A new candidate for the regulation of erythropoiesis

Insulin-like growth factor I

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The effect of pure human insulin-like growth factor I (IGF I) on the colony formation of late stage erythroid precursor cells (CFU-e) from fetal mouse liver and adult bone marrow was studied in a serum-free culture system. We found that IGF I in physiological concentrations stimulated erythroid colony formation. The combined effect of IGF I and erythropoietin was smaller than the sum of their single effects. The number of colonies induced by IGF I was linearly dependent on the number of plated cells. Our results indicate that IGF I is the first clearly defined mitogen that stimulates the late stages of erythroid differentiation independently of erythropoietin.

Insulin-like growth factor *Somatomedin* *Erythroid differentiation* *Erythroid precursor cell* *Cell structure*

1. INTRODUCTION

Insulin is capable of stimulating the growth of late erythroid precursor cells (CFU-e) [1]. This growth-enhancing effect of insulin did not require the presence of the glycoprotein hormone erythropoietin (Ep) that is thought to be indispensable for the proliferation of CFU-e [2]. The stimulating effect of insulin on erythroid colony formation could only be observed at supraphysiological concentrations of the hormone, the concentration for the half-maximal effect being 5 orders of magnitude higher than the physiological insulin concentration. Such a mitogenic effect of high concentrations of insulin has been demonstrated for various other cell types in vitro [3]. In fibroblast cultures [4] the growth enhancing capacity of insulin is mediated by receptors which bind insulin with a low affinity but have a high affinity for the insulin-like growth factors (IGF I and IGF II) [5]. To test the hypothesis that the effect of insulin on colony formation of CFU-e is due to a direct effect of IGF we have examined the influence of IGF I on the growth of CFU-e in a serum-free culture system. We found that IGF I in the physiological concentration range greatly enhances erythroid colony formation in fetal mouse liver and adult-mouse bone marrow independently of Ep.

2. MATERIALS AND METHODS

2.1. *Erythroid colony assay*

Effects of IGF I and Ep were studied using the in vitro bioassay for Ep. Liver cells were obtained from 13-day fetal NMRI mice (Dr Ivanovas, Kisslegg). Bone marrow cell suspensions were prepared from femurs of adult female mice. The test procedure was essentially that in [6] except that fetal bovine serum was replaced by a serum-free incubation medium according to [7]. Cells were cultured in 4-well dishes (Greiner/FRG). Dishes were incubated for 48 h at 37°C in a humidified atmosphere containing 20% O_2, 5% CO_2, balance N_2, using O_2- and CO_2-controlled incubators (Heraeus, Hanau). Cultures were evaluated as in [8]. Each experiment represents the mean of 3 replicate wells. Repetition of experiments was done with different cell preparations.

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2.2. *Erythropoietin*

Human urinary Ep, supplied by the National Institutes of Health (MD), was standardized against the International Reference Preparation B in the exhypoxic polycythemic mouse assay for Ep.

2.3. *IGF I*

A stock solution (10 μg/ml) of pure human IGF I in Iscoves modified Dulbecco medium (Gibco) containing 1% deionized albumin was stored at −60°C until use. IGF I was kindly provided by Professor E.R. Froesch (Zürich).

3. RESULTS AND DISCUSSION

Fig. 1 shows the effect of Ep and IGF I on the growth of 2 day erythroid colonies (CFU-e) from fetal mouse liver and adult bone marrow in a serum-free culture medium. The molar concentrations of Ep and IGF I were calculated using M_r 40000 and spec. act. 70000 U/mg for Ep [9] and M_r 7500 for IGF I [10]. It can be seen that IGF I stimulated the growth of erythroid colonies in a dose-dependent manner. The percent increase of the number of colonies upon raising the molar concentration of IGF I was similar for CFU-e from both adult and fetal erythroid tissue. This parallels the similar responsiveness of CFU-e towards Ep from adult and fetal erythroid tissue in a serum-free culture system (fig. 1, left curve). The dose–response curve of Ep on CFU-e colony formation in adult bone marrow fits very well with the curve in [7] obtained with the same culture system.

