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Good prognosis cytogenetics in B-cell chronic lymphocytic leukemia is associated *in vitro* with low susceptibility to apoptosis and enhanced immunogenicity

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Chromosomal abnormalities in B-cell chronic lymphocytic leukemia (B-CLL) have been shown to correlate with prognosis. Little is known about the relationship between chromosomal abnormalities and biological behavior of B-CLL cells *in vitro*. The present study was designed to explore the impact of chromosomal abnormalities determined by interphase fluorescence *in situ* hybridization (FISH) on the *in vitro* survival and immunogenicity of B-CLL. Considerable heterogeneity was noted in the *in vitro* survival and expression of costimulatory, adhesion, and antigen-presenting molecules by B-CLL cells. Spontaneous apoptosis of B-CLL cells *in vitro* was significantly lower in samples with good prognosis cytogenetics when compared to samples with poor prognosis cytogenetics. In contrast, B-CLL cells from samples with good prognosis cytogenetics exhibited higher basal expression of molecules involved in costimulation, cellular adhesion, and antigen presentation, and induced significantly more T-cell proliferation in mixed lymphocyte cultures. We conclude that chromosomal aberrations of B-CLL cells correlate with the *in vitro* biological behavior of B-CLL. Our data indicate that good prognosis cytogenetics correlates with less spontaneous apoptosis but greater *in vitro* immunogenicity. These findings could have significant implications on the design of future therapeutic approaches in patients with CLL, and the likelihood of response based on cytogenetics.
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Introduction

Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia in the world, and is characterized by the slow accumulation of malignant lymphocytes, which are B cells in 98% of cases.[1] B-CLL is associated with a variety of chromosomal aberrations that can be characterized with interphase fluorescence *in situ* hybridization (FISH).[2,3] These aberrations are most commonly found on chromosomes 6, 11, 12, 13, and 17. An association has been found between chromosomal abnormalities and clinical prognosis or disease progression. Aberrations in chromosome 13 are the most common, and are associated with the best clinical prognosis. Aberrations in chromosome 17p are associated with aberrations in p53, are less common, and have a much poorer prognosis.[3–5] In a study of 325 patients, the median treatment-free interval, a good indicator for disease progression, for patients with 13q deletion as single aberration and normal karyotype was 92 and 49 months respectively. In contrast, the treatment-free interval was shorter in patients with 17p deletion (9 months), 11q deletion (13 months), or trisomy 12 (33 months).[2]

A number of biological mechanisms may be responsible for the striking differences in clinical behavior in B-CLL. Bcl-2 is known to confer chemoresistance in a variety of cancers including hematological malignancies. Bcl-2 is overexpressed in the majority of B-CLL cells,[6] and it has been reported to be associated with poor prognosis.[7] On the other hand, Bcl-2 overexpression does not appear to predict a poor response to purine analogs.[8–10] Therefore, overexpression of Bcl-2 alone with resulting resistance to apoptosis may not be the primary factor responsible for poor outcome and prognosis of B-CLL.

Little is known about the importance of B-CLL immunogenicity in disease progression and treatment-free interval. B-CLL cells can be immunogenic and induce leukemia-specific T cells;[11–13] however, it is not clear whether this immunogenicity impacts on prognosis or whether enhancement of immunogenicity will be beneficial therapeutically. A variety of immunotherapeutic approaches designed to render B-CLL cells more immunogenic have been developed recently. These approaches include gene therapy[14,15], CD40 ligation, and[14–17] treatment with cytokines[18] or immunostimulatory oligonucleotides (CpG ODN).[19–22]

Based on this background, and the fact that FISH can be performed easily in B-CLL, we explored the relationship between the cytogenetic status of B-CLL cells and their tendency to undergo apoptosis *in vitro*. We also explored the impact of cytogenetic status on the immunogenicity of the B-CLL cells.

Materials and methods

Patients and cell culture

Peripheral blood samples from 22 subjects with B-CLL were used for this study under a protocol approved by the Institutional Review Board of the University of Iowa. Informed consent was obtained from each subject. The diagnosis of CLL was determined according to NCI Working Group criteria.[23] B-CLL subjects were not under treatment at the time the samples were obtained. Peripheral blood mononuclear cells (PBMC) were isolated as described previously.[24] For *in vitro* culture, cells were suspended in RPMI 1640 (Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated (56°C) FCS (HyClone, Logan, UT, USA), 1.5 mM L-glutamine (Gibco BRL), 100 U/ml penicillin, and 100 μg/ml streptomycin (complete medium). Cells were incubated in 96-well plates (1×10^6 cells/ml).

