higher in less differentiated cells,[6] and document that the production of sCD44 follows an inverse pattern. The expression of CH44H correlates with cell binding to HA, since KG1a and KG1 (see the legend to Table 1) but not HL-60 cells, adhere to immobilized and soluble HA. The effect of HA on CD44H expression, cell proliferation and differentiation was analyzed after exposing cells (for up to 4 days) to hyaluronan. As seen in Table 1,B, HA exposure resulted in a modest increase in the expression of tmCD44H in KG1a, but not in KG1 and HL-60 cells. In turn, production of sCD44 was greatly increased (13-fold) in KG1a, but not in KG1 or HL-60 cells. The effect of HA on cell proliferation is shown in Figure 1. While HA decreased the proliferation of KG1a in a time-dependent mode, it was without effect in KG1 cells. Moreover, cell cycle studies revealed for KG1a, but not for KG1 cells, a HA-dependent increase in the fraction of cells at Go/G1, with a concomitant decrease in the fraction of active proliferating (S+G2/M) cells (Figure 1, inset). In these cells, differentiation (as judged by morphologic and histochemical analysis) does not occur after HA-exposure. In HL-60 cells, HA does not affect proliferation, cell cycle parameters or differentiation.

Our results show that in immature progenitors (KG1a), hyaluronan modulates the level of transmembrane and soluble CD44H in a fashion which resembles the effect elicited by anti-CD44 monoclonal antibodies.[7] Concomitantly, cell proliferation is decreased. The modulatory effect of HA seems to require a specific CD44H-hyaluronan interaction, since other CD44 ligands,[8] such as chondroitin sulfate or heparin were found to be ineffective. Moreover, as expected HA regulates neither transmembrane nor soluble expression of CD54 in KG1a cells. In myeloid cells, the latter is modulated by cytokines.[9] Thus, in immature myeloid progenitors, the interaction of CD44H with its ligand not only delineates a specific cell-matrix adhesive system,[1] but a signal to diminish cell proliferation.

Carlos J. Moreno, María J. Epuñan,° José J. Minguell°*

**Department of Immunology, King's College School of Medicine & Dentistry, Bessemer Road, London SE5 9PJ, London, UK; °Unidad de Biología Celular, INTA, Universidad de Chile, Santiago, Chile*

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Correspondence

José J. Minguell, Ph.D., Unidad de Biología Celular, INTA, Universidad de Chile, Casilla 138, Santiago 11, Chile. Phone: international +56-2-6781400 – Fax: international +56-2-2214030 – E-mail: jminguel@uec.inta.uchile.cl

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Isolated asymptomatic severe neutropenia as the presentation of myeloid/natural killer cell acute leukemia

Myeloid/natural killer cell acute leukemia is a poorly known hematologic malignancy. We describe a case presenting as isolated severe asymptomatic neutropenia, and show the evolution of flow cytometry markers in two separate bone marrow samples.

Sir,

CD56+ malignancies include some distinct clinical entities that have been recently characterized.[1] Some diseases[1-4] seem to be of T-cell lineage but others[1,5-7] involve the natural killer cell (NKC) precursors. We present the case of a patient with an unusual myeloid/natural killer cell acute leukemia (M/NKCAL).

Diffuse abdominal pain and mild weight loss led a 75-year old woman to consult her gastroenterologist, who ordered a routine analytic evaluation. The hematologic findings started additional investigations. Total leukocyte count (TLC) was 3.5×10^9/L, absolute neutrophil count (ANC) 0.042×10^9/L, red blood cell count 3.69×10^{12}/L, hemoglobin 122 g/L, hematocrit 36.2%, MCV 97.9 fL, reticulocytes 2.2% and platelet count 174×10^9/L. Routine biochemistry tests and coagulation times were normal; D-dimer was negative. Ten percent of circulating leukocytes were immature, with hypogranular cytoplasm and nuclear irregularity. We found gingival hypertrophy but no lymphadenopathy or hepatosplenomegaly. Abdominal ultrasonography was normal.

