CD10 is a marker for cycling cells with propensity to apoptosis in childhood ALL

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Malignant B cells from the majority of childhood acute lymphoblastic leukaemia (ALL) cases are characterised by the surface expression of CD10, previously known as common acute lymphoblastic leukaemia antigen or CALLA (as reviewed in LeBien and McCormack, 1989; Shipp and Look, 1993; Weir and Borowitz, 2001). Although initially considered a tumour specific antigen, CD10 was subsequently detected on a variety of normal cells of haemopoietic and non haemopoietic origin. CD10, also called neutral peptidase 24.11 or NEP, possesses a well-defined enzymatic activity, but its function in the physiology of lymphoid cells is largely unknown. From the standpoint of childhood ALL, CD10 expression represents a favourable, although not independent, prognostic marker (Pui et al, 1993; Consolini et al, 1998). The reason as to why a surface molecule has impact on prognosis is not known, although a number of observations on other cell types suggest that CD10 may mark cells with special cycling and apoptotic abilities. For example, CD10 was found on the surface of cells that are particularly prone to apoptosis such as normal germinal centre B cells (Liu et al, 1992) or malignant cells from Burkitt’s Lymphomas (BL) (Rowe et al, 1987). Moreover, it was observed that CD10 was expressed by T cells induced into apoptosis by a variety of means in vitro (Cutrona et al, 1999). Finally, c-myc upregulation not only induces cell entry into the early phases of cell cycle, but also renders the cells apoptosis-prone and concomitantly induces CD10 expression (Cutrona et al, 1995, 2000).

Based on the above observations, we reasoned that expression of CD10 by malignant B cells from ALL might indicate a special cycling ability as well as a propensity to undergo apoptosis. Indeed, in this study we demonstrate that expression of CD10 marks malignant ALL cells that are apoptosis-prone and actively cycling and express high levels of the c-myc oncogene. By contrast, CD10-negative ALL cells have lower c-myc levels and inferior cycling and apoptotic properties under the same in vitro conditions. CD10-positive ALL comprise different subgroups characterised by distinctive cytogenetic abnormalities. Nevertheless all of these cells appear to share the same cycling/apoptotic features, which can represent a common prognostic factor.

MATERIALS AND METHODS

Patients

Bone marrow aspirates of 28 cases of childhood pre-B ALL (mean age of 6.8 years) were obtained from hospitals affiliated with the
Italian Association of Pediatric Hematology and Oncology (AIEOP). Diagnosis of B-ALL was based on morphological analysis of bone marrow aspirates according to the French–American–British (FAB) guidelines (Bennett et al., 1981), and on cytogenetical and phenotypic features of the leukaemia cells. Such features include positive nuclear staining for terminal deoxynucleotidyl transferase, negative staining for myeloperoxidase, expression of B-cell differentiation markers such as CD19 and cytoplasmic Ig µ chains and absence of surface Ig. Karyotype analysis did not reveal any chromosomal abnormality in which the c-myc oncogene could be involved, i.e. t(8;14), t(2;8), or t(8;22) (MAGRATH, 1990). At the time of study, ALL patients were at the onset of disease and were untreated. All the bone marrow samples were stored in liquid nitrogen (-180°C) until tested. In the experiments with PNA (see below), freshly prepared cells from cases #655, 657, 659, 660 and 661 were employed.

**Cyto genetic analysis**

Bone marrow aspirates were processed as previously described (Sainati et al., 1997). The cyto genetic studies were performed using Trypsin–Giemsa banding technique. Chromosomes were identified and assigned according to the International System for Human Cytogenetic Nomenclature (Mitelman, 1995).

**Detection of specific chromosome gene aberration by RT–PCR**

Total RNA was isolated by using the RNaZol-B reagent according to the manufactory protocol (Duotech srl Milan, Italy). Two micrograms of total RNA from each specimen was reversed transcribed using the SuperScript reverse transcriptase (Life Technologies Milan, Italy) and random exmers: PCR amplification was performed using AmpliTaq polymerase (Applied Byosistem) according to BIOMED-1 (van-Dongen et al., 1999) protocols. A screening for the following fusion gene transcript t(1;19) with E2A-PBX1, t(4;11) with MLL-AF4, t(9;22) with BCR-ABL p190 and 661 were employed.