Phenotypic determination

At the time points indicated, cells were washed in ice-cold PBS and stained for expression of various antigens as described previously.[25] Monoclonal antibodies (mAB) against CD3

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(Cy-Chrome, UCHT1), CD5 (PE, UCHT2), CD8 (PE, RPA-T8), CD19 (Cy-Chrome, HIB19), CD54 (PE, HA-58), CD80 (FITC, L307.4), and CD86 (FITC, FUN-1), MHC class II (FITC, G46-6) as well as appropriate isotype controls (all from BD Biosciences, San Diego, CA, USA) were used. For intracellular staining of Bcl-2 (Bcl-2/100, BD Biosciences, San Diego, CA, USA), cells were fixed and permeabilized according to the manufacturer's protocol (Fix & Perm, Caltag, Burlingham, USA). Cells were run on a FACScan (BD Immunocytometry Systems, San Jose, CA, USA), and data were analyzed with the FACS evaluation program FlowJo (version 4.5, Tree Star Inc., Stanford, CA, USA). In most experiments, B-CLL cells were identified using anti-CD19 only, since more than 95% of CD19+ PBMC were CD5+.

Apoptosis and cell survival assay

Cells were harvested and stained with Annexin V (BD Biosciences, San Diego, CA, USA), PI, and mABs for CD5 and CD19. A predetermined number of calibration beads (CaliBRITE™ Beads, BD Biosciences, San Diego, CA, USA) were added to allow for normalization of cell counts. Viable cells were counted rather than apoptotic cells. Survival was expressed as percentage of viable cell counts relative to initial plating counts.

FISH

Unstimulated PBMC from subjects with B-CLL were cultured for 24 h in RPMI media containing 15% FCS and antibiotics. Slides were made after the cells were processed and fixed in 3:1 methanol and acetic acid fixative. The FISH studies were performed using a CLL probe panel (VYSIS, Downers Grove, IL, USA) that includes two cocktail probes. One set contains ATM (11q22.3)/17p13.1 (p53) and the second set has 12cen (CEP12)/13q14.3 (D13S319)/13q34. These studies were carried out using standard methods as specified by the manufacturer. A total of 300 interphase nuclei were analyzed for each probe set. The cutoff for positive values was 2.2% for trisomy 12 and 2.7, 6.4, 6.0, and 4.4% for ATM, p53, D13S319, and 13q34 respectively.

CFSE staining

CFSE (5- (and 6-) carboxyfluorescein diacetate succinimidyl ester; Molecular Probes, USA) was used to evaluate cell proliferation. CFSE is a fluorescein-derived intracellular fluorescent label, which is divided equally between daughter cells upon cell division. Staining of cells with CFSE allows both quantification and immunophenotyping of proliferating cells in a mixed cell suspension. The technique is described in detail by Lyons and Parish.[26]

Mixed lymphocyte culture

T cells were isolated from a buffy coat of a healthy donor using Ficoll and a Pan T cell kit (Miltenyi Biotec, Auburn, CA, USA), labeled with CFSE, and resuspended in complete medium at 1×10^6/ml. A 100 μl portion was plated on a 96-well plate. Cryopreserved PBMC from subjects with B-CLL were thawed, washed, and diluted to 0.2×10^6 live cells/ml. A 100 μl portion was added to the previously plated CFSE-stained T cells. These cocultures were incubated for 5 days at 37°C. Cells were then stained with CD3-CyChrome and CD8-PE and analyzed by flow cytometry.

Statistical analysis

Data are expressed as means ± s.e.m. To determine statistical differences in the mean of two data columns, the paired or unpaired *t*-tests were used as appropriate. Pearson's correlation was used to examine the relationship between two groups of variables. Statistical analysis was performed using the SAS System for Windows (SAS, version 8.2, 2001, SAS Institute, Cary, NC, USA).