Bone marrow (BM) was infiltrated with 80% immature cells with finely granular Sudan black-B staining and atypical promyelocyte appearance. In flow cytometry analysis (Figure 1) 79% of these cells were $CD45^+$ $HLA\text{-}DR^-$ $CD13^+$ $CD33^+$ $CD117^{+\,weak}$, and some also expressed CD56 (22% of BM cells). The malignant clone lacked CD1, CD2, CD4, CD7, CD10, CD11b,

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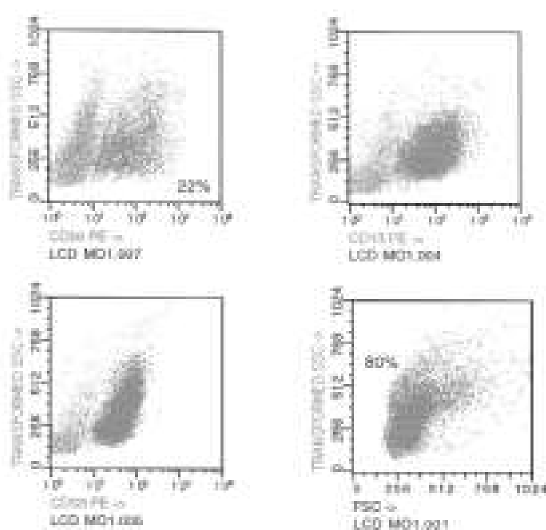

Figure 1. Antigen expression of bone marrow cells at diagnosis.

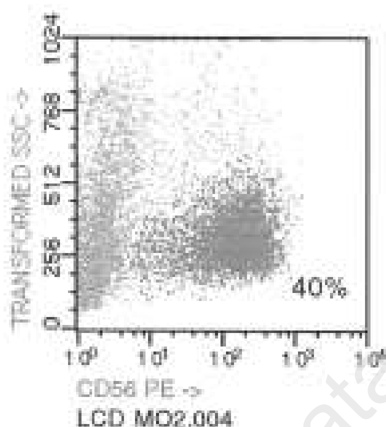

Figure 2. CD56 expression of bone marrow cells at relapse.

CD14, CD15, CD19, CD22, CD34, CD41, CD42b and glycophorin-A. The BM karyotype was normal.

With a reduced-dose acute myeloid leukemia induction schedule, the patient developed subclinical disseminated intravascular coagulation syndrome (DICS) with high titer D-dimer and mild prolongation of coagulation times. After moderate aplasia, there was excellent ANC recovery to normal values. In a new BM sample obtained one month after, flow cytometry (Figure 2) showed that the total immature population ($CD13^+$ $CD33^+$ HLA-DR-) formed 65% of BM cells, but that two-thirds (40% of BM cells) expressed CD56. Neutropenia was again the unique cytopenia present (TLC 2.5×10^9/L with ANC 0.375×10^9/L).

M/NKCAL seems to be a malignant acute proliferation of the NKC precursor. It is relatively frequent and could be misdiagnosed as acute promyelocytic leukemia microgranular variant (APL-M3v)[5,6] because cells have some morphologic and cytochemical properties of atypical promyelocytes. NKC and some myeloid surface antigens are present but HLA-DR and CD34 expression is variable.[7-10] M/NKCAL cells lack T-cell antigens, although some aggressive disorders of $CD56^+$ cells also express them,[3] representing the neoplastic counterpart of γ/δ-TCR^+ cell precursors.

Our patient had unique interesting features. First, the initial presentation as a severe selective neutropenia, as occurs in some chronic T-lymphoproliferative disorders, suggests that NKC and T-$CD56^+$ cells could share a common intermediate precursor. Second, the absence of anemia and thrombocytopenia in the various phases of the disease indicates that NKC precursor effects (cytokines?), and not predominantly BM infiltration, induced the persistent neutropenia. Third, chemotherapy caused subclinical DICS, similar to that occurring in APL-M3v. Fourth, the natural history of the disease or the low-dose chemotherapy provoked a clonal evolution with increasing expression of CD56. Clinical and immunophenotypic data from M/NKCAL patients could yield information about the origin, differentiation and regulatory properties of natural killer cells and their precursors.

Martin Rubio-Batllés, Adela Castañeda de la Mata, Juan Ramón Romero Macias, Antonio Rubio Vitaller**

*Hematology Department, Hospital "Santa Barbara", Puertollano, Ciudad Real; *Flow Citometry Section, Hematology Department, Hospital General, Albacete, Spain*

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Correspondence

Martin Rubio, M.D., Hospital "Santa Barbara", Puertollano, 13500 Ciudad Real, Spain. Phone: Hospital: international +34-926-421100 (Internal phone 486 or 489) or 610-547973 – Fax: international +34-926-431616 – E-mail: mrubiob@aehh.org

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Liposome encapsulated daunorubicin (daunoxome) for acute leukemia

Daunoxome (DNX, Nexstar) was given, as a single agent, to 11 patients with very poor-risk acute leukemia. Pharmacokinetic data were also obtained from 9 of the 11 cases. This small pilot study show that the toxic profile of this liposomal-encapsulated anthracycline is low.

