fetal erythroblasts exceeded the density of adult erythroblasts in adult spleen. These findings differ from our results, in which the numbers of F-blasts were consistently less than the numbers of A-blasts. Specificity of the antibody against HbF may have differed between the studies. We also speculate that in our study the proportion of F-blasts might have been underestimated, because there may be a lot of GPC-positive cells that do not show a detectable level of hemoglobin label.

In conclusion, HbF production is increased during EMH but is not restricted to fetal erythropoietic organs. An extramedullary hematopoietic milieu does not necessarily impose HbF synthesis in adulthood.

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Altered constitutive and activation-induced expression of CD95 by B- and T-cells in B-cell chronic lymphocytic leukemia

Expression of CD95, a molecule involved in activation-induced cell death (AICD), might contribute to explain accumulation of leukemic B-cells and functional impairment of T-cells in B-cell chronic lymphocytic leukemia (B-CLL). Therefore, we compared constitutive and activation-induced expression of CD95 and CD69 by B- and T-cells in CLL patients and in healthy donors.

We examined 19 untreated B-CLL patients (13M, 6F; mean age 66.5 years) and 14 sex- and age-matched healthy subjects by using murine monoclonal antibodies conjugated to FITC, PE or PerCP and specific for CD3, CD19, CD5, CD23, CD10, CD69, CD95, CD38, CD25, CD71, CD11c, CD80, CD86 and κ/λ immunoglobulin light chains (Becton Dickinson, Mountain View, CA, USA). Staining was performed both on fresh whole blood samples and on cultured isolated peripheral blood mononuclear cells (PBMCs). Multiparametric analysis was performed on a FACScan cytometer equipped with CellQuest software® (Becton Dickinson). The percentages of cells and their mean fluorescence intensity (MFI) were calculated on *gated* $CD19^+$ or $CD3^+$ lymphocytes coexpressing CD69[1] or CD95.[2]

Isolated PBMCs from nine B-CLL patients and nine healthy donors were cultured for 24-72 hrs in complete medium (RPMI 1640 with 10% fetal bovine serum, antibiotics, L-glutamine), with or without PMA (5 ng/mL) + ionomycin (250 ng/mL), and then FACS-analyzed. The Student's t-test was used for analysis of differences in surface molecule expression in the two examined groups; linear regression analysis was used to correlate CD95 and CD69 expression.

Leukemic B-cells were positive for CD19, CD5, CD23, with poor expression of surface Ig, negative for CD10, and variably positive for CD38, CD25, CD71, CD11c, CD80 and CD86.[3,4]

As shown in Figure 1, freshly examined B-cells from CLL patients expressed more CD69 than those from controls (%= 26±20.7 vs 5±2.3, p=0.005; MFI=215±61 vs 147±17, p=0.003), while no significant difference was observed in CD95 expression. After 24-hr-stimulation with PMA + ionomycin, normal B-cells showed a significantly correlated (r=0.802; p=0.017) increase in CD69 (%=99.2±0.6 vs 21.4±5.9, p<0.001; MFI=614±74 vs 204±39, p<0.001, in stimulated vs unstimulated cultures, respectively) and CD95 expression (%=73.8±14.9 vs 20.7±7.6, p<0.001; MFI=386±54 vs 198±46, p<0.001), with no further increase at 72 hours. In contrast, PMA + ionomycin-stimulated CLL B-cells displayed a marked increase in CD69 (%=92.8±16.4 vs 46.4±33.5, p=0.01; MFI=544±119 vs 284±95, p=0.001, in stimulated vs unstimulated cultures, respectively), but not in CD95 expression (%=26.2±16.6 vs 14.5±13.8, p>0.05; MFI= 227±57 vs 180±57, p>0.05) after 24 hrs of culture. These results demonstrate a constitutively higher CD69 expression on B-cells in CLL patients compared to in normal subjects, while, in contrast with what is commonly assumed,[5] and in agreement with Molica *et al.*,[6] no significant difference was observed in CD95 constitutive expression. However, after activation, while no difference was observed in CD69 upregulation, CD95 expression was significantly lower in CLL B-cells compared to in B-cells from normal subjects (p<0.001). No difference was observed in activation-induced CD69 expression.