Results

Spontaneous in vitro *survival of malignant B cells from subjects with B-CLL is highly heterogeneous and does not correlate with the percentage of non-B-CLL cells in the peripheral blood or prior therapy*

Although B-CLL cells appear to be quite resistant to apoptosis *in vivo*, their survival *in vitro* without further stimulation (spontaneous survival) is limited. For most B-CLL cases, cell viability was greater than 90% on day 0, but decreased significantly after 7 days of culture. The survival varied strongly from sample to sample. Samples from a minority of subjects demonstrated a stable or even slight increase of viable cell count over 7 days. As outlined in Table 1, the *in vitro* survival of B-CLL cells (CD19+ cells) after 7 days did not correlate with the percentage of non-B-CLL cells present in the blood at the time the samples were obtained ($R = 0.05$; Table 1). Seven subjects had received prior therapy for their CLL, four of them within 1 month prior to sampling and three of them 5 months or more before sampling. Comparison of *in vitro* survival of samples from recently or previously treated subjects was not statistically different from that of subjects who had not received therapy (Table 1). Given the small number of subjects who had received prior therapy, and the heterogeneity of the therapy they received, it is not possible to reach any conclusions related to *in vitro* survival and prior treatment.

Cytogenetic status correlates with B-CLL cell survival, intracellular Bcl-2 levels, and patients' serum lactic dehydrogenase levels

Several investigators have reported that the cytogenetic status of B-CLL correlates with clinical prognosis. We therefore evaluated whether the spontaneous survival of B-CLL cells (CD19+ cells) *in vitro* also correlates with cytogenetic status. Samples studied included B-CLL cells with a normal karyotype ($n = 4$), 13q deletion as single aberration ($n = 7$), 17p deletion ($n = 5$), and other poor prognosis aberrations including trisomy 12 ($n = 5$) and 11q deletion ($n = 1$) (Table 1 and supplementary Table 2). B-CLL cells with normal karyotype and 13q deletion showed comparably high survival, with 67 ± 12% of cells from subjects with normal karyotype and 83 ± 12% of cells from subjects with 13q deletion remaining viable after 7 days of *in vitro* incubation (Figure 1). In contrast, the spontaneous survival of B-CLL cells from subjects with 17p deletion, trisomy 12, or 11q deletion was relatively low after 7 days (17p deletion: 39 ± 6%; trisomy 12: 32 ± 13%; 11q deletion: 43%) (Figure 1).

Table 1 Clinical data of B-CLL subjects included in the study

Sample no.	*Age*	*Sex*	*Spontaneous survival at day 7 (%)*	*WBC (1000/mm^3)*	*Non-B-CLL cells (%)*	*Rai Stage*	*Cytogenetics*[a]	*LDH (U/l)*[b]	*CD38 expression (MFI)*	*Time since most recent treatment*	*Most recent therapeutic regimen*
1	67	F	66	13	>50	0	Normal karyotype	150	1.32	Untreated	Untreated
2	79	F	97	33	21	0	Normal karyotype	NA	0.76	Untreated	Untreated
3	71	F	54	29	6	4	Normal karyotype	251	NA	5 months	Cyt
4	56	M	51	17	28	0	Normal karyotype	131	1.07	Untreated	Untreated
5	63	M	65	11	>50	0	13q14.3 del	171	1.27	Untreated	Untreated
6	76	M	79	15	16	1	13q14.3 del	132	2.24	Untreated	Untreated
7	71	M	118	56	5	4	13q14.3 del	NA	1.55	3 years	Flu
8	59	F	124	19	13	0	13q14.3 del	NA	1.12	18 months	Flu
9	52	M	43	188	15	2	13q14.3 del	183	1.65	Untreated	Untreated
10	72	F	66	49	18	2	13q14.3 del	148	1.21	Untreated	Untreated
11	51	F	89	24	38	0	13q14.3 del, 13q34 del	NA	NA	Untreated	Untreated
12	77	F	24	10	17	3	17p13.1 del	324	1.46	Untreated	Untreated
13	76	M	42	87	4	4	17p13.1 del; 13q14.3 del	154	NA	3 weeks	Pre, Chl, BMT
14	64	F	54	160	11	4	17p13.1 del; 13q14.3 del	NA	NA	2 weeks	Flu, Cyt, Rit
15	53	F	48	14	29	1	17p13.1 del; 13q14.3 del	171	2.98	Untreated	Untreated
16	63	M	29	39	30	4	17p13.1 del; 13q14.3 del	211	NA	1 month	Flu, Pre
17	51	M	25	150	5	3	Trisomy 12; 13q14.3 del	248	1.96	Untreated	Untreated
18	80	F	53	45	15	1	Trisomy 12; 13q14.3 del	184	NA	Untreated	Untreated
19	55	M	4	184	14	0/1	Trisomy 12; 13q14.3 del	195	NA	Untreated	Untreated
20	66	F	65	12	43	0	Trisomy 12; 13q14.3 del	177	1.39	Untreated	Untreated
21	69	M	14	18	31	0	Trisomy 12; 13q14.3 del	186	37.04	Untreated	Untreated
22	78	F	43	284	<1	4	11q22.3 del; 13q14.3 del	263	1.94	2 weeks	Cyt, Pre

