Bone Marrow Stromal Cells in Myelodysplastic Syndromes and Acute Nonlymphocytic Leukemia

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A. Introduction

Despite some recent insights into the structural composition and function of hemopoietic stroma, there are still many open questions. One of these is whether there is any connection of stromal cells with the process of leukemic transformation in patients with acute leukemia.

We investigated whether bone marrow cells (BMCs) from patients with myelodysplastic syndromes (MDS) or acute nonlymphocytic leukemias (ANLL) are altered in their ability to form adherent stromal layers with active hematopoiesis in vitro and whether this depends on the stage of disease.

B. Material and Methods

BMCs were obtained from 24 normal volunteers, 28 patients with ANLL in different stages of the disease, and 9 patients with various forms of MDS: 4 with refractory anemia (RA), 4 with refractory anemia with excess of blast cells (RAEB), and 1 with RAEB in transformation. For studying the hematopoietic stroma we used modified Dexter liquid culture (Fig. 1).

C. Results

There were no differences between the stromal layers of patients with ANLL in complete remission (CR) and those of normal volunteers after 2 weeks of cultivation (Fig. 2). In most cases, however, both BMCs from patients with ANLL before treatment and BMCs from patients in relapse formed poorly adherent stromal layers. In 6 cases (4 with RA and 2 with RAEB) of 9 we observed the normal stromal grade in liquid culture of BMCs from patients with MDS (Fig. 3). It is of interest that the patient with RAEB in transformation showed no adherent layer in our system.

Growth characteristics of BMC from patients with ANLL and MDS in liquid culture are grouped in Fig. 4.

Bone marrow cultures from patients with ANLL in CR and those from patients with MDS, who established stromal layers, had quantitatively normal growth characteristics in liquid culture. However, there were qualitative differences in the nonadherent cell population between normal volunteers and ANLL patients in CR. In most cases of patients with ANLL in CR we found morphologically recognizable erythroid cells after 2 weeks culture in the non-adherent cell

Table 1. Growth characteristics of bone marrow cells in Dexter liquid culture

SG:	Stromal grade. Each Petri dish was assigned a score from 1 to 4, corresponding to a stromal layer covering from 25% to 100% of the area of the culture dish.
NAC:	Nonadherent cells (trypan-blue – negative)
F:	Fibroblast-like cells
CS:	"Cobble stone" (active hematopoietic areas)

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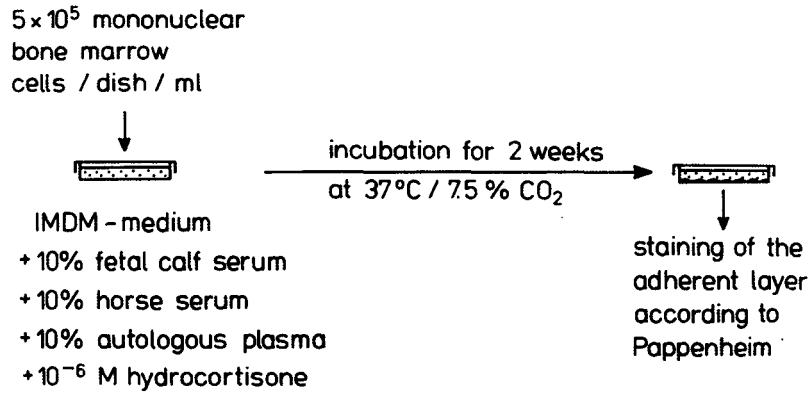