**Cells and cell cultures**

Mononuclear cell fractions were purified from bone marrow aspirates by centrifugation on Ficoll–Hipaque gradients (Seromed, Biochrom KG, Berlin, Germany). All of the suspensions were comprised of more than 90% leukaemia cells except for cases #738 and 702, in which there were 55 and 69% leukaemic cells, respectively. Where indicated, special staining procedures had to be employed for these cases.

The LAM C4 cell line, derived from a Burkitt lymphoma patient, with the typical (t8;14) translocation and c-myc over-expression was used as a positive control in the studies on c-myc expression (Roncella et al., 1993).

The culture medium used throughout was RPMI 1640 (Seromed) supplemented with 10% FCS (Seromed).

**Flow-cytometry**

The following mAbs were used for immunofluorescence staining: anti CD10 (J5) (Coulter Corp., Hielah, FL, USA), and anti CD19 (Leu-12) (Becton Dickinson & Co., Sunnyvale, CA, USA). Both of these mAbs were used in indirect immunofluorescence. The secondary FITC- or PE-conjugated antibodies to the appropriate murine Ig isotype were from Southern Biotechnology (Birmingham, AL, USA). Permeabilised cells were stained for indirect immunofluorescence with a murine anti Myc mAb (6E10 clone, Cambridge Research Biochemicals, Cheshire, UK), an anti Ki67 mAb (DAKOPATTS, Glostrup, Denmark), and an anti Ig µ chains (Becton Dickinson & Co.) as previously reported (Cutrона et al., 1997). The samples were analysed by flow cytometry (FACS Calibur, Becton Dickinson & Co.). Data were expressed as histograms of fluorescence intensity vs cell number or as relative fluorescence intensity (MRFI) calculated according to the following formula: mean fluorescence intensity of cells stained with the mAb/mean fluorescence intensity of control cells treated with an unrelated mAb.

Two–colour analysis of 5-bromo-deoxyuridine (BrdUrd) (Sigma Aldrich, Milan, Italy) incorporation and DNA content was performed according to a modification of the method of Dolbeare et al. (1985) as previously described (Dolbeare et al., 1985; Cutrона et al., 1997).

CD10 positive and negative cells from CD10-negative ALL cases were separated by cell sorting (FACS Calibur, Becton Dickinson & Co.). The two populations were gated on the basis of CD10 expression and the forward light scatter parameter.

Measurements of ploidy (DNA index, DI) was carried out by staining samples with a hypotonic solution of PI using an automated DNA staining device (DNA-prep reagents; Coulter) and analysed by flow cytometry (Epics XL; Coulter). DNA histograms were obtained by a cell cycle analysis program (Multicyle; Phoenix, San Diego, CA, USA). Leukaemic cells were classified as diploid when the DI was between 0.9 and 1.09; as hyperdiploid when the DI was 1.8 and less then 1.80; and as hypodiploid when DI was <0.9. The DI was established from the ratio of the modal channel number of the G0/G1 peak of neoplastic cells to that of normal cells.

**Western blot**

Western blot analysis for MYC protein was performed as previously described (Cutrона et al., 1995). The nitrocellulose membranes were immunoblotted with an anti Myc hybridoma supernatant (9E10 clone, kindly provided by Dr R Sitia, San Raffaele Institute, Milan, Italy) or a rabbit antiserum to histone 2b (H2b) (kindly provided by Dr U Pfeffer, CBA, Genoa, Italy).

**PCR methodologies**

The PCR technique for analysis of V(D)J rearrangements and of HCDR3 length was performed as previously described (Dono et al., 1996). A RT–PCR methodology was used to detect c-myc and gyceraldehyde 3-phosphate dehydrogenase (GAPDH) RNA expression by the leukaemia cells treated in different manners (Cutrона et al., 1997).