BMT: bone marrow transplantation (autologous); 2-CDA: 2-chlorodeoxyadenosine; Chl: chlorambucil; Cyt: cytoxan; Flu: fludarabine; MFI: median fluorescence intensity; NA: not available; Pre: prednisone; Rit: rituxan.

[a]See supplementary Table 2 for a more detailed information on FISH.

[b]Normal LDH range: 135–225 U/l.

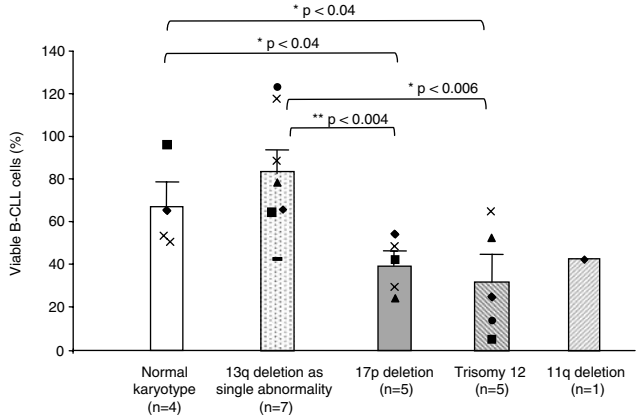


Figure 1 Impact of cytogenetic status on B-CLL cell survival. B-CLL cells from 22 subjects were cultured *in vitro* and the percentage of viable B-CLL cells (CD19+ cells) on day 7 was determined by Annexin V/PI staining. Average and individual survival data of five distinct cytogenetic groups are compared. Error bars indicate s.e.m.

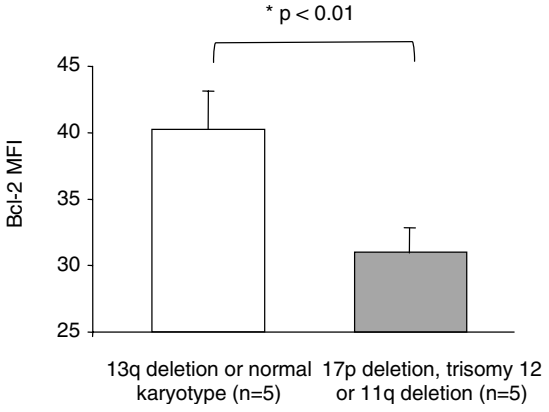
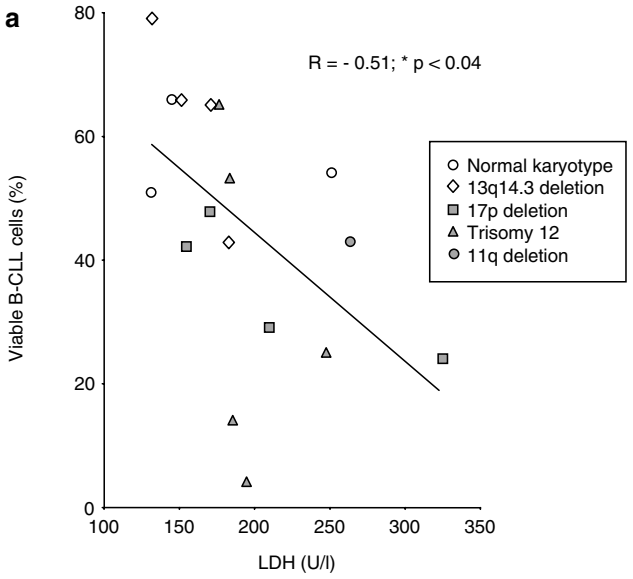


Figure 2 Correlation of basal Bcl-2 expression with cytogenetic status of B-CLL cells. Bcl-2 expression of 10 different B-CLL samples was determined using intracellular staining and flow cytometry. All experiments were run in duplicate, and standard deviation within the same sample was lower than 5% in each case. The average Bcl-2 expression (*y*-axis) in B-CLL with good and poor prognosis cytogenetics is compared. Error bars indicate s.e.m.