Fig. 1. Schematic representation of the method

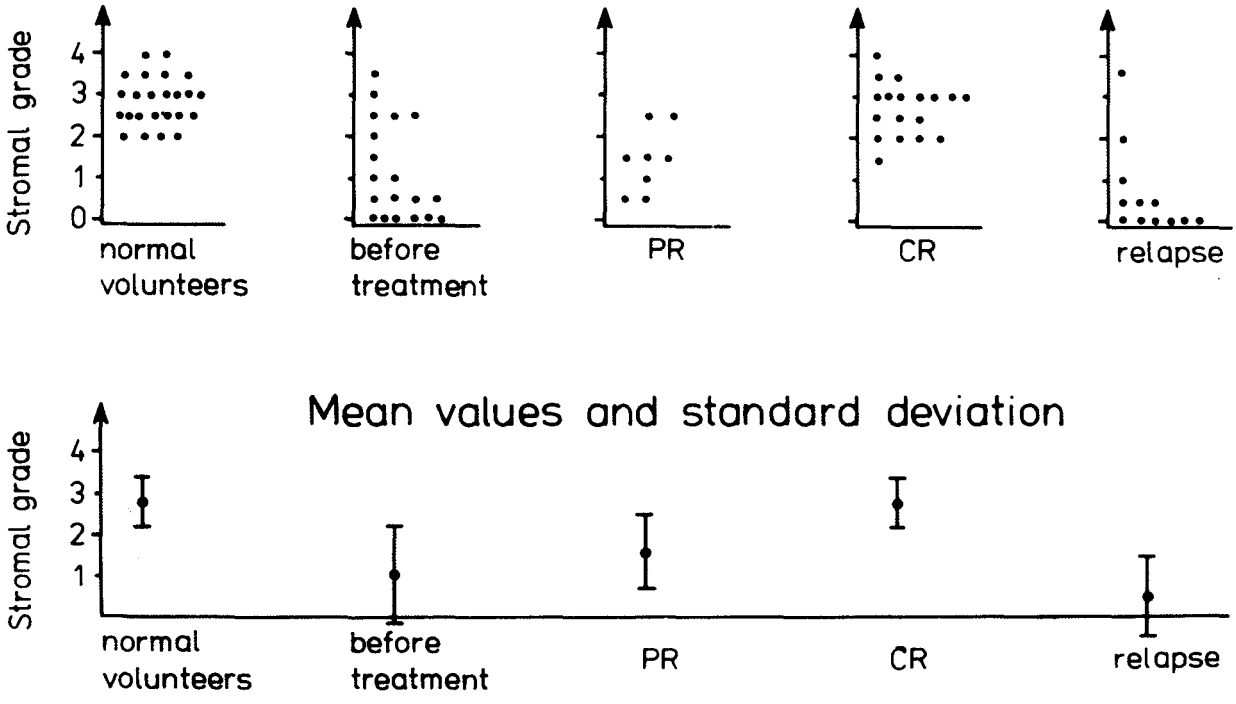

Fig. 2. Adherent layer in Dexter liquid culture (day 14) from bone marrow cells of patients at different stages of acute nonlymphoblastic leukemia

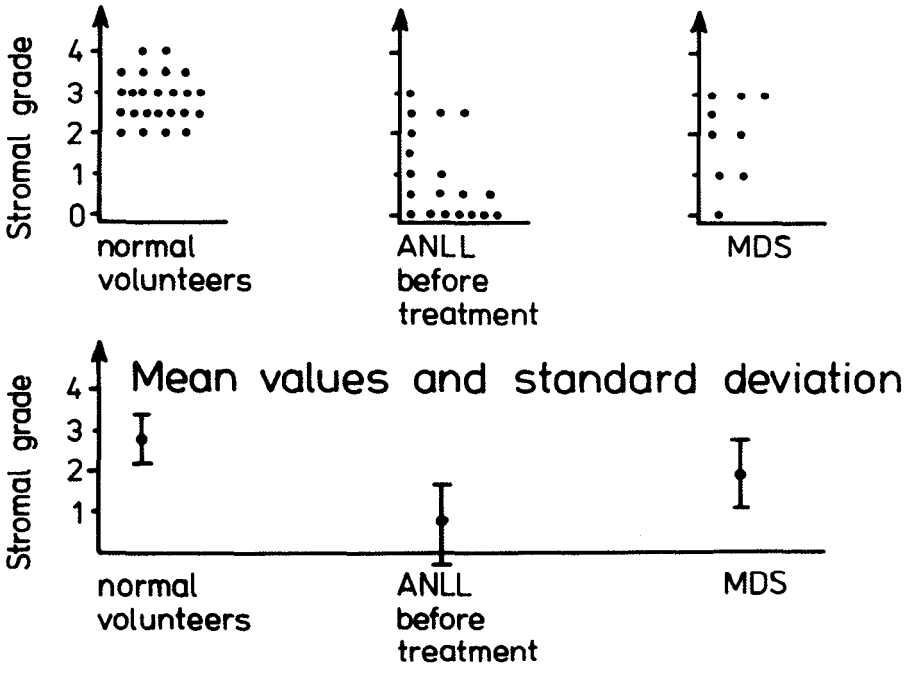

Fig. 3. Adherent layer in Dexter liquid culture (day 14) from bone marrow cells of patients with myelodysplastic syndromes (MDS) and acute nonlymphocytic leukemia (ANLL) before treatment

