that malignancy increased its expression.[9] Despite the extensive research performed, there is little evidence that either supports or refutes the role of fragile sites in cancer. Our results suggest that the origin of Ph[1] chromosome is independent of spontaneous instability or fragile site expression, because bands 9q34 or 22q11 were not observed in our series either spontaneously or by induced breakage. The proximity between BCR and ABL genes in specific cell cycle phases may explain the genesis of the translocation.[10] The increased chromosome instability affecting specific bands could be a systemic manifestation or a consequence of the leukemic process, possibly due to certain unknown clastogenic factors of the neoplastic cells.

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Key words

Chromosome instability, spontaneous breakage, fragile site expression, chronic myeloid leukemia.

Funding

This work was supported by grants from the National Agency of Scientific and Technological Promotion (ANPCyT), the National Research Council (CONICET) and the National Academy of Medicine, Argentina.

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References

1. Limoli CL, Kaplan M I, Corcoran J, Meyers M, Boothman DA, Morgan W F. Chromosomal instability and its relationship to other end points of genomic instability. Cancer Res 1997; 57:5557-63.
2. Ribas M, Miro R, Gelabert A, Egozcue J. Chromosome instability in lymphocytes from two patients affected by three sequential primary cancers: the role of fragile sites. Cancer Genet Cytogen 1999; 110:133-5.
3. Kolialexi A, Mavrou A, Tsenghi C, et al. Chromosome fragility and predisposition to childhood malignancies. Anticancer Res 1998; 18:2359-64.
4. Fundia AF, Larripa IB. Coincidence in fragile site expression with fluorodeoxyuridine and bromodeoxyuridine. Cancer Genet Cytogen 1989; 41:41-8.
5. Huret JL, Tanzer J, Henry-Amar M. Aberrant breakpoints in chronic myelogenous leukaemia; oncogenes and fragile sites. Hum Genet 1986; 74:447-8.
6. De Braekeleer M. Aberrant breakpoints in chronic myelogenous leukemia: oncogenes and fragile sites. Hum Genet 1988; 78:199.
7. Stopera SA, Ray M, Riordan D, Christie N, Wickstrom D. Variant Philadelphia translocations in chronic myeloid leukemia: correlation with cancer breakpoints, fragile sites and oncogenes. Cancer Lett 1990; 55:249-53.
8. Heim S, Billström R, Kristoffersson U, Mandahl N, Strömbeck B, Mitelman F. Variant Ph translocations in chronic myeloid leukemia. Cancer Genet Cytogen 1985; 18:215-27.
9. Morgan R, Morgan SS, Hecht F. Enhanced expression of chromosome fragile site 10q25 in chronic myelogenous leukemia. Am J Med Genet 1985; 21:347-50.
10. Neves H, Ramos C, da Silva MG, Parreira A, Parreira L. The nuclear topography of ABL, BCR, PML and RARa genes: evidence for gene proximity in specific phases of the cell cycle and stages of hematopoietic differentiation. Blood 1999; 93:1197-207.

Vascular endothelial growth factor isoforms 121 and 165 are expressed on B-chronic lymphocytic leukemia cells

We used flow cytometry to analyze the expression of vascular endothelial growth factor (VEGF) on leukemic cells of 11 B-CLL patients using a monoclonal antibody directed against the 121 and 165 isoforms. All patients tested displayed a positive reaction for VEGF. Interestingly, mean fluorescence intensity (MFI) of cases with a progressive pattern of disease was higher than MFI of patients with stable disease. Cellular VEGF-expression may be involved in disease progression.

Sir,

An increasing body of evidence has been accumulated which suggests a central role for angiogenesis in the pathophysiology of hematopoietic malignancies.[1-6] Although most information comes from patients with multiple myeloma (MM) it has been recently shown that acute myeloid leukemia (AML) cells express vascular endothelial growth factor (VEGF), a potent inductor of angiogenesis.[7] Furthermore, elevated levels of basic fibroblastic growth factor (b-FGF) were detected in the urine of patients with acute lymphoblastic leukemia (ALL) and associated with increased bone marrow microvessel density.[6] In B-cell chronic lymphocytic leukemia (CLL), evidence for increased angiogenesis has been demonstrated by Kini *et al*.[8] and clinico-prognostic implications of of such a feature have been investigated by our group in a series of CLL patients with early disease.[9]

With respect to the source and mechanisms of production of serum VEGF in CLL much remains unproven. Chen *et al*.,[10] on the basis of results obtained in RT-PCR and Slot-blot analysis, showed that B-CLL cells express VEGF m-RNA and identified in VEGF 121 and VEGF 165 the two isoforms produced. We studied 11 B-cell CLL patients using flow cytometry and a monoclonal antibody anti-VEGF whose specificty covered

Table 1.

