We previously reported that the risk of BCP ALL was greater in DPB1*0201 homozygotes than heterozygotes (Taylor et al, 2002), suggesting that BCP ALL might be the rare ‘down-side’ of the advantage that MHC-heterozygosity confers on immune responses to infection. Although evolution of HLA allelic diversity is thought to favour heterozygotes (Takahata and Néi, 1990), a recent study suggests that this advantage may be allele-specific (Lipsitch et al, 2003). Our finding that only one (DP2/DP4) of 15 heterozygous supergenotypes (GEG/GKD) is associated with BCP ALL fits this model.
Using DPB1*0201 peptide-binding data and molecular modelling (Diaz et al., 2005), it is possible to make predictions about the amino acid anchors at P1, P4, and P6 of peptides binding to DP2. Pocket 4 of DP2 is deeper, more negatively charged than DP4 (Diaz et al., 2003), giving it a greater affinity for positively charged nonpolar aromatic residues, such as glutamine (Q), arginine (R), and lysine (K). Furthermore, glycine (G) makes pocket 1 (β84) and pocket 6 (β11) deep and hydrophilic, preferentially-binding hydrophobic and aromatic amino acids, notably phenylalanine (F), and tyrosine (Y) (Berretta et al., 2003; Diaz et al., 2003, 2005). This predicts that infectious peptides with an FXXKFXXAX/V motif (where X is unknown, and P9 can be A or V) are likely to bind to DP2.

In this context, Van Steensel-Moll et al. (1986) reported a negative (protective) association between childhood ALL and infections in the first year of life, and Rosenbaum et al. (2005) documented a weak negative association between childhood ALL and bronchiolitis and pneumonia. Roman et al. (2007) found a slight deficit in lower respiratory tract infection in the first year of life of UKCCS ALL cases diagnosed at 2–5 years. Together these findings suggest that the immune response to RSV infection may be a factor in BCP ALL. RSV is a highly contagious, weakly pathogenic, but strongly immunogenic virus that is widely distributed in the childhood population (Handforth et al., 2000; McNamara and Smyth, 2002). The G protein of RSV elicits CD4+ T-cell responses (De Graaf et al., 2004; De Waal et al., 2004), the peptide 162D-N179 containing two overlapping T-cell epitopes, 162FHVFNFPV1271 and 162FHVFNFPVPC1773 that are restricted by DPB1*0401 (DP4), and DPB1*0201 (DP2) (De Graaf et al., 2004). Both peptides have F at P1 and P6 suggestive of binding to GEG (DP2) and KGK (DP4), consistent with the association of BCP ALL with DP2/DP4. While this conclusion is speculative it points to a need for detailed sero-epidemiological studies of RSV in BCP ALL.

ACKNOWLEDGEMENTS

This study was funded by grants from the Kay Kendall Leukaemia Fund (to GMT and MFG), by support from Cancer Research UK (JMB, TE), and by the Leukaemia Research Fund (MFG). We are indebted to the children and families who took part in the UK Childhood Cancer Study. We thank J Simpson and Professor E Roman at the Epidemiology and Genetics Unit, University of York for providing diagnostic and other patient information, Mrs R Carter for blood sample documentation and the midwives at St Mary’s Hospital, Manchester for cord blood samples. We are grateful to MD Robinson, Dr C Watson, Dr GA Gokhale, S P Dearden for sample processing, and DPB1 typing. A complete list of UKCCS investigators and sponsors is given in: UK Childhood Cancer Study Investigators (2000).

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