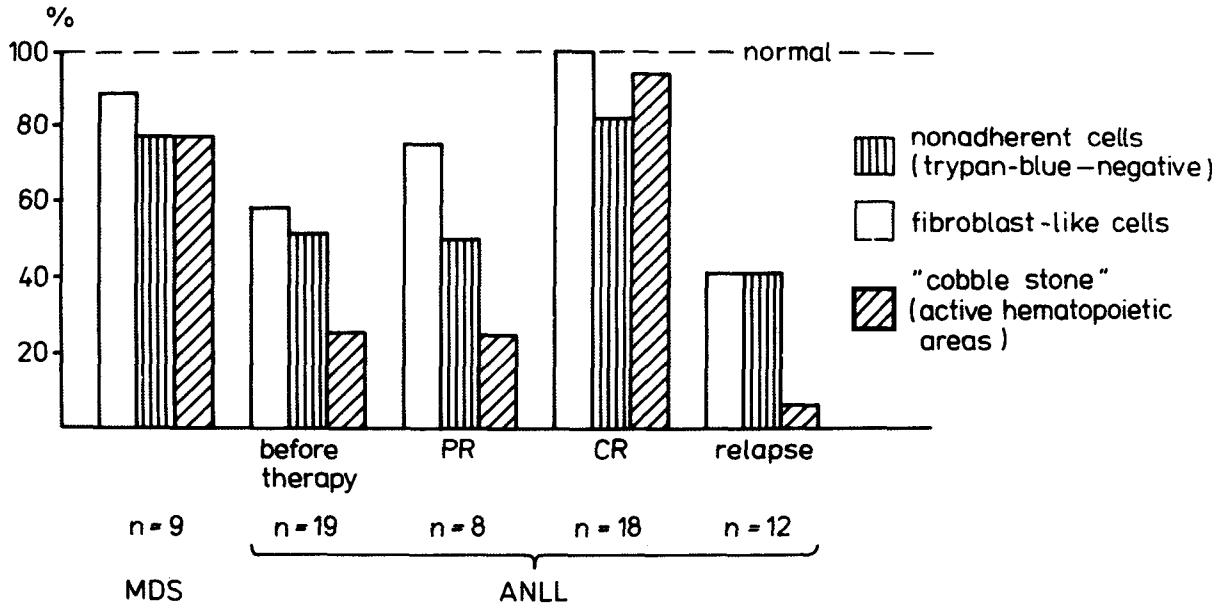

Fig. 4. Growth characteristics of bone marrow cells from patients with myelodysplastic syndromes (MDS) and acute nonlymphoblastic leukemia at different stages of disease in Dexter liquid culture (day 14)

population, which were not seen with normal volunteers. The growth characteristics of patients with ANLL in relapse and patients before therapy showed not only a poor formation of stromal layers, but there were also predominantly blast aggregations in the nonadherent cell population.

D. Discussion

Our results show that the ability of BMCs from patients with ANLL to form adherent stromal layers with active hematopoiesis depends on the stage of disease. Before therapy and in relapse we observed poor formation of stroma in most cases. In the nonadherent cell population there were predominantly blast aggregations in the 2-week culture. Nagao et al. reporting on CFU-F in patients with acute and chronic myelocytic leukemias, observed significant suppression of fibroblast colony formation at the time of diagnosis. The suppression was relieved during chemotherapy-induced remission. However, during relapse the level of CFU-F was again low. There is a close relationship between the leukemic disease and the functional state of bone marrow stroma [1]. At present, the cause of marrow stromal deficiency in leukemia remains unclear. Cocultivation of normal BMCs and leukemic cells reveals an inhibitory activity of leukemic blasts on CFU-F from normal marrow. Inhibition of CFU-F from normal marrow was also induced by leukemic-cell conditioned medium [2].

Bone marrow cells from ANLL patients before therapy are able to form a normal stromal layer with active hematopoiesis in more than four-week-old Dexter culture [3]. Normal stromal cells are present even in a marrow with 80%–90% leukemic blast cells. It is of interest that in our system BMCs showed a higher capacity to form stroma with active hematopoiesis before therapy (25%) than they did in relapse (6%). This finding suggests that in relapse of ANLL the normal stromal progenitors are either strongly reduced, absent, or changed, probably by the chemotherapy. It is known that chemotherapeutic agents impair stroma function [4–6]. We observed a qualitative difference between normal volunteers and some ANLL patients in CR in the nonadherent cell population