**Apoptosis assays**

Five x 10⁵ per ml cells were cultured in RPMI medium (Seromed) supplemented with 10% FCS (Seromed). The cultures were harvested at different intervals and the apoptotic cells were detected by using Annexin-V conjugated with FITC (Apoerlot™ Apoptosis Kit, Gentaclab Inc., Palo Alto, CA, USA), by PI staining, or by DNA laddering, as previously described (Cutrона et al., 1999).

**PNA anti-gene**

A 17-mer anti myc PNA, complementary to a unique sequence located at the beginning of the second exon of the c-myc oncogene (TCA ACG TTA GCT TCA CC) was used. This PNA was modified by addition of a Nuclear Localization Signal peptide (NLS) PNA-
mynuc-NLS as reported (Cutrona et al., 2000). A PNA characterised by a 3-base substitution (TTA ACG CTA GCT TTA CC), but unchanged purine/pirimidine ratio (PNA-mycmut-NLS) was used as control. The PNAS described were purchased from TIB MOLBIOL (Berlin, Germany).

Statistical methods
Mean, standard deviation, and standard error were calculated for each of the markers determined for the two groups of patients investigated. Mean differences by groups of patients were evaluated via the Student’s t-test and, when necessary (i.e., skewness and/or heteroscedasticity in the biomarker distributions), statistical testing was applied on long transformed variables (Armitage and Berry, 1987). All statistical comparisons and related P-values were two tailed. Mean differences were considered significant when P-values were less than or equal to 0.05.

RESULTS
Definition of CD10-positive and CD10-negative ALL
The cells from the bone marrow of 28 ALLs of B cell origin were simultaneously stained for CD10 and CD19. As shown in Figure 1, the ALL cases were subdivided into two groups: one (17 cases) with a high CD10 expression (>80% CD19-expressing cells were CD10 positive, MRFI 211 ± 82), and one (11 cases) with low CD10 (<20% CD19-expressing cells were CD10 positive, MRFI 10 ± 7). Both these differences were highly significant (P<0.001 for both percentage positive cells and MRFI values). Cases belonging to the first group, i.e. high CD10 expression, were defined as CD10-positive, while cases falling in the second group, i.e. low CD10 expression, were considered as CD10-negative.

Next, we investigated whether correlations existed in the different cases between the presence of certain chromosome abnormalities and CD10 expression. The 28 ALLs of B cell origin were analysed for the fusion gene transcripts t(1;19) with E2A-PBX1, t(4;11) with MLL-AF4, t(9;22) with BCR-ABL p190 and BCR-ABL p210, t(12;21) with TEL-AML1 by RT-PCR. As shown in Table 1 only one out of 14 CD10-positive and two out of 10 CD10-negative ALL cases displayed the t(4;11) translocation, three out of 14 CD10-positive ALL cases displayed the t(12;21) rearrangement and t(9;22) was negative in all of the cases analysed. Notably, because of the study design, all of the cases tested did not display any of the typical translocations of BL (Magrath, 1990).

Differences in spontaneous apoptosis in vitro
Here, we investigated whether the malignant cells from CD10-positive ALL had higher spontaneous apoptotic capacities than those from CD10-negative cases. Apoptosis was measured by PI staining in cells taken ex vivo or after 24 h in culture. While the proportion of apoptotic cells in the suspensions ex vivo was very low in the two groups (mean 4 ± 3.8) (Figure 2), significant differences were noted after culture. Apoptotic cells in the CD10-positive ALL group were 66.7 ± 9.1% vs 22.3 ± 10.5% in the other group (P<0.001). These differences were further confirmed by measuring the DNA laddering (Figure 2).

Differences in cell cycle status
Next, we investigated the cell cycling abilities of CD10-positive and CD10-negative ALL cells. Eight CD10-positive and seven CD10-negative ALL cases were stained for Ki67, which is expressed from the late G1 phase of the cell cycle (Gerdes et al, 1984). The proportion of Ki67-expressing cells was significantly higher in the CD10-positive than in the CD10-negative ALL cases (63.66 ± 23% vs 27.37 ± 9.4%, P=0.009, see Figure 3A). Figure 3A also shows typical flow cytometry profiles where a bimodal distribution of Ki67-positive cells is evident for each group.