Sir,

Daunoxome (DNX, Nexstar) is a preparation of daunorubicin (DNR) with remarkable physical stability that is encapsulated into small liposomes and is registered for the treatment of AIDS-related Kaposi's sarcoma.[1,2] Since *in vitro* studies have shown that DNX can be at least as effective as free DNR against leukemic cells[3-5] and since DNX is likely to be less toxic than free DNR, we planned a systematic study of DNX in the treatment of acute leukemia. The first part of the study was accomplished by evaluating the effects of DNX alone in 11 patients with very poor-risk acute leukemia (2 cases of advanced blastic phase of chronic myeloid leukemia, 2 cases of acute lymphocytic leukemia (ALL) in 2nd relapse, and 7 cases of ANLL in 2nd or subsequent relapse or primary refractory). The age range of the patients was 39 to 71 years, mean 60±9, median 58.

DNX was given alone, as a single agent, in three doses of 60 mg/m^2 each (days 1, 3 and 5). The infusion was given through a central venous catheter and lasted 1 hour. A complete remission (CR) was obtained in the 2 cases of ALL; one case relapsed after 4 months of unmaintained CR and the other died in CR of a Gram negative septicemia. A partial response with a marked and stable regression of splenomegaly was obtained in the two cases of CML in blastic phase. Both patients were alive 6 and 12 months after treatment. The 7 cases of ANLL failed to achieve CR. Fever developed in 8 of 11 cases. In 4 of 8 cases, the etiology of the fever was bacterial, Gram positive bacteremia in 2 cases, Gram positive and Gram negative bacteremia in 1 case, and fatal Gram negative septicemia in 1 case (after achieving CR). Hospital stay was 15 to 45 days (median 20, range 15 to 45). Blood transfusion support included red cells (median 12 units, range 0 to 25) and platelets (median 3 units, range 0 to 7). Oral mucositis, intestinal toxicity and liver toxicity were not seen. Anti-emetic medications (ondansetron, tropisetron or granisetron) were used prophylactically in 3 cases, but no patient complained of nausea or vomiting.

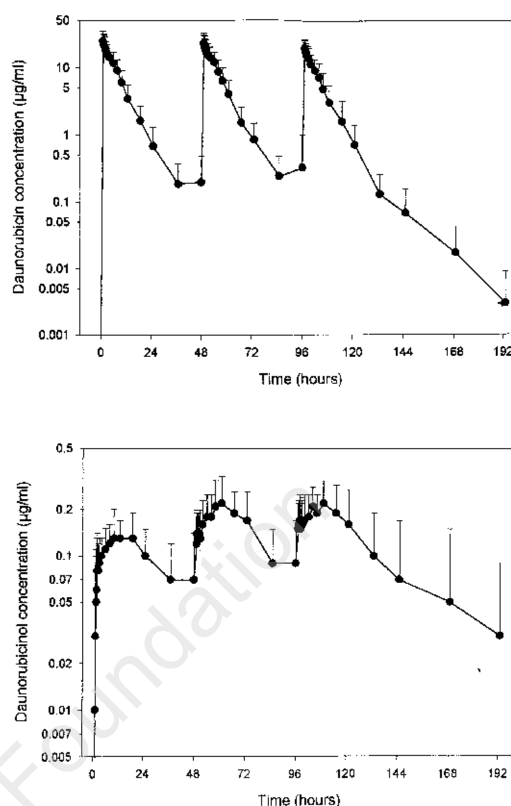

Figure 1. Mean (SD) serum daunorubicin and daunorubicinol concentrations–versus–time profiles following 1-hour i.v. infusion of daunoxome (60 mg/m^2, days 1, 3 and 5).

Pharmacokinetic data were obtained from 9 of the 11 cases by high pressure liquid chromatography. Figure 1 shows plasma concentrations of DNR and its main metabolite, daunorubicinol. The half-life of DNR was 4.65±1.09 hours, with C^{max} ranging between 20 and 25 μg/mL and C^{min} ranging between 0.1 and 0.2 μg/mL. The area-under-the-curve was 472 ±214 μg/mL/h for DNR and 18±8 μg/mL/h for daunorubicinol. All these values are more than a hundred fold higher than those expected with free DNR.[6]

This small pilot trial of DNX alone in patients with very poor-risk leukemia has shown that the toxic profile of this liposomal-encapsulated anthracycline is low, suggesting that it is worth testing higher doses of DNX alone or in combination with other drugs in the treatment of acute leukemia.

Anna Ermacora, Mariagrazia Michieli,* Federico Pea,° Giuseppe Visani,# Alessandro Bucalossi,@ Domenico Russo**

**Division of Hematology and °Institute of Clinical Pharmacology and Toxicology, Udine University Hospital; #Institute of Hematology "L. and A. Seràgnoli", Bologna University Hospital; @Division of Hematology, Siena University Hospital, Italy*