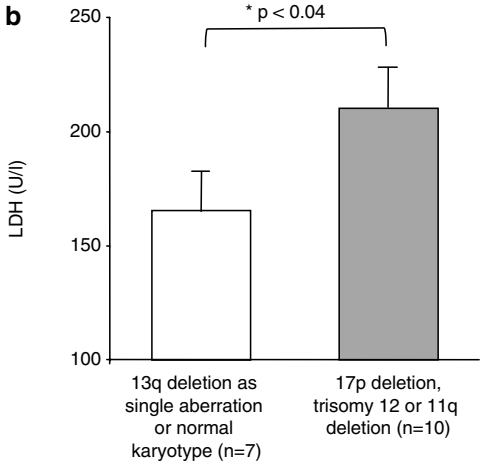


Figure 3 Correlation of serum LDH levels with spontaneous B-CLL cell survival and cytogenetics of B-CLL. Survival of B-CLL cells from 17 subjects after 7 days in culture was correlated with the subjects' serum LDH levels at the time the blood was drawn. (a) B-CLL cell viability and LDH levels are negatively and significantly correlated as determined by Pearson's correlation analysis. (b) The average LDH level (*y*-axis) in subjects with good and poor prognosis cytogenetics is compared. Error bars indicate s.e.m.

Based on the known correlation of cytogenetics and clinical prognosis, and the *in vitro* data above, we classified the samples as good prognosis cytogenetics (13q deletion as single aberration and normal karyotype) and poor prognosis cytogenetics (all others) for subsequent analyses. The regulation of B-cell survival is known to be influenced by Bcl-2. We therefore examined whether samples from the two groups varied in expression of Bcl-2. Intracellular staining of cells from 10 subjects demonstrated higher Bcl-2 expression in B-CLL cells with good prognosis cytogenetics than in B-CLL cells with poor prognosis cytogenetics ($^*P<0.01$; Figure 2).

Because serum lactic dehydrogenase (LDH) levels are a clinical prognostic factor in B-CLL, we evaluated whether clinical serum LDH levels from the day the B-CLL cells were obtained correlated with *in vitro* spontaneous survival or cytogenetic status. Interestingly, we found that better spontaneous survival correlated with a lower LDH level in the blood at the time the samples were drawn ($R=-0.51$, $^*P<0.04$; Figure 3a). In addition, we found lower serum LDH levels in samples with good prognosis cytogenetics as compared to samples with poor prognosis cytogenetics ($^*P<0.04$; Figure 3b). Spontaneous B-CLL cell survival *in vitro* did not significantly correlate with other clinical prognostic factors such as white blood cell count, Rai stage, CD38 expression, beta-2-microglobulin level, or platelet count (Table 1 and data not shown).

B-CLL cell expression of molecules involved in T-cell/B-cell crosstalk correlates with cytogenetic status

Unstimulated B-CLL cells are considered poorly immunogenic and appear to induce only limited T-cell responses. Expressions of costimulatory, adhesion, and antigen-presenting molecules are among the factors that determine whether or not antigen-presenting cells can induce a T-cell response. We therefore

