Comparison of protein synthesis profiles in chronic lymphocytic leukaemia cells and B-lymphocytes from peripheral blood, cord blood and tonsil

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Abstract. 2D-gel electrophoresis was used to investigate protein synthesis in leukaemic cells from a series of 15 chronic lymphocytic leukaemia (CLL) patients, and in non-malignant B-cell populations from different sources. The protein synthesis profiles of CD5+ B-cells from umbilical cord blood and from tonsil were determined, and the levels of expression of their proteins were observed to be similar to the CLL cells. The CD5− cells from cord blood resembled peripheral blood B-lymphocytes, and the protein synthesis profile of CD5− cells from tonsils was very complex. One protein was also identified which consistently appeared to be synthesised at a low level in CD5+ B-cells from tonsil but which was always more prominant in CLL cells and other non-malignant B-lymphocytes. On the basis of these data it is possible that the closest non-malignant counterpart to CLL is the CD5+ B-lymphocyte from cord blood.
Key words. CLL; 2D-gel; protein; CD5+ve; B-lymphocyte.

Chronic lymphocytic leukaemia is the most common form of leukaemia, and accounts for 30% of all leukaemias in the U.K.[1]. It is characterized by a clonal proliferation of small lymphocytes[2], with bone marrow infiltration. Most cases are of B-cell origin[3], which interestingly express a pan T-cell marker, CD5[4,5]. Progression of CLL is variable and a high proportion of cases remain stable for many years[6], though about 10% of cases transform into a more aggressive form of the disease; usually an immunoblastic or a prolymphocytoid transformation[7].

Although CLL is a major form of leukaemia, there are still important aspects of this disease that are unclear. One of these is the identity of the non-malignant counterpart of CLL cells. We have recently reported that comparison of CLL cells with peripheral blood B-cells revealed a number of differences in protein synthesis between the leukaemic and non-malignant cells, although the protein synthesis profiles of CD5+ and CD5− B-lymphocytes appeared to be essentially the same[8]. Several groups have further investigated the origins of CLL by analysing various aspects of the functions and gene expression of CLL cells and comparing them to different populations of B-cells. In terms of CD23 mRNA expression and response to IL-4, CLL cells resemble umbilical cord blood CD5+ cells rather than peripheral blood B-cells[9]. However expression of *BCL-2*, and a range of cytokines, in CLL cells were found to be more reminiscent of B-lymphocytes isolated from tonsil[10,11].

The application of large format 2D-gel electrophoresis to the analysis of normal and malignant lymphoid cells is likely to reveal how closely related CLL cells are to different populations of B-lymphocytes. In these studies we have therefore compared the protein synthesis profiles from both CD5+ and CD5− populations of B-cells that were obtained from adult peripheral blood, umbilical cord blood and tonsil tissue. It was hoped that such an approach might indicate which B-lymphocyte population was most likely to represent the non-malignant equivalent of CLL.

Materials and methods

Blood samples were obtained from 15 CLL patients. Their clinical details as well as their staging are published elsewhere[12]. Cord blood was collected from 4 individuals at parturition, and 3 samples of tonsil tissue were obtained after tonsilectomy. 10 ml samples of cord blood and adult peripheral blood (from both normal donors and CLL patients) were collected in Heparin tubes and the mononuclear cells isolated using J-prep gradients (Techgen). Cells from CLL patients were then washed in RPMI-1640 (Gibco-BRL) and resuspended in labelling medium (RPMI 1640-methionine deficient, supplemented with 1% foetal calf serum) at 2×10^6 cells/ml. Tonsils were dissociated using forceps in a petri dish containing 10 ml PBS (supplemented with heparin and DNAse). The resulting cell suspension was then washed with PBS. The non-malignant B-cells from tonsil tissue, peripheral blood of normal donors,

or umbilical cord blood were sorted on a FACS. The mononuclear cells were incubated at 5×10^6 cells/ml PBS with 1/100 dilutions of FITC-conjugated CD5 and RPE-conjugated CD19 antibodies (Dako), and sorted using a FACS 420 (Becton and Dickinson) with CONSORT:30 and IBM DACS accessory computers (Applied Cytometry Systems Ltd, Dinnington, S. Yorkshire, U.K.). In this way populations of CD19+, CD5+ and CD19+, CD5− cells were obtained.