In another experiment, we investigated BrdUrd incorporation by the CD10-positive and CD10-negative ALL following 1 h in culture. The incorporation of BrdUrd was much higher in the CD10-positive than in the CD10-negative ALLs (average 47.52 ± 21.27% vs 7.66 ± 7.84%, P=0.001) (Figure 3B). Figure 3B also shows a typical flow cytometry profile obtained in a test where the cells were pulsed with BrdUrd for 1 h, PI stained and analysed by flow cytometry. Most of the cells from the CD10-positive ALL were in the G1-S phase, whereas the majority of the cells from the CD10-negative ALL were in the G0-G1 phase of the cell cycle, a finding which is in agreement with the results of the Ki67 staining.

The cells from five CD10-positive ALL cases (775, 743, 1022, 745 and 657) were pulsed with BrdUrd for different time intervals before PI staining and flow cytometry analysis. Most of the cells were already in the S phase of the cell cycle after 1 h. At later times, there were very few cells in the M phase, whereas the majority of the cells underwent apoptosis from the G1-S phase of the cell cycle, as shown by the finding that virtually all of the hypodiploid cells seen at 48 h had also incorporated BrdUrd (Figure 3C).
Differences in Myc expression

Cell undergoing apoptosis from the G1-S phase of the cell cycle often overexpress c-myc (Hueber and Evan, 1998). To explore this issue further, CD10-positive and CD10-negative ALL were analysed for the expression of Myc protein by immunofluorescence. Single staining was carried out in all cases in which there were greater than 80% neoplastic cells (i.e., CD19+/CD10+ or CD19+/CD10- large blasts), while in two cases, where the proportion of malignant cells was lower (#738 and 702), double staining for surface CD19 and intracytoplasmic Myc was carried out. The data, summarised in Figure 4A, show a significant difference in Myc expression. CD10 positive ALL cases had an average MRFI of 69.24 ± 25.3 vs 14.98 ± 6.8 of CD10-negative ALL cases (P < 0.001). These findings were confirmed by Western blot analysis (Figure 4B). The percentages of cells positive for Myc were not determined since the cells of both groups displayed some positive fluorescence, as indicated by the typical profiles shown in the inset of Figure 4A. It could be argued that because of its relatively short half life (Hann and Eisenman, 1984), Myc expression was influenced by the cryopreservation of the cells. However, extensive comparative tests carried out

Table 1 Cytogenetic analysis of CD10-positive and CD10-negative B-ALL

| Patient | CD10 %a | Cariotypeb | Ploidy | t(12;21) | t(4;11) | t(1;19) | t(9;22) P190 | t(9;22) P210 |
|---------|---------|------------|--------|----------|---------|---------|-------------|-------------|
| 745     | 95.9    | 46, XY     | diploid| +        | −       | −       | −           | −           |
| 738     | 55.1    | 46, XY     | hyperdiploid| +       | −       | −       | −           | −           |
| 743     | 93.6    | nd         | diploid| −        | −       | −       | −           | −           |
| 775     | 94.1    | nd         | nd     | nd       | nd      | nd      | nd          | nd          |
| 702     | 69.1    | 46, XY     | nd     | −        | −       | −       | −           | −           |
| 1022    | 96.6    | nd         | diploid| −        | −       | −       | −           | −           |
| 1422    | 92.6    | 46, XY, der 3, der 12 | diploid| +        | −       | −       | −           | −           |
| 996     | 87.6    | nd         | hyperdiploid| −       | −       | −       | −           | −           |
| 1307    | 86.8    | 45, X, (10%)45, XX, del(9)(p21), −20(90%) | diploid| −        | −       | −       | −           | −           |
| 1059    | 98.3    | nd         | nd     | −        | −       | −       | −           | −           |
| 1348    | 91.95   | 46XX, (15%)47, XXY, t(4;11)(q21;q23)(85%) | hyperdiploid| −       | +       | −       | −           | −           |
| 1089    | 93.4    | 45, XX, −20 | hypodiploid| −        | −       | −       | −           | −           |
| 635     | 82      | nd         | nd     | nd       | nd      | nd      | nd          | nd          |
| 675     | 95      | 46, XY     | hyperdiploid| −        | −       | −       | −           | −           |
| 659     | 98      | nd         | nd     | nd       | nd      | nd      | nd          | nd          |
| 660     | 93      | 46, XX     | diploid| −        | −       | −       | −           | −           |
| 661     | 93.9    | nd         | hyperdiploid| −        | −       | −       | −           | −           |
| 282     | 7       | 46, XX, t(4;11)(q21;q23) | diploid| −        | +       | −       | −           | −           |
| 748     | 4       | 46, XX, t(4;11)(q21;q23) | diploid| −        | +       | −       | −           | −           |
| 441     | 7.9     | 46XX, 9p−/−48, XX, 9p−, +8, +12 | nd     | nd       | nd      | nd      | nd          | nd          |
| 841     | 15.7    | 46, XY     | diploid| −        | −       | −       | −           | −           |
| 571     | 16.2    | 46, XY     | diploid| −        | −       | −       | −           | −           |
| 1110    | 5.6     | 46, XY     | hypodiploid| −        | −       | −       | −           | −           |
| 1384    | 10      | 47, XX, +21 | nd     | −        | −       | −       | −           | −           |
| 470     | 18.5    | nd         | nd     | −        | −       | −       | −           | −           |
| 621     | 12.3    | nd         | nd     | −        | −       | −       | −           | −           |
| 1372    | 17.8    | 46, XX     | diploid| −        | −       | −       | −           | −           |
| 570     | 17.8    | 46, XY     | diploid| −        | −       | −       | −           | −           |

