In Vitro Effect of GM-CSF and IFN-γ on the Establishment of Stromal Layer and Hemopoiesis in Human Dexter Culture

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Introduction

Recently, the phenomenon of communication and interaction between stromal cells and hemopoietic stem cells has become the focus of interest. The questions of what role colony-stimulating factors (CSF) play in this respect and whether it may be possible to manipulate this process are important for the development of new therapeutic strategies.

The aim of our study was to test the influence of recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) (100 IU/ml), alone or in combination with recombinant human interferon-γ (rhIFN-γ), (10 IU/ml) on the establishment of the stromal layer and on the proliferation and differentiation of hemopoietic cells in 10-day Dexter culture of normal bone marrow cells.

Materials and Methods

Light-density mononuclear cells were isolated from iliac bone marrow aspirates diluted [1:1 with Iscove's modified Dulbeco's medium (IMDM) containing preservative-free heparin (G. Richter, Hungary] by Ficoll-Visotrast gradient separation at a density of 1.077 g/ml.

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The cells were tested for colony-forming unit – granulocyte-monocyte (CFU-GM) growth, cytomorphological composition (staining according to Pappenheim), expression of HLA-DR and CD14 antigens [fluorescence activated cell sorting (FACS) analysis after incubation with monoclonal antibodies: L234 (HLA-DR) derived from hybridomas (ATCC, Rockeville, CA), and LeuM3 (CD14) (Becton and Dickinson, Heidelberg, FRG)].

The Dexter liquid culture [1–3] introduced for human bone marrow cells [7] was slightly modified [6]. Briefly, 5×10^5 cells/ml culture medium (70% IMDM, 10% horse serum, 10% fetal calf serum, 10% autologous bone marrow plasma, 10^{-6} M hydrocortisone sodium succinate) were incubated in 7.5% CO_2 at 37 °C. At the start of the culture 100 IU rhGM-CSF/ml (Behring, FRG) and 10 IU rhIFN-γ/ml (Boeringer, FRG) were added alone or in combination. After 10 days in Dexter liquid culture, stromal formation was evaluated by the expansion of the adherent cell layer (stromal grades 1–4 correspond to an area covered by adherent cells of 25%–100%). The establishment of active hemopoiesis was evaluated by the presence of hemopoietic islands. Adherent cells were removed from the culture dishes with a cell scraper. After having been washed twice, the whole cell population (adherent and nonadherent cells) was tested again for CFU-GM growth, cytomorphology, and expression of HLA-DR and CD14.

The estimation of CFU-GM counts was performed in a monolayer system

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with soft agar, IMDM supplemented with 20% fetal calf serum, 20% mixed culture conditioned medium and $5 \times 10^{-5} M$ mercaptoethanol.

Results and Discussion

Exogenous rhGM-CSF caused an increase in both stromal grade (Fig. 1) and activity of hemopoiesis (number and size of hemopoietic islands). Cultures with 10 IU rhIFN-γ/ml showed almost the same degree of stromal formation as the control cultures. The composition of hemopoietic cells was influenced by the factors added (Fig. 2). rhIFN-γ (10 IU/ml) alone caused a switch in the differentiation of hemopoietic cells to monocytes and macrophages. There was an increase in the number of immature cells in cultures with rhGM-CSF, both alone and in combination with rhIFN-γ.

The CFU-GM derived from the Dexter cultures were more numerous than those from the controls without precultivation in suspension (Fig. 3). An additional increase in the CFU-GM count was seen in all Dexter cultures with rhGM-CSF, both alone and in combination with rhIFN-γ. This elevation in CFU-GM count was mainly due to an increase in the number of small compact aggregates. This is expressed by an elevated cluster/colony ratio (Fig. 3).

Our preliminary data seem to indicate that exogenous rhGM-CSF affects CFU-GM as well as stromal precursor cells in Dexter liquid culture.

GM-CSF added to a preestablished Dexter culture had no effect [4, 5, 12]. In our study, however, we examined the important early period of the process of establishing the microenvironment.

Exogenous rhGM-CSF accelerates the maturation of monocytes to macrophages and, therefore, shortens the time which these cells need to acquire the ability to function within the network of stromal cells.

Another humoral mediator for hemopoietic regulation is IFN-γ. It exerts an inhibitory action on CFU-GM growth [8, 9, 11] and is able to induce myeloid progenitor cells to differentiate towards the monocytic lineage [10]. In our cultures, there was no effect of rhIFN-γ

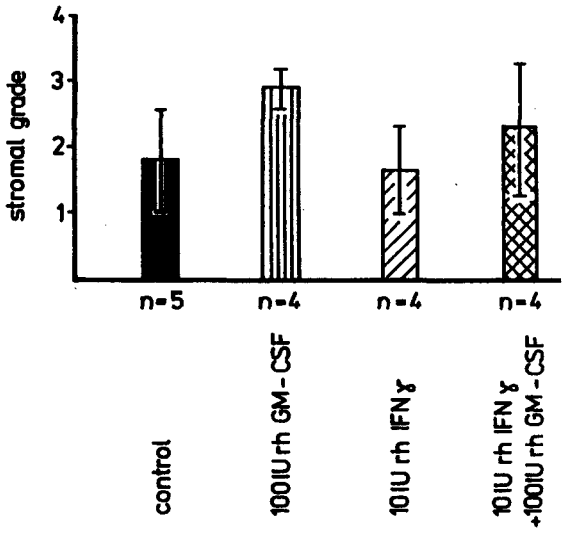

Fig. 1. Stromal formation of normal human bone marrow cells after 10 days in Dexter liquid culture