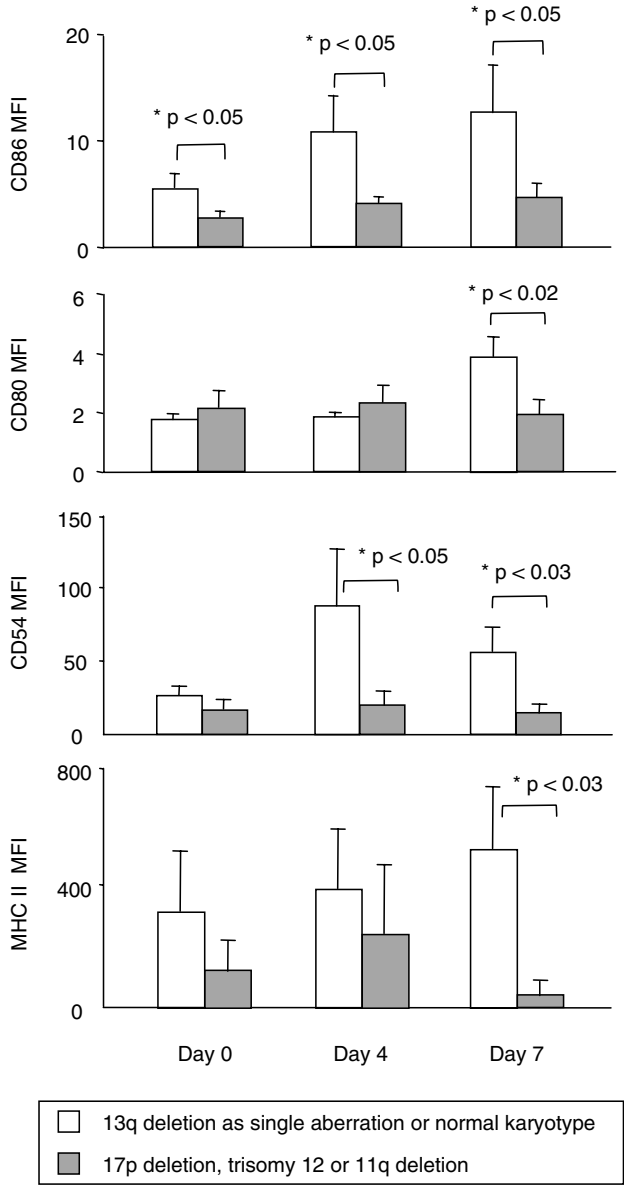


Figure 4 Correlation of costimulatory molecule expression with cytogenetic status of B-CLL cells. B-CLL cells from 11 subjects with poor prognosis cytogenetics and 10 subjects with good prognosis cytogenetics were cultured for up to 7 days *in vitro*. Expression of CD86 ($n \geqslant 10$), CD80 ($n \geqslant 6$), CD54 ($n \geqslant 8$), and MHC class II ($n = 5$) on days 0, 4, and 7 was determined by FACS analysis. All samples studied expressed CD80, CD86, MHC class II, and CD54. The average expressions of these markers on B-CLL cells from different cytogenetic groups are compared. Error bars indicate s.e.m.

evaluated whether CD80, CD86, CD54, and MHC class II expression by B-CLL cells correlated with cytogenetic status. All samples studied expressed CD80, CD86, MHC class II, and CD54. Basal CD86 expression was significantly higher in the group with good prognosis cytogenetics as compared to the group with poor prognosis cytogenetics on day 0 ($^*P < 0.05$; see Figure 4). Basal expression of CD80, CD54, and MHC class II did not differ significantly between samples with good prognosis or poor prognosis cytogenetics on day 0; however, the potential to increase spontaneously the expression of these molecules during culture was particularly strong in the good prognosis cytogenetic samples. This resulted in significantly higher expression of each of these molecules on day 7 in the group with good prognosis cytogenetics as compared to the group with poor prognosis cytogenetics (CD80: $^*P < 0.02$; CD86: $^*P < 0.05$; CD54: $^*P < 0.03$; MHC II: $^*P < 0.03$).

Allogeneic T-cell response to B-CLL cells depends on their cytogenetic status

Based on these findings, we hypothesized that the capacity of B-CLL cells to stimulate T cells might also depend on their cytogenetic status. We therefore studied the ability of B-CLL cells (CD19 + cells) to stimulate T cells in a mixed lymphocyte culture (MLC). It is known that patients with B-CLL can have variable degrees of T-cell dysfunction. Because we wished to evaluate the immunostimulatory capacity of B-CLL cells (rather than the ability of an individual subject's own T cells to respond), we utilized responder T cells from normal donors in an allogeneic system. The studies involved three B-CLL samples with 13q deletion as single aberration, three samples with trisomy 12, and one sample with a 17p deletion. In the absence of stimulator cells, no T-cell proliferation was observed. As illustrated in Figures 5a and b, the B-CLL samples with 13q deletion induced greater proliferation of T cells compared to samples with poor prognosis cytogenetics.

Discussion

As outlined above, B-CLL is characterized by the slow accumulation of a clonal population of malignant B-lymphocytes, thought to result from defective apoptosis resulting in prolonged survival. In contrast, spontaneous apoptosis occurs rapidly in most, but not all, B-CLL samples that are cultured *in vitro*. Survival of cells *in vivo* and apoptosis *in vitro* have been attributed to a variety of external humoral[27–32] and cellular[28,33–35] factors. Less is known about how cytogenetic status and other endogenous factors within the B-CLL cells themselves impact on cell survival. The cytogenetic status of B-CLL cells is known to impact on the expression of a number of molecules that play a central role in B-CLL growth, survival, and apoptosis (Kienle *et al*, Annual Meeting of the American Society of Hematology, 2003; 102: 187a–188a; abstract). However, little has been reported to date regarding the impact of cytogenetic status on the immunological and biological behavior of B-CLL cells. Such information is important, since it may help us understand why the prognosis of B-CLL varies with chromosomal status and could lead to the development of new therapeutic approaches for different B-CLL groups. The work described here represents an early step toward understanding the biologic behavior of B-CLL with different chromosomal patterns, and demonstrates that cytogenetic status indeed correlates with the *in vitro* biological behavior of B-CLL including both spontaneous apoptosis and immunogenicity.