*aPercentage of CD10 positive leukaemic cells was determined by immunofluorescence and flow cytometry analysis; bCariotype analysis was assessed by Trypsin-Giemsa banding technique; cPloidy was defined based upon DI values (see Materials and methods); d+ and − indicate presence or absence of fused gene transcripts by PCR analysis, respectively; e nd=not determined.

Figure 2 Apoptotic capacities of CD10-positive and CD10-negative ALL cells. Cells from the indicated cases were tested for apoptosis by PI staining either immediately after isolation or following 24 h in culture (left). Apoptotic capacities were confirmed by measuring DNA laddering (two typical experiments are shown on the right).
in case 657 demonstrated that cryopreservation did not affect Myc expression. Moreover, cases (655, 657, 659, 660, 661) were also tested using freshly drawn cells.

Features of CD10-positive cells isolated from CD10-negative ALL cases

Next, we investigated the characteristics of the few CD10-positive cells detected in the CD10-negative ALL cases. The cells from six cases (571, 1372, 621, 1384, 470 and 843) were stained with CD10 mAb and the CD10-positive cells were FACS-sorted. Sufficient quantities of CD10-positive and CD10-negative cells could be isolated for further analyses. As shown in Figure 5 (which reports one representative experiment on cells from patient #571), CD10-positive cells were actively cycling, with high levels of c-myc and a special propensity to undergo apoptosis in vitro, whereas CD10-negative cells had low c-myc levels and low cycling and apoptotic capacities (Figure 5). These data demonstrate a different functional status in two cell populations that originated from the same clone, as shown by the analyses of their V(D)J rearrangements (see Figure 5).

Role of c-myc in apoptosis, cell cycle and CD10 expression

Here, we tested the hypothesis that the high level of c-myc conferred to CD10-positive ALL cells both a superior cycling capacity and a more pronounced propensity to undergo apoptosis. To this end, c-myc expression was blocked using a PNA anti-c-myc anti-gene. PNAs are synthetic structural homologues of nucleic acids in which the phosphate-sugar polynucleotide backbone is replaced by a flexible polyamide (Egholm et al, 1992). They can bind to the complementary DNA sequences more stably than DNA itself (Egholm et al, 1993). However, to be effective on intact cells in vitro, PNAs must be coupled to a vector, which allows their transport to the cell nuclei (Nielsen, 1999). In this study, we used PNA with complementarity to a sequence of the second exon of the c-myc oncogene. When coupled to an appropriate nuclear localisation sequence (NLS), this PNA-mycwt-NLS is able to penetrate cell nuclei and to selectively block the expression of c-myc (Cutrona et al, 2000). A PNA that differed for the presence of three mutations in the sequence complementary to the c-myc sequence was used as control (PNA-mycmut-NLS).
Cells from CD10-positive ALL were incubated with medium, 10 μM PNA-mycwt-NLS, or PNA-mycmut-NLS for 24 h. The cultured cells were harvested and checked for c-myc expression by flow cytometry (Figure 6A), RT–PCR (Figure 6B) and Western blot (Figure 6C). As shown in Figure 6, a mean reduction in MRFI of 54 ± 18% was noticed by immunofluorescence and flow cytometry. This reduction in c-myc expression was confirmed by the two other methods. Inhibition of c-myc expression was accompanied by a significant decrease of BrdUrd incorporation by the cells (Figure 6D), indicating a substantial decline in their cycling abilities.

