Inherited predisposition to CLL is detectable as sub-clinical monoclonal B-lymphocyte expansion

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ABSTRACT

Monoclonal CLL-phenotype cells are detectable in 3.5% of otherwise normal individuals using flow cytometric analysis of CD5/CD20/CD79b expression on CD19-gated B-cells. To determine whether detection of such CLL-phenotype cells is indicative of an inherited predisposition, we examined 59 healthy first-degree relatives of patients from 21 CLL families. CLL-phenotype cells were detected in 8/59 relatives (13.5%), representing a highly significant increase in risk (p=0.00002). CLL-phenotype cell levels were stable with time, and had the characteristics of indolent CLL. Both indolent and aggressive clinical forms were found in family members, suggesting that initiation and proliferation involves distinct factors. The detection of CLL-phenotype cells provides a surrogate marker of carrier status, potentially facilitating gene identification through mapping in families and direct analysis of isolated CLL-phenotype cells.
INTRODUCTION

The etiology of CLL is largely unknown, however, several studies have reported families with an increased risk of CLL, and other non-CLL lymphoproliferative disorders, indicative of an inherited predisposition 1-4. The detection of a CLL-associated marker in healthy relatives of familial CLL cases may provide a surrogate marker of inherited predisposition, assisting in the identification of causative gene mutations. It is possible to identify markers of some B-cell malignancies in healthy individuals, including the follicular lymphoma-associated t(14;18) translocation 5,6, and the neoplastic plasma cells common to both myeloma and Monoclonal Gammopathy of Undetermined Significance (MGUS) 7,8. We have previously reported a flow cytometry technique for quantifying CLL cells when they represent as few as 0.5% of B-cells, utilising the higher CD5 and lower CD20/CD79 expression by the neoplastic cells 9. Application of this technique to 910 individuals with normal haematological parameters and no evidence of malignant disease detected CLL-phenotype cells in 3.5% 10. We report here the frequency of CLL-phenotype cells in 59 healthy first-degree relatives of affected individuals in 21 CLL families.
METHODS

Twenty-one families with two or more cases of CLL were ascertained through UK clinicians. Diagnoses of CLL were based on standard criteria. Fifty-nine healthy first-degree relatives of a family member with CLL were studied, with repeat samples assessed in 38/59 cases. Median age of relatives was 47 years, range 23 - 86. Samples were also examined from 23 healthy spouses, median age 45 years, range 23-79. The prevalence of CLL-phenotype cells in familial CLL relatives was compared to a sample of 910 individuals reported previously. The age-adjusted odds ratio was derived from logistic regression analysis, and distribution of continuous variables was compared with Wilcoxon or Mann-Whitney tests, using STATA v6.0 (Stata Corporation, Texas, USA). Samples were obtained with informed consent and Ethical Review Board approval.

CLL-phenotype cells were enumerated as reported. Briefly, $10^6$ leucocytes isolated from peripheral blood were incubated with: i) CD20 FITC*, CD79b PE†, CD19 Cy5/PE‡ & CD5 APC‡; ii) anti-kappa FITC‡, anti-lambda PE‡, CD19 Cy5/PE‡ & CD5 APC‡. Total B-lymphocytes were identified by their CD19 and light scatter characteristics. Samples containing cells that represented more than 50 events in all three “CLL” regions were subjected to extended phenotyping. Cells were incubated with CD19 Cy5/PE‡, CD20 APC¥, CD5 PE‡ or FITC‡ and either CD11a FITC‡, CD22 PE¥, CD23 PE‡, CD27 FITC¥, CD38 PE¥, kappa FITC‡, lambda PE‡, or FMC7 FITC§. Antibodies were either prepared in-house (‡) or supplied by BD Biosciences, Oxford, UK (¥); Immunotech, Marseilles, France (*); Serotec, Oxford, UK (†);
or Chemicon, Harrow, UK, (§). Samples were only classified as having a population of CLL-phenotype cells if the phenotype was consistent with clinical CLL for the antigens: CD5 (positive), CD20 (weak or negative), CD79b (weak or negative), CD22 (weak), FMC7 (weak) ¹².
RESULTS AND DISCUSSION

CLL-phenotype cells were detected in 8 of the 59 relatives from seven families (Table 1). Absolute numbers were on average a thousand-fold lower than the levels required for a clinical diagnosis of CLL (median 5, range 3 – 127 cells/µL), and were similar to the levels detected in the outpatient survey (median 13, range 2 – 1458 cells/µL, P=0.07)\(^{10}\). The observation that 13.5% of relatives harbor CLL-phenotype cells translates to a 7-fold increase in risk of sub-clinical disease (OR=6.6; 95% CI: 2.7-16.0; p=0.00002, Figure 1). CLL-phenotype cells were only detected in 1/23 unrelated family members - a prevalence not significantly different to that in the outpatient group (P>0.1). This individual came from a different family to the affected relatives. The highly significant increase in risk for family members indicates that CLL-phenotype cells represent a surrogate marker of carrier status in CLL families.