Pts	VEGF⁺/CD19⁺	VEGF (MFI)	Disease-status
1	65	143	PD after FLUDA
2	72.1	129	PD after FLUDA
3	86.4	108	PD after CLB
4	60.2	99	SD (Stage A)
5	56.1	105	SD (Stage A)
6	51	128	SD (Stage A)
7	48	144	PD after CLB
8	97.7	134	PD after FLUDA
9	97.3	148	SD (Stage A)
10	70.5	156	PD after CLB
11	37.2	109	SD (Stage A)

MFI = mean fluorescence intensity; PD = progressive disease; SD = stable disease.

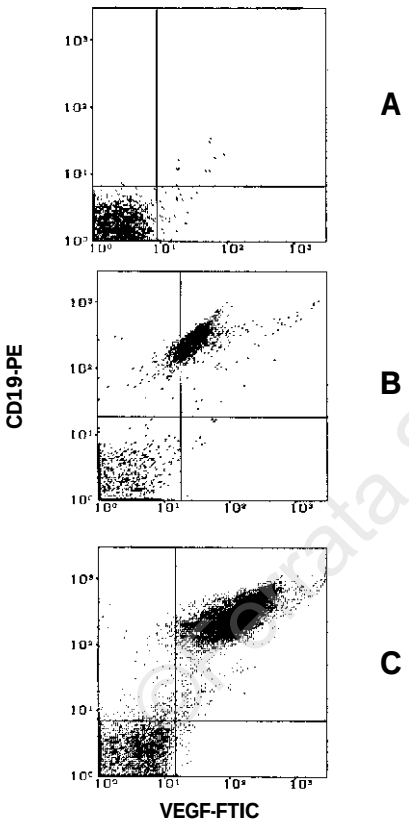

Figure 1. VEGF-FTIC / CD19-PE double staining: A) negative control; B) patient with stable disease; C) patient with progressive disease.

either the 165 or 121 isoforms (Clone, 26503; mouse IgG 2b; R & D Systems Inc.). In all instances diagnosis of typical B-cell CLL relied on either cytomorphologic or immunologic analysis ($CD5^+$, $CD23^+$, $CD22^{+/-}$, $FMC7^-$, $CD79b^-$ and dim light chain Sm Ig expression). All experiments were carried out in double staining according to previously reported methods.[9]

All patients tested displayed a positive reaction for VEGF (Table 1) and the percentage of leukemic cells reactive to VEGF ranged between 37.2% and 97% (median, 62.5%). Interestingly, mean fluorescence intensity (MFI) was higher in patients with a progressive pattern of disease than in patients with stable disease at the time of sampling (median MFI, 138.5 versus 109; p=0.170) (Figure 1).

In lymphoma and in other types of human cancers neoplastic cells produce VEGF, although several accessory cells such as macrophages, peripheral blood T-lymphocytes and platelets also contain appreciable amounts of VEGF.[1] Fiedler *et al*.[5] have shown that AML cells express VEGF as well as VEGF receptors (VEGFR-1 and VEGFR-2). These findings raise the possibility that VEGF may play a role as an autocrine growth factor for AML cells. Bellamy *et al*.[4] studied different hematopoietic cell lines and found that they all expressed VEGF, whereas only 50% of them expressed b-FGF. In B-cell CLL studies dealing with either cellular or soluble VEGF are limited.[8-10] Our results, though based on a small number of patients, lend further support to previous studies suggesting a role of angiogenesis in CLL. Interestingly, the VEGF density changed as a function of disease status, thus supporting a potential involvement of such a protein in the mechanisms of disease progression. Finally, the flow cytometric identification of the isoforms produced raises the possibility of using specific angiogenesis inhibitors as a novel therapeutic stategy for CLL.

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Key words

Chronic lymphocytic leukemia, VEGF-expression, disease-progression.

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References

1. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other diseases. Nat Med 1995; 1:27-31.
2. Vacca A, Ribatti D, Roncali L, et al. Bone marrow angiogenesis and progression in multiple myeloma. Br J Haematol 1994; 87:503-8.
3. Vacca A, Ribatti D, Roncali L, Dammacco F. Angiogenesis in B cell lymphoproliferative diseases. Biological and clinical studies. Leuk Lymphoma 1995; 20:27-38.
4. Bellamy WT, Richter L, Frutiger Y, Grogan TM. Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. Cancer Res

1999; 59:728-33.
5. Fiedler W, Graeven U, Ergun S, et al. Vascular endothelial growth factor, a possible paracrine growth factor in human acute myeloid leukemia. Blood 1997; 89:1870-5.
6. Perez-Atayde A, Sallan S, Tedrow U, Connors S, Allred E, Folkman J. Spectrum of tumor angiogenesis in the bone marrow of children with acute lymphoblastic leukemia. Am J Pathol 1997; 150:815-21.
7. Hussong JW, Rodgers GM, Shami PJ. Evidence of increased angiogenesis in patients with acute myeloid leukemia. Blood 2000; 95:309-15.
8. Kini AR, Peterson LC, Kay NE. Evidence for abnormal angiogenesis in the bone marrow of patients with B-cell chronic lymphocytic leukemia. Blood 1998; 92 (Suppl 1):717a.
9. Molica S, Vitelli G, Levato D, Gandolfo GM, Liso V. Increased serum levels of vascular endothelial growth factor predict risk of progression in early B-cell chronic lymphocytic leukaemia. Br J Haematol 1999; 107: 605-10.
10. Chen HJ, Treweeke AT, Till KJ, et al. Vascular endothelial growth factor (VEGF) is produced by CLL cells and stimulates angiogenesis within lymphoreticular tissues. VIII International Workshop on CLL; Paris 29-31 October 1999; Abstract Book, pag. 43.