In another series of experiments, freshly prepared cells were incubated with PNA-mycwt-NLS, PNA-mycmut-NLS or medium for 24 h. Subsequently, apoptosis was measured by PI or Annex-
inhibition (mean reduction 48 ± 8, in the four cases that were investigated, there was a substantial concomitant reduction in CD10-expression. As shown in Figure 8, assessed by flow cytometry (Figure 8).

This study demonstrates that malignant B cells of CD10-positive ALL are mostly actively cycling, and are apoptosis-prone, whereas the cells of CD10-negative ALL are not actively cycling, and do not undergo apoptosis readily in vitro. In addition, the present data show a close correlation between Myc levels and the cycling and apoptotic capacities of the cells. Finally, these studies indicate that CD10 expression distinguishes cells with different properties. Various results from this study point to correlations between CD10 expression and the cells’ biological behaviour in vitro. The few CD10-positive malignant cells, present in those cases classified as CD10-negative ALL, shared all of the characteristics of the malignant cells found in the CD10-positive ALL cases, including elevated c-myc levels and the propensity to undergo spontaneous apoptosis. Furthermore, the block imposed on c-myc expression by PNA myc wt-NLS was followed by inhibition of cell cycling, as well as of the apoptotic capacities of the ALL cells. Upon c-myc inhibition, there was also a substantial reduction of CD10 expression, thus confirming the value of CD10 as a marker for cycling and/or apoptosing ALL cells.

A wide range of results for Ki67 staining and BrUrd incorporation in ALL cases was reported in previous studies, but these features were not correlated with the immunophenotype of the malignant cells (Tsurusawa et al, 1995; Salomons et al, 1999).

Admittedly, most of the cases reported could have been CD10-positive. This raises the questions as only in those cases the proliferative capacities of the leukaemic cells were in general lower than those observed for CD10-positive ALL cases in the present study. Although there are no apparent explanations for these differences, one should, however, point out that the flow cytometry method used here may have increased the ability of the techniques to detect cycling cells.

The present study indicates a fundamental role of c-myc in the apoptosis of ALL cells, since spontaneous cell death occurred in vitro only in those cells that displayed elevated c-myc levels. Whether this correlation holds true also for other types of apoptosis (e.g. drug or radiation induced) has to be further investigated, although studies on different cell types overexpressing c-myc would predict this to be the case (Rowe et al, 1987; Cutrona et al, 1995, 1997, 1999; de-Saint-Vis et al, 1995; Martinez-Valdez et al, 1996; Mueller et al, 1997; Guedez et al, 1998a,b). A close correlation between c-myc expression and apoptosis was first indicated by studies on murine fibroblasts transfected with the c-myc oncogene (Evan et al, 1992). Recent studies on cells induced to overexpress c-myc in vitro have demonstrated that these cells have difficulties in
completing cell cycle and usually either undergo apoptosis from the late G1 phase or remain arrested in the G2 phase. These arrested cells have a tendency to become aneuploid (often hyperdiploid) (Felsher et al, 2000). The latter mechanism observed in vitro may suggest an appealing model to explain the frequent hyperdiploidy seen in CD10-positive ALL cells. In this respect, it is of note that hyperdiploidy was detected in 41.7% of the CD10-positive ALL cases studied (five out of 12, Table 1) (see also reviews Look, 1997; Faderl et al, 1998; Ito et al, 1999).