Proteins were labelled with ^{35}S by incubating the cells for 24 h in labelling medium supplemented with 185 kBq of ^{35}S-methionine/ml (Amersham). After labelling, the cells were washed and lysed in sample buffer (9 M urea, 5% NP40, 5% DTT, 2% ampholine pH 3.5-10). 2D-gel electrophoresis was carried out using large format gels (Millipore) to improve resolution[13] 19 cm long gels were poured in 1 mm wide glass tubes using Ampholines with an expanded pH midrange (Millipore). The gels were prefocussed for approximately 90 min prior to loading the sample in a maximum volume of 40 µl ($2-5 \times 10^5$ cells). Isoelectrical focussing was carried out for 17 h at 1,000 V, followed by 30 min at 2,000 V. The tube gels were extruded, equilibrated in SDS, and loaded onto 10% SDS-acrylamide gels which were run until the dye front was 2 cm from the bottom of the gel (5–6 h). The gels were fixed in 10% acetic acid, 40% methanol for 30 min and prepared for fluorography using 1:4 diphenyloxazole (Sigma):acetic acid[14]. The dried gels were exposed to preflashed Hyperfilm (Amersham) for 1–3 weeks at −70 °C. Alternatively the fixed gels were dried onto Whatmann 3M filter paper and exposed directly to unflashed film. This resulted in slightly better resolution but decreased sensitivity.

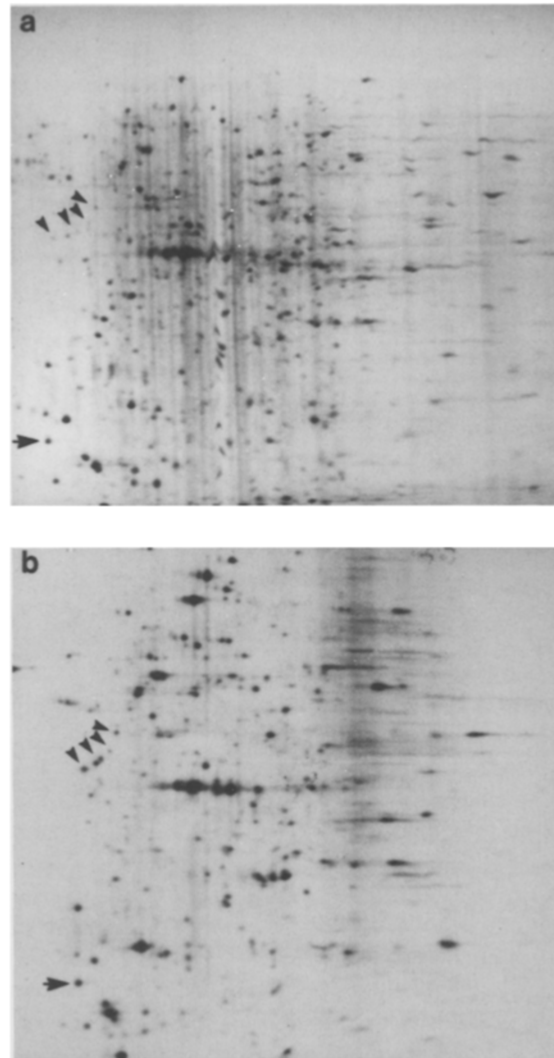

Figure 1. 2D-gel analysis of ^{35}S-methionine labelled proteins isolated from *a* CLL cells, and *b* CD5+ B-lymphocytes from adult peripheral blood. The autoradiographs are orientated with the acidic proteins to the left side. The small arrows indicate the position of the 50–60 kD group of proteins, and the large arrow indicates the 30 kD protein.

Results

The analysis of such a large number of proteins will of course reveal a number of polymorphic differences between the samples under investigation and, as the sample sizes were relatively small in this study, we concentrated only on those changes that appeared to be consistent events. We have analysed 15 CLL samples and an example of one of these samples is shown in figure 1a. It was apparent that the majority of proteins present were expressed at similar levels in the CLL samples and in the non-malignant CD5+ B-cells from peripheral blood (fig. 1b). However, one group of proteins (approximate size 50–60 kD) were consistently prominent in gels of the peripheral blood CD5+ B-lymphocytes, but not in the CLL cells. These proteins were also prominent in CD5− B-lymphocytes from peripheral blood (data not shown).

We analysed FACS sorted CD5+ and CD5− populations of B-lymphocytes from 4 cord blood samples. The protein synthesis profiles were similar to those of peripheral blood B-cells, but the CD5+ population from cord blood exhibited a variable and generally lower level of synthesis of the 50–60 kD group of proteins (fig. 2a) than the CD5− population (fig. 2b). As such these CD5+ B-cells appeared to resemble the CLL cells (table).