Expansion of CD3+CD56+ cytotoxic cells from patients with chronic lymphocytic leukemia: *in vitro* efficacy

Cytokine-induced killer cells were expanded from 12 patients with chronic lymphatic leukemia. In these cultures, T-cells increased significantly from less than 10% to 56.3± 29.4% after 14 days. Similarly, the percentage of cells expressing the natural killer-cell marker CD56 increased significantly to 31.8±26.3%.

Sir,

Cytokine-induced killer (CIK) cell cultures were generated from 12 patients with chronic lymphatic leukemia (CLL) and assayed for their expression of various cell surface markers by flow cytometry. On day 0 all patients had at least 90% CD19 positive lymphocytes in their blood. After two weeks of culture CD19 positive cells had decreased significantly to 33.3±30.5% with the range being 1.5 and 78.6% (p = 0.02; Figure 1). In contrast, fewer than 10% of the lymphocytes were $CD3^+$ on day 0 of culture. Expression of CD3 increased to 56.3±29.4% after two weeks of culture (p = 0.03). Similarly, CD8 positive cells increased to 53.8±31.4% after two weeks (p = 0.08). The percentage of CD56 positive cells increased significantly to 11.0±11.1% after one week of culture and to 31.8±26.3% after two weeks (p = 0.01; Figure 1). CD56 positive cells co-expressed CD3. Next, we tested the cytotoxic activity of CIK cells using a ^{51}Cr release assay. Fourteen-day old CIK cells were tested using autologous or allogeneic leukemia cells as targets. In the autologous setting CIK cells were unable to lyse leukemia cells. However, CIK cell lysis could be increased by addition of anti-CD3 monoclonal antibody. Addition of anti-CD32 antibody did not abolish this effect. In contrast, addition of anti-CD19 antibody did not produce an increase in cytotoxicity. In the allogeneic setting CIK cells showed a weak cytotoxic effect on leukemia cells. Again, this effect could be increased by addition of anti-CD3 antibody. This effect was not abolished by addition of anti-CD32 (Figure 2).

CLL cells are resistant to T-lymphocytes.

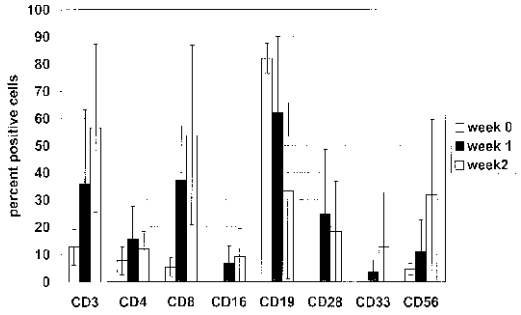

Figure 1. Flow cytometric analysis of various cell surface markers on CIK cell cultures derived from CLL patients. Expression was analyzed by flow cytometry as described elsewhere.[3] Data are shown as the mean from twelve separate experiments. Please note that data on day 0 are derived from three patients only and that CD16, CD28 and CD33 were not determined on day 0.

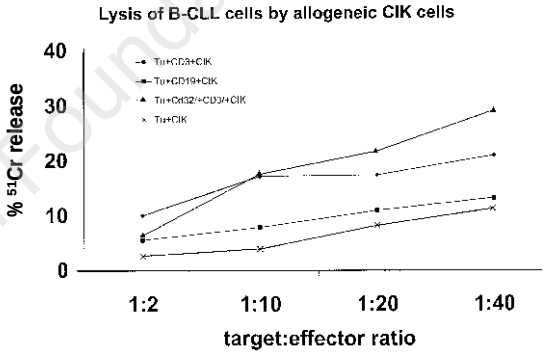

Figure 2. Cytotoxic activity of CIK cell cultures derived from CLL patients. CIK cells were generated from CLL patients as described before.[4-7] After 14 days of culture, cells were assayed in a ^{51}Cr release cytotoxic assay with or without addition of anti-CD3 monoclonal antibody at various effector to target cell ratios in an allogeneic setting. In the control experiment anti-CD19 antibody was used instead of anti-CD3. In addition, anti-CD32 was added to anti-CD3 in some experiments. Results shown represent data from two separate experiments. Data are shown as means.