The finding of elevated c-myc levels raises questions regarding the mechanisms involved in c-myc overexpression in CD10-positive ALL cells. Chromosomal translocations typical of BL and L3 leukaeasias, which juxtapose c-myc to the Ig gene loci and cause c-myc overexpression, were excluded in all of the ALL cases selected for this study. Therefore, other options have to be considered. One possibility is that, because of the neoplastic transformation, the CD10-positive ALL cells were frozen at a maturational stage characterised by a physiological c-myc elevation and active cycling capacities. Studies on murine B cells have demonstrated that under particular circumstances, pre-B cells may proliferate autonomously in vitro (Rolink et al, 2000) and conceivably also in vivo (Carsetti, 2000). Moreover, there is evidence in man that most CD10-positive pre-B cells are actively cycling in vivo (Ghia et al, 1996). Another plausible, and not mutually exclusive, option is that alterations of oncogenes other than c-myc could be responsible for the malignant transformation and for the upregulation of c-myc (and cell proliferation). Finally, it is possible that in ALL cells the c-myc gene presented a number of mutations which prolong the intracellular half-life of its product, similar to that reported in certain BL cells (Bahram et al, 2000; Fais et al, 2000). Central to this issue is the problem of whether ALL cells express physiological (i.e. similar to those seen in proliferating cells) or pathological (i.e. similar to levels of those of BL) c-myc. Definitive proof for one or other hypothesis awaits further studies.

Previous investigations on cells with elevated c-myc levels have indicated that the choice between apoptosis or cell proliferation is often dictated by the presence of additional signals capable of promoting or preventing apoptosis (Bissonnette et al, 1992; Fanidi et al, 1992). For example, B cells in which the c-myc oncogene has been transected and hyper-expressed would die in low serum concentrations in vitro unless exposed to CD40L or to an agonistic CD40 mAb which are both capable of inducing the upregulation of bcl2 and possibly of other anti-apoptotic genes (Cutrona et al, 1995). These observations suggest that a number of signals, delivered either by contact with stromal cells or by cytokines, prevent the apoptosis of CD10-positive ALL cells in vitro and facilitate their proliferation (Welch et al, 1990; Manabe et al, 1992, 1994; Saeland et al, 1992; Campana et al, 1994; Murti et al, 1996; Nishigaki et al, 1997). In this regard, it is of note that previous studies demonstrated that the ability of ALL cells to survive when cultured on stromal cells was an accurate predictor of a negative clinical outcome irrespective of the cell proliferative ability in vitro, possibly underlying the value of the anti-apoptotic properties of the stromal cells in the survival/expansion of the malignant ALL cells (Campana et al, 1993; Coustan-Smith et al, 1996; Kumagai et al, 1996).

Both CD10-positive and CD10-negative ALL are comprised of a heterogeneous group of diseases characterised by different cytogenetic abnormalities. Although data on such abnormalities are not available for all of the cases studied here, observations on a proportion of the CD10-positive ALL points to their heterogeneity. Thus, the t(4;11) translocation, which involves MLL-AF4 gene (Pui et al, 1991), was found in only one out of 14 CD10-positive and 2/10 CD10-negative ALL cases. Likewise t(12;21) causing TEL-AML1 (Romana et al, 1995; McLean et al, 1996; Borkhardt et al, 1997) rearrangement was seen in three out of 14 CD10-positive ALL cases.

The latter findings perhaps suggest a marginal involvement of these translocations in the control of c-myc expression. On the other hand, the intrinsic properties of the cells from the CD10-negative ALL do not prevent them from expressing CD10 as shown by the data in Figure 5, which reinforced the concept that CD10 expression is linked to the cycling/apoptotic properties. This consideration may in part explain why CD10 expression represents a non-independent prognostic marker of ALL. Thus, the superior...
cycling and apoptotic features of CD10-positive ALL cells may render them more susceptible to the external signals including chemotherapeutic agents. However, additional features independent of the cycling/apoptotic capacities of the cells may also greatly influence the course of the disease.

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