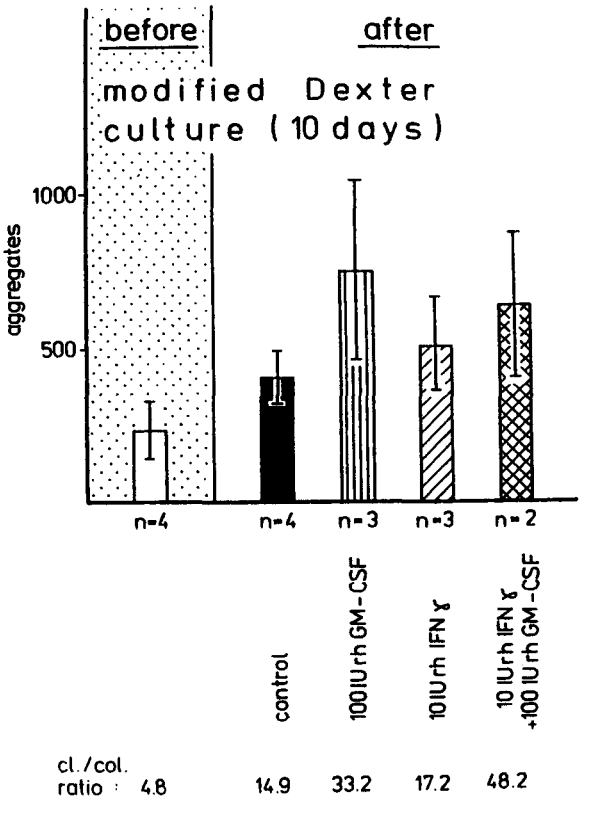

Fig. 2. CFU-GM counts and cluster/colony ratios derived from Dexter cultures after 10 days of cultivation

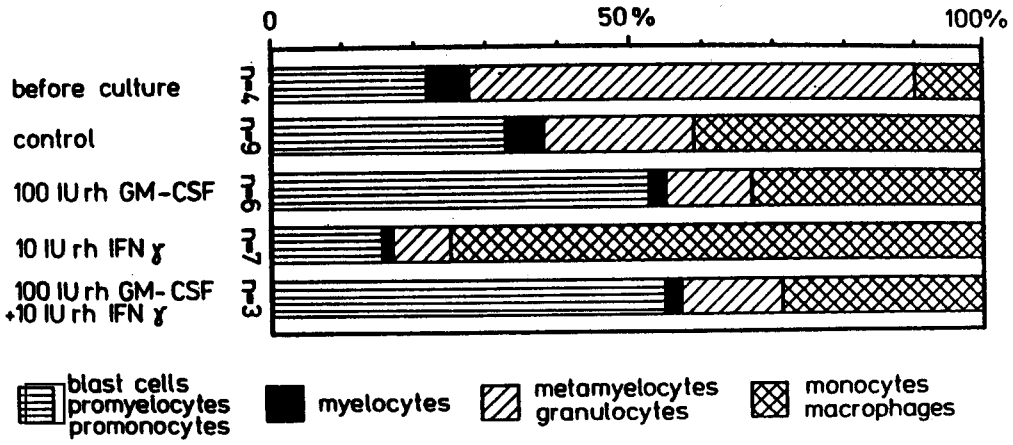

Fig. 3. Distribution of cytomorphologically differentiated subpopulations of hemopoietic cells derived from Dexter cultures after 10 days of cultivation

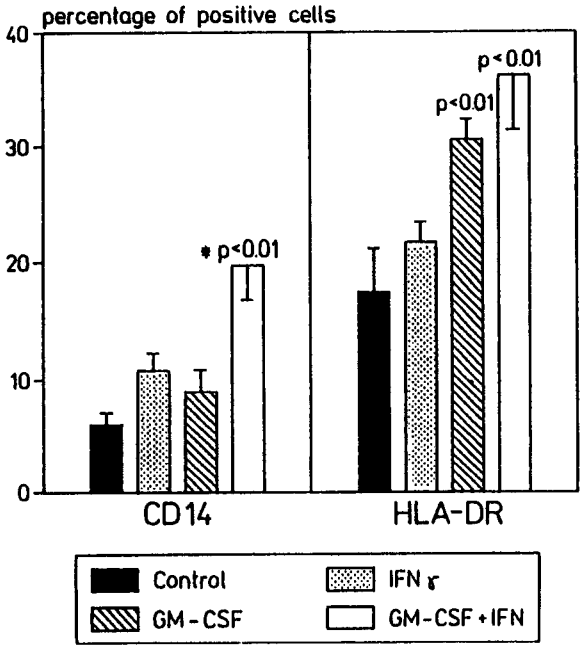

Fig. 4. The influence of rhGM-CSF and rhIFN-γ on the expression of the CD14 and DR antigen on bone marrow cells derived from 10-day Dexter liquid cultures

(10 IU/ml) on stromal formation and a minimal enhancing effect on the CFU-GM count. A combination of rhIFN-γ and rhGM-CSF had the same stimulatory effect on CFU-GM derived from Dexter cultures as rhGM-CSF alone. Simultaneously, an expansion of immature cells (blasts, promyelocytes, promonocytes) was observed, which correlated with elevated HLA-DR expression (activation marker). It is of special interest that further specification of the cells by FACS analysis revealed elevated expression of the monocytic marker CD14 after the combined application of rhGM-CSF and rhIFN-γ, but not after rhGM-CSF alone (Fig. 4).

We assume that the addition of both factors to the Dexter culture stimulates the establishment of marrow stroma and also the proliferation of hemopoiesis with a switch in the differentiation towards monoblasts.

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