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Expression of transmembrane and soluble forms of CD44H in human myeloid cell lines and its regulation by hyaluronic acid

Using myeloid cell lines, it has been demonstrated that hyaluronan modulates transmembrane and soluble CD44H expression in immature (KG1a) but not mature (KG1) progenitors. Concomitantly, cell proliferation is decreased. This effect suggests that the specific interaction of CD44H with its ligand not only delineates a specific cell-matrix adhesive system, but also a proliferation signal.

Sir,

CD44, a protein involved in many cell to matrix adhesive events, exists as transmembrane (tm) and soluble (s) isoforms. In myeloid cells, CD44H (the common isoform) is the receptor for several ligands, including hyaluronate (HA). HA in addition, is a differentiation and activation signal in a variety of cells.[1-4] Since, marrow stromal cells produce and organize HA for deposit and matrix assembly,[5] myeloid progenitors while in the marrow stroma should be continuously exposed to an HA-enriched environment. By using established myeloid cells lines, expressing defined differentiation patterns (KG1a, KG1 and HL-60), we tested whether cell exposure to HA may affect CD44H expression and release and cause a concomitant effect on cell proliferation and differentiation.

As judged by flow cytometric analysis, the three cell lines uniformly express tmCD44H (Table 1,A); however, variants CD44v4/5 are not expressed and CD44v6 is expressed with only low intensity (MFI=5). In turn, production of sCD44H was lower in KG1a, as compared to KG1 and HL-60 (Table 1, A). Thus, these results confirm that tmCD44H expression is

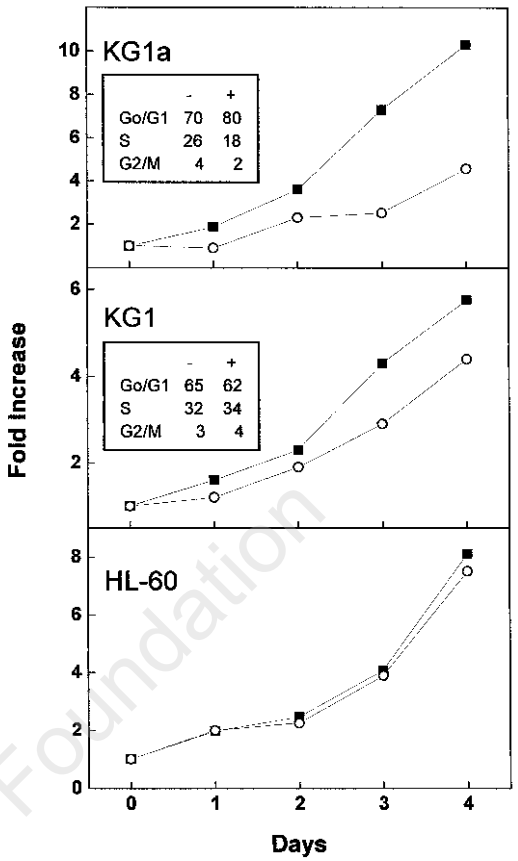

Figure 1. Effect of hyaluronan on proliferation and cell cycle status of myeloid cells. Cells (20×10³ cells/mL) were seeded in culture medium alone (■) or containing 10 µg/mL HA (○). At the indicated culture times, the total number of viable cells was counted and expressed as fold increase over the respective starting cell number, which was set to 1. Data shown are representative of 4 experiments. SEM (not shown), was in all cases less than 5 %. Insets show percentages of control (–) or HA-exposed (+) cells at the indicated cell cycle phase, as determined (day 4) by flow cytometric analysis of DNA content, after propidium iodine and RNAase treatment.

Table 1. Expression of transmembrane and soluble CD44H in myeloid cells and the effect of hyaluronan.

	A: control cells		*B: HA-treated cells*	
	tmCD44 (MFI)	*sCD44 (ng/10⁶ cells)*	*tmCD44* (ratio)*	*sCD44° (ratio)*
KG1a	80	1.3±0.1	1.6±0.5	13.4±1.7
KG1	65	3.7±1.6	1.0±0.4	2.2±0.2
Hl-60	8	5.5±1.4	1.0±0.1	2.0±0.5

*Cells were cultured (3 days) in medium (RPMI 1640 containing 10 % fetal calf serum) (control) or containing 10 µg/mL hyaluronic acid (HA-treated). Transmembrane CD44H (tmCD44) was estimated by flow cytometry after labeling cells with anti-human CD44H or FITC-conjugated mouse IgG_{wm}. Data shown is the mean fluorescence intensity (MFI), which was calculated as the ratio of MFIs for first antibody/control antibody. Soluble CD44H (sCD44) in the culture medium, was measured by an immunoenzymometric assay (Parameter, R&D Systems). Values are mean±SEM for 3 separate experiments performed in duplicate. *Ratio of MFI for HA-treated to control cells. °Ratio of sCD44 produced by HA-treated to control cells. Control cells were also assessed (3) for their capacity to bind to immobilized or soluble hyaluronic acid. For KG1a and KG1 respectively, the values were: to immobilized HA: 39 and 37% (± 10 %); to soluble HA: 3.4 and 4 (relative shift in MFI)*

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.

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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,