If spontaneous apoptosis of B-CLL cells plays an important role in determining disease progression, and *in vitro* apoptosis corresponds to *in vivo* cell death, we would expect a lower rate of spontaneous apoptosis, and more viable cells, in samples with poor prognosis cytogenetics. On the other hand, it is known that a high level of serum LDH, which is a measure of tumor burden and turnover, is associated with rapid disease progression and worse clinical prognosis in B-CLL.[2,36,37] In our studies, spontaneous *in vitro* apoptosis was higher in samples with poor prognosis cytogenetics. In addition, spontaneous

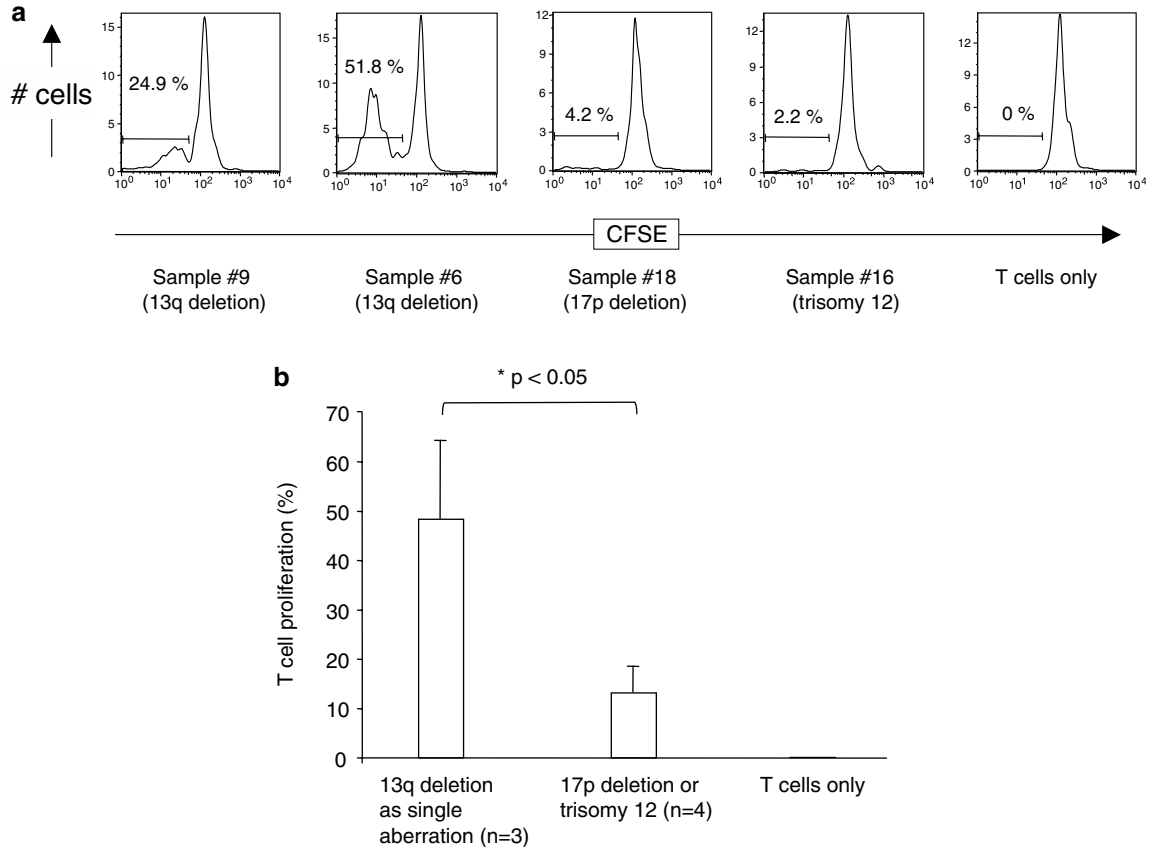


Figure 5 T-cell proliferation in allogeneic B-CLL-cell/T-cell cocultures. CD3 + T cells from a buffy coat of a healthy blood donor were isolated by negative selection using magnetic beads. These allogeneic CD3 + T cells were CFSE-labeled and cocultured with B-CLL cells (CD19 + cells) from seven subjects at a stimulator:responder ratio of 1:5. CFSE fluorescence of T cells on day 5 was analyzed using flow cytometry. All cocultures were run in duplicate, and standard deviation was <5% in each case. Similar results were obtained with allogeneic T cells from a different donor. (a) CFSE histograms of four different samples and controls with T cells only. Percentages given indicate CFSE-low T cells, that is, T cells that have undergone proliferation. (b) Average T-cell proliferation of cells added to B-CLL cell samples with good and poor prognosis cytogenetics. Error bars indicate s.e.m.

apoptosis *in vitro* correlated with higher clinical levels of serum LDH, suggesting that the *in vitro* observation of higher cell death is not simply artifact, but is likely occurring *in vivo* as well. Taken together, the finding that poor prognosis cytogenetics is associated with a higher rate of spontaneous apoptosis *in vitro*, and with elevated LDH *in vivo*, suggests that such patients have a greater degree of B-CLL cell neogenesis (with higher turnover), and resistance to apoptosis may not be the primary explanation for their poorer prognosis. One molecule known to play an important role in determining the sensitivity of cells to apoptosis is Bcl-2. As would be expected, we found higher levels of Bcl-2 in samples with low spontaneous apoptosis. In contrast, the correlation of high Bcl-2 with good prognosis cytogenetics was unexpected given the association between Bcl-2 expression and inhibition of apoptosis.[6,7,38] Again, this points to more rapid turnover, and not resistance to apoptosis, as being a key factor in the poor prognosis associated with certain cytogenetic patterns.