We also analysed lymphocytes isolated from three tonsil samples. In these samples the CD5+ population resembled the CD5+ B-lymphocytes from cord blood (fig. 3a), and hence were more similar to CLL cells than were the CD5+ B-cells from peripheral blood. However we noted that one protein (approximate size 30 kD) was faint in all of the tonsil CD5+ samples (fig. 3a), but was much more prominent in the other B-cell populations that we had studied (figs 1, 2). The protein synthesis profile of the tonsil CD5− population, although similar to the CLL cells in terms of the levels of expres-

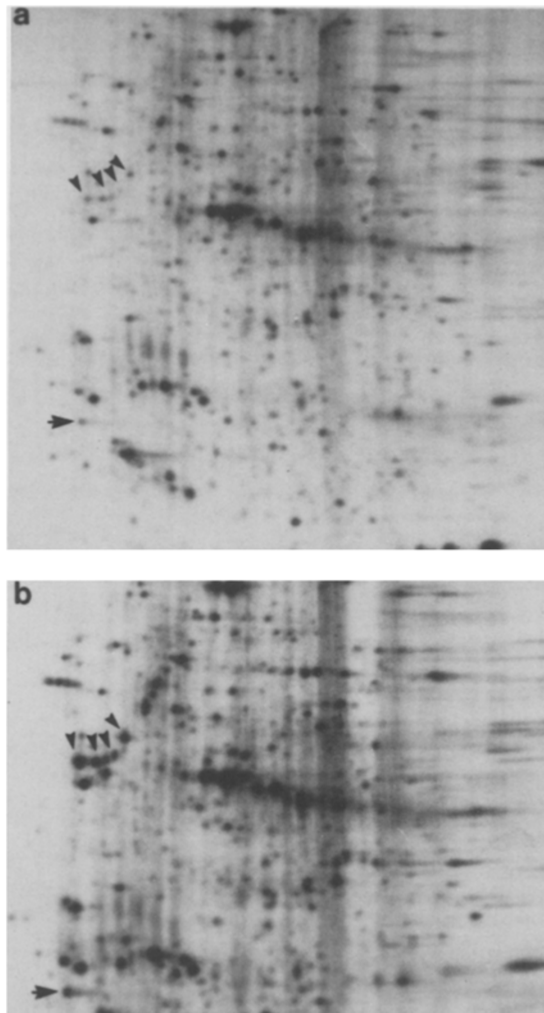

Figure 2. 2D-gel analysis of ^{35}S-methionine labelled proteins isolated from umbilical cord blood cells: *a* CD5+ B-lymphocytes, *b* CD5− B-lymphocytes. The autoradiographs are orientated with the acidic proteins to the left side. The small arrows indicate the position of the 50–60 kD group of proteins, and the large arrow indicates the 30 kD protein.

sion of the 30 kD and 50–60 kD group of proteins (table), was much more complex than in any other B-lymphocyte population (fig. 3b).

Discussion

2D-gel electrophoresis of radio-labelled cellular proteins offers the opportunity of separating many of the proteins that are synthesised in a cell, to produce a pattern that is characteristic of a particular cell type, and hence is a powerful tool for evaluating cell lineage relationships. It is thought that at any one time a cell is likely to express approximately 5,000 proteins[15], 70–80% of which are housekeeping genes which are expressed, albeit at varying levels, in all cell types. The remainder of the genes expressed will be specific to the cell type or lineage[15]. In a previous study we had labelled the cells with ^{14}C-leucine and used a procedure which allowed for the resolution of 300–400 proteins[8],

Comparison of the expression of selected proteins in CLL and non-malignant B-lymphocyte populations

B-lymphocytes	Low level of expression of the 50–60 kD protein group	Low level of expression of the 30 kD protein
CLL	15/15	0/15
Peripheral blood CD5+	0/4	0/4
Peripheral blood CD5−	0/4	0/4
Cord blood CD5+	4/4	0/4
Cord blood CD5−	0/4	0/4
Tonsil CD5+	3/3	3/3
Tonsil CD5−	3/3	0/3

however, in the present paper we present data based on the ^{35}S-methionine labelling of proteins separated using larger format gels which enabled up to 1,000 proteins to be resolved. As the number of spots detected on the gels presented in this paper was up to 1,000, this was a good representation of protein synthesis in the leukaemic and non-malignant lymphoid cells.