Figure 2 shows that T-cells in CLL-patients constitutively expressed more CD69 (%=8.0±5.7 vs 3.0±1.3, p=0.01; MFI=197±20 vs 175±17, p=0.02) and CD95 (%=82.6±14.1 vs 59.3±9.3, p=0.001; MFI=415±59 vs 341±38, p=0.009) than con-

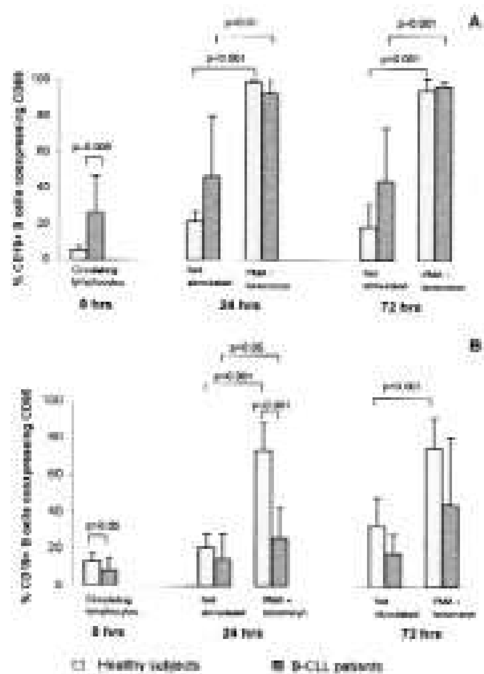

Figure 1. Constitutive (0 hrs) and *in vitro* (24 and 72 hrs) expression of CD69 (A) and CD95 (B) by CD19+ B-cells from normal donors (n. = 9) and B-CLL patients (n. = 9).

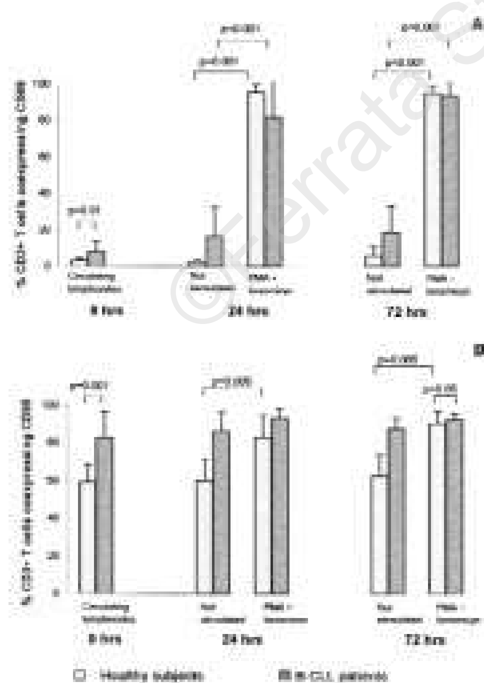

Figure 2. Constitutive (0 hrs) and *in vitro* (24 and 72 hrs) expression of CD69 (A) and CD95 (B) by CD3+ T-cells from normal donors (n. = 9) and B-CLL patients (n. = 9).

trol T-cells.

In unstimulated cultures, T-cells from normal subjects and B-CLL patients did not show significant changes in CD69 and CD95 expression after 24 and 72 hrs. However, the higher CD69 and CD95 expression on T-cells from B-CLL patients remained confirmed. In PMA + ionomycin-stimulated cultures, T-cells from normal subjects and CLL patients showed a significant increase in CD69 expression compared to unstimulated cells (%= 95.5±4.1 vs 2.5±0.9, $p<0.0001$; MFI=655±96 vs 163±16, $p<0.0001$, for normals); (%= 81.8±30.2 vs 16.0±16.0, p=0.001, MFI= 519±147 vs 225±57, p=0.001, for B-CLL patients).

T-cells stimulated for 24 hrs from normal subjects had stronger CD95 expression than unstimulated T-cells (%=83.0± 11.4 vs 60.3±11.2, p=0.005; MFI= 402±44 vs 342±40, p=0.03), whereas T-cell expression, which was constitutively upregulated on T-cells from B-CLL patients, did not further increase after stimulation (%=93.2±4.8 vs 86.4±9.9, p=n.s.; MFI=466±28 vs 433±42, p=n.s.). The significant difference in CD95 expression observed on freshly analyzed T-cells in the two groups remained up to 24 hrs of culture (p=0.04 for percentage; p=0.006 for MFI) but was lost after 72 hrs.