Another key finding of our study was that expression of molecules involved in T-cell/B-cell crosstalk and the ability of B-CLL cells to induce proliferation of allogeneic T-cells in an MLC were higher in B-CLL samples with good prognosis cytogenetics compared to B-CLL cells with poor prognosis cytogenetics. This raises the possibility that immune responsiveness to B-CLL plays a significant role in slowing disease progression in subjects with good prognosis B-CLL, whereas evasion from immune surveillance could be a major factor contributing to disease progression in subjects with poor prognosis B-CLL. There may also be a connection between immunogenicity and proliferation. More rapidly dividing cells (ie those with poor prognosis cytogenetics) might have additional mutations that result in a higher plasticity of their antigenic pattern or that contribute otherwise to resistance to an active immune response. Further studies are needed to evaluate these hypotheses. These studies should include evaluation of whether immunogenicity decreases as B-CLL progresses across the spectrum of cytogenetic groups.

Our studies, and the conclusions we are able to reach from them, are limited by a number of factors. We divided our samples into good and poor prognosis groups based on cytogenetics, but we do not have information on mutational status or adequate follow-up on these subjects to know their actual clinical course. It will be interesting to see whether *in vitro* apoptosis or immunogenicity does, indeed, correlate with clinical outcome. Our approach to measuring sensitivity to apoptosis (by culturing cells *in vitro*) and immunogenicity (by measuring allogeneic response) is clearly imperfect, and future studies will need to focus on evaluating more physiologic apoptotic systems and autologous T-cell responses. Despite these limitations, we believe that the studies outlined above provide evidence for two basic hypotheses related to B-CLL that

deserve further evaluation. First, that poor prognosis cytogenetics in B-CLL is not associated with resistance of the cells to apoptosis, but to more rapid cell turnover, and second, that B-CLL immunogenicity could play an important role in determining prognosis.

In conclusion, the results outlined above confirm that cytogenetic status correlates with the biological behavior of B-CLL *in vitro*. Poor prognosis cytogenetics was associated with more rapid spontaneous apoptosis *in vitro* and lower immunogenicity, while good prognosis cytogenetics was associated with less spontaneous apoptosis, higher Bcl-2 levels, and stronger immunogenicity. While *in vitro* culture undoubtedly introduces significant artifact, our results suggest that differences in prognosis associated with cytogenetic status may not be due solely to a tendency to undergo apoptosis. Other factors such as immunogenicity may also play a role.

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Supplementary Information

Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu).

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