In some respects CLL is a heterogeneous group of haematological malignancies[16,17], nevertheless, we have reported a considerable similarity in the protein synthesis profiles from different CLL samples[8]. In a subsequent study we have examined changes in protein synthesis profiles in CLL as a function of stage[12]. The later study revealed that there were a small number of proteins that showed consistent changes in expression as a function of stage of disease. These proteins were different, however, to the proteins identified in the present study that were characteristic of CLL or non-malignant lymphocytes. This approach to the classification of cells should allow for the identification of a cell type which might represent a non-malignant counterpart to CLL, and thus provide a suitable comparison for identifying alterations in gene expression. When analysing a large number of proteins there will of course be a number of polymorphic differences observed between the samples under study, as we have observed in our samples. However, certain changes can be identified as consistent events and it is these that could represent useful cell lineage markers. We had previously observed that by metabolically labelling cells with ^{14}C-leucine, a group of proteins of 50–60 kD, could be identified as showing a consistently lower level of expression in CLL cells than in either CD5+ or CD5− B-lymphocytes from peripheral blood[8]. Our present study which utilized ^{35}S-methionine, confirmed the initial observation concerning the reduced levels of expression of the 50–60 kD group of proteins in CLL. This group of proteins therefore became our prime candidates for evaluating the relationship with other non-malignant B-cell populations to CLL.

As CD5+ B-cells, identified in foetal spleen[18,19] and in umbilical cord blood[9], have been reported to resemble

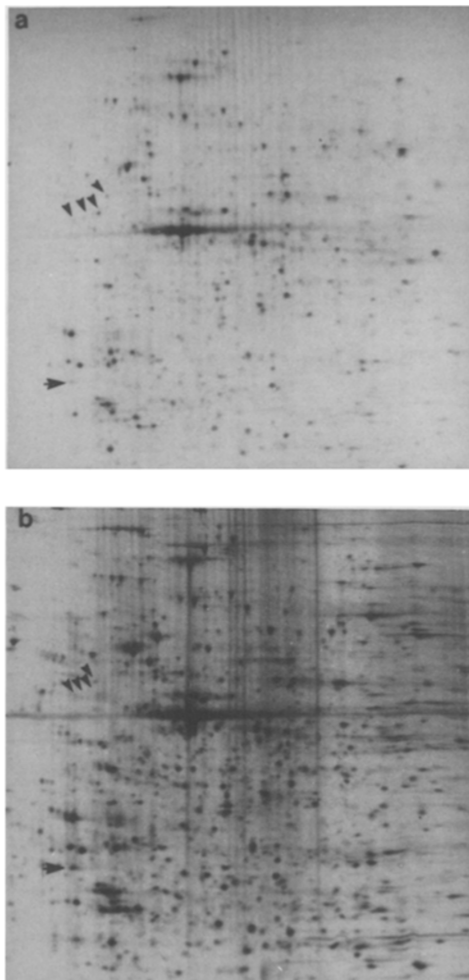

Figure 3. 2D-gel analysis of ^{35}S-methionine labelled proteins isolated from tonsil: *a* CD5+ B-cells, *b* CD5− B-cells. The autoradiographs are orientated with the acidic proteins to the left side. The small arrows indicate the 50–60 kD group of proteins, and the large arrow indicates the 30 kD protein.

CLL cells, we decided to analyse such cells by 2D-gel electrophoresis to compare them to CLL cells. We found that the CD5+ B-cells from cord blood resembled CLL cells, in that they also exhibited low levels of synthesis of the 50–60 kD protein group, in contrast to the CD5+ peripheral blood B-lymphocytes. We were unable to identify any obvious and consistent differences between the CLL cells and CD5+ cord blood cells.

The expression of a range of genes in CLL cells has also been reported to be similar to B-cells isolated from tonsils[10,11]. Our analysis of the tonsil CD5+ B-cells demonstrated that they had protein synthesis profiles similar to both the cord blood CD5+ B-lymphocytes and the CLL cells, especially with regard to the low level of expression of the 50–60 kD group of proteins. By contrast the CD5− B-cells from tonsil exhibited a much more complex protein synthesis profile, which perhaps reflected the various maturation stages of these cells present within the tonsil.

The observation of a low level of synthesis of a protein, approximate size 30 kD, in the tonsil CD5+ B-cells was interesting because in our other studies, the only other sample in which we had observed a similarly low level of expression of this protein was a leukaemic phase sample that had evolved from a low grade follicular non-Hodgkin's lymphoma (unpubl. observ.). These data therefore indicated that a low level of expression of this protein could be a marker for a follicular cell phenotype. This observation also suggested that the CD5+ B-cells from cord blood and tonsil might be distinguished, and thus indicated that the cell which was most likely to be the non-malignant counterpart of CLL was the foetal CD5+ B-lymphocyte.

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