Repeat analysis of the majority of the relatives was performed in a blinded fashion to examine if aberrant cells were persistently detectable. Median time between sampling was 18 weeks (range 13 – 25). Six of the eight relatives with detectable CLL-phenotype cells were re-assessed, and all were positive at second assessment. The level of CLL-phenotype cells were not different between the two time points (P=0.1) with the second assessment level representing a median 85% of the initial level (range 30 – 112%). Long-term follow-up is required to determine whether the relationship between the CLL-phenotype cells and clinical disease follows a similar pattern to that seen in MGUS and myeloma\(^{13,14}\). However, the fact that the levels are stable with
time suggests that the generation of CLL-phenotype B-cells is governed by different genetic mechanisms to their proliferative potential.

CLL-phenotype cells were not detectable in 38 of the relatives assessed at both time points. However, in 2 relatives, CLL-phenotype cells were detectable at either first or second assessment only. In both cases, levels were low (1.4/µL and 1.9/µL respectively), below the range found in the outpatient study. As these individuals were not categorized as having CLL-phenotype cells, our estimate of the prevalence of this phenotype in relatives is therefore likely to represent an underestimate of the true risk.

Indolent forms of clinical CLL are characterized by a high degree of IgH hypermutation and low level of CD38 expression 15-18. CLL-phenotype cells present in otherwise normal individuals show these characteristics 10. CD38 expression was also undetectable on the CLL-phenotype cells from the affected relatives. CD38+ CLL-phenotype cells are presumably not seen in sub-clinical form because such clones would expand rapidly and present as clinical disease. However, both aggressive CD38+ and indolent CD38- forms of clinical disease were present in the familial patients with clinical disease. This also indicates that proliferative potential is regulated separately from the oncogenic process, and may relate to the stage of differentiation in the B-cell undergoing neoplastic transformation. Recent data has shown that CLL cells are most similar to memory B-cells 19. As in clinical disease, the CLL-phenotype cells in normal individuals and familial-CLL relatives all express CD27, which is normally restricted to B-cells that are post-germinal centre20. In addition, the level of bcl-2 expression in the CLL-phenotype cells is equivalent to that of normal circulating memory B-cells, a level approximately
twice that of naïve B-cells (median 1.9-fold higher, P=018). This supports the hypothesis that all CLL-phenotype cells are derived from memory B-cells. Further studies are warranted to determine whether clinical features relate to particular memory B-cell subsets from which the neoplastic cells are derived.

In addition to the 8 cases with a CLL-phenotype, a non-CLL-phenotype monoclonal B-cell population was detected in a relative from another family, with an extended phenotype suggestive of marginal zone lymphoma. Non-CLL phenotype monoclonal B-cell expansions were also detected in 9/910 outpatients. The detection of sub-clinical disease in myeloma, follicular lymphoma, and now in CLL as well as other B-cell disorders suggests that all common chronic lymphoproliferative disorders have a pre-malignant counterpart. Furthermore, the finding that all these lymphoproliferative disorders appear within certain families at both the clinical and sub-clinical level raises the possibility of a common genetic predisposition to the development of B-cell malignancies. As in MGUS and myeloma, comparison of cells from individuals with sub-clinical, indolent, and progressive disease should allow identification of some of the genetic factors responsible for oncogenesis and disease progression 21-23. Application of our observation should facilitate identification of CLL genes through mapping in families and direct analysis of isolated CLL-phenotype populations.
Table 1. Age, sex and haematological parameters of relatives with detectable B cell expansions

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Phenotype</th>
<th>Age</th>
<th>Monoclonal B cell count (cells/µL)</th>
<th>Leucocyte count (10⁹/L)</th>
<th>Haemoglobin (g/dL)</th>
<th>Platelet count (10⁹/L)</th>
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<td>20.8</td>
<td>6.8</td>
<td>15.0</td>
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Figure 1 Prevalence of monoclonal CLL-phenotype cells in relatives of familial CLL index cases compared to the general population. Figure 1a shows the highly significant overall difference (P value age-adjusted). Figure 1b demonstrates that the increase in prevalence in the familial relatives is seen at all age groups, although not significant due to the numbers of relatives available for assessment.
REFERENCES


sensitive flow cytometric assay improves the prediction of outcome and can be used to optimize therapy. Blood 98:29, 2001