In conclusion, the presented data demonstrate that T- and B-cells in CLL constitutively expressed more CD69 than their normal cell counterparts, while CD95 expression did not significantly differ in the two groups. However, the *in vitro* activation-induced CD95 upregulation shown by normal B-cells was strongly impaired in leukemic B-cells. Furthermore, constitutive CD95 expression was significantly higher on T-cells in CLL than on cells from healthy subjects and could not undergo further upregulation after *in vitro* activation.

These abnormalities in CD95 expression by B- and T-cells in B-CLL might contribute to explain prolonged survival of leukemic B-cells[7] and functional defects of T-cells.[8,9] We suggest that in CLL B-cell molecular mechanisms leading to CD95 upregulation and AICD might be defective.[10] Concomitantly, in B-CLL patients, residual T-cells might be chronically activated by the expanding B-cell clone, upregulate CD95, and undergo AICD more easily and rapidly.

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Key words: B-CLL, CD95, CD69, T-cells, B-cells, apoptosis.

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Advantages of using thalidomide for the management of patients with refractory myeloma

A group of 11 heavily pretreated patients receiving low-dose thalidomide was compared with a similar group of 10 patients with refractory myeloma treated with a conventional oral chemotherapy. This study shows that thalidomide is not only effective in controlling the neoplastic clone but moreover, thanks to its low toxicity, allows out-patient management of these subjects.

Thalidomide has recently been adopted in the treatment of refractory multiple myeloma patients. From 30 to 50% of patients achieve a response at the dose of 200-800 mg/day.[1-3] Other studies report that thalidomide is also effective at lower dosages (50-100 mg/day) with fewer side-effects.[4-6]

We retrospectively compared two similar groups of patients with refractory myeloma treated with low-dose thalidomide (group I) or with the oral combination regimen CAVD (CCNU, alkeran, vepeside, dexamethasone) (group II). The aim of the study was to evaluate the impact of thalidomide on the management of refractory myeloma patients.

Group I was formed of 11 patients enrolled in a study whose criteria of inclusion were: age less than 75 years, at least two previous lines of therapy, kidney and liver-function tests no more than twice the upper limit of normal levels, no evidence of neuropathies, life expectancy of at least 6 weeks. Thalidomide, kindly supplied by Grünenthal (Aachen, Germany), was administered at a starting dose of 100 mg and escalated, according to tolerance, after two weeks to 200 mg /day.

The dosage of 200 mg/day was reached by all patients, but only 5 maintained this dose for more than 3 months. To improve control of symptoms, four patients also received dexamethasone at the dosage of 20 mg/day for two days every two weeks for the first two months.

Group II comprised 10 patients treated with the oral regimen CAVD, conceived in order to manage them as outpatients. Inclusion criteria were the same as those adopted for the group receiving thalidomide except for presence of neuropathies. The schedule was: CCNU (80 mg/m^2 *per os*) day 1, and melphalan (5 mg/m^2/day *per os*), VP16 (60 mg/m^2/12h *per os*), dexamethasone (8 mg/day *per os*) for 5 days. The chemotherapy was repeated every 4-6 weeks.

The characteristics of the patients of both groups, recorded at

Table 1. Characteristics of MM patients at starting thalidomide or CAVD treatment, and type of response to therapy.

	Thalidomide	*CAVD*
No. of patients	11	10
Median age (range)	59 (52-67)	61 (46-76)
Hb <9 g/dL	6/11 (54%)	3/10 (30%)
Bone marrow plasma cells >50%	6/11 (54%)	6/10 (60%)
β2 microglobulin >3 mg/L	9/11 (82%)	8/10 (80%)
>3 sites of osteolysis	3/11 (27%)	7/10 (70%)
Previous therapy:		
Conventional	7/11 (64%)	5/10 (50%)
High-dose	4/11 (36%)	5/10 (50%)
Type of response		
CR	1/11 (9%)	–
PR	4/11 (36%)	6/9 (66%)
MR	6/11 (55%)	–
NR	0	3/9 (33%)

Table 2. Therapy-related toxicity in patients treated with thalidomide or CAVD.

	Thalidomide	*CAVD*
Need for hospitalization	1/11 (9%)	7/10(70%)
Grade 3-4 infections	1/11 (9%)	3/10(30%)
Transfusion requirement	0	4/10(40%)
Grade 3-4 extra-hematologic toxicity	1/11 (9%)	0
Grade 4 therapy-related neutropenia	0	5/10 (50%)

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