The dose–response curve for IGF I was only half

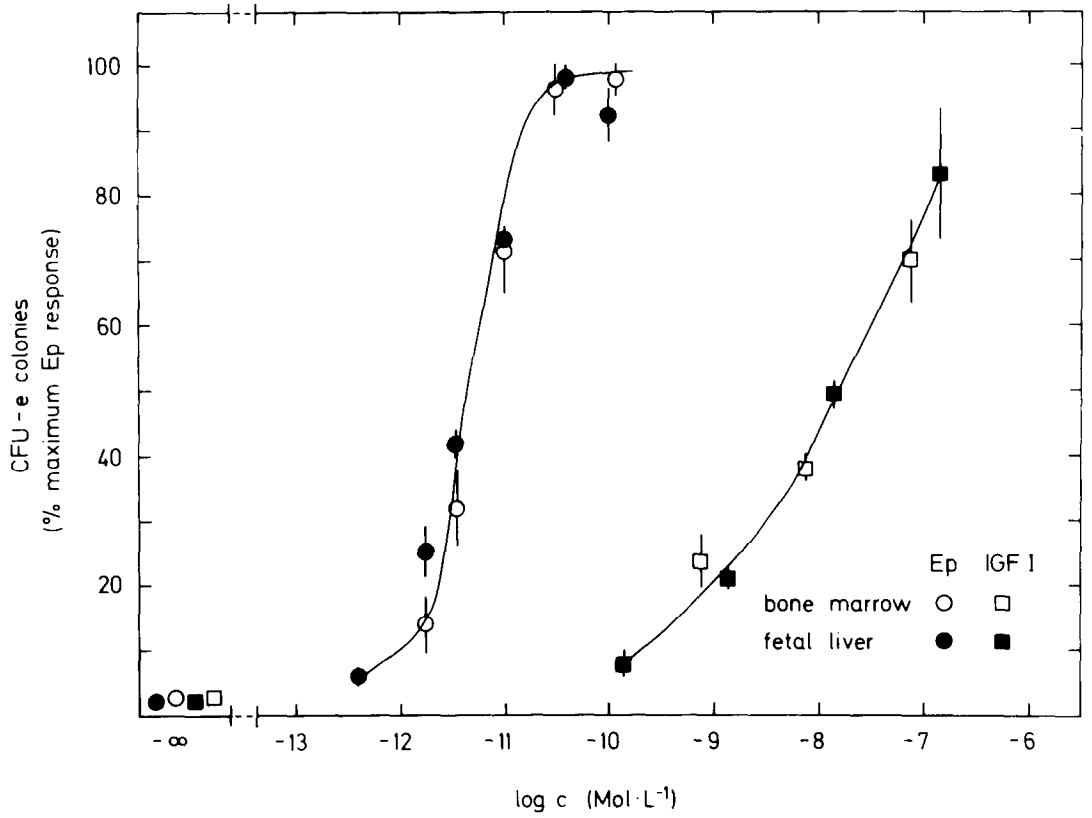

Fig. 1. Formation of erythroid colonies from fetal mouse liver cells and adult bone marrow cells grown for 2 days in vitro in the presence of Ep or IGF I. The concentrations of the hormones are given on a molar basis. The number of CFU-e colonies is expressed as a percentage of the maximum response to Ep (2530 ± 120 colonies/10^5 cells, mean ± SEM of 13 obs. for fetal liver cells and 203 ± 60 colonies/10^5 cells, mean ± SEM of 3 obs. for adult bone marrow cells) in the same experiment.

as steep as for Ep (fig. 1). This could be due to the formation of IGF-dimers at higher concentrations of the mitogen, which in analogy to the dimer formation of insulin [11], might lead to a diminished biological activity.

A comparison of the molar concentrations of Ep and IGF I which give rise to 50% of the maximum number of colonies, shows that the potency of Ep is by 4 orders of magnitude greater than that of IGF I. However, in doing such comparisons it should be noted that the plasma concentrations of Ep and IGF I under normal conditions are about 10^{-11} M [12] and 10^{-7} M [13], respectively. Therefore, both substances are likely to be about equally effective in stimulating late erythroid differentiation under physiological conditions.

Furthermore, the number of erythroid colonies stimulated by IGF I was linearly dependent on the number of plated cells. This finding supports the idea that there is a direct effect of IGF I on CFU-e which is not mediated by co-cultivated cells.

The combined effect of Ep an IGF I was smaller than the sum of the single effects of Ep and IGF I (table 1). In the presence of saturating concentrations of Ep, addition of IGF I did not lead to a further increase in the number of colonies. This indicates that both hormones act on the same cell population. IGF I is strongly related to the family of somatomedins [14], a group of mitogens which are known to mediate the effects of the growth hormone (GH) [15,16]. In view of these connections the effect of IGF I on colony formation from CFU-e could explain various reports demonstrating a stimulation of erythropoiesis by GH [17,18].

It has become increasingly apparent that the late stages (CFU-e) of erythroid differentiation are enhanced by growth factors other than Ep. The sources of these factors comprise medium from lectin-stimulated mouse spleen cells [19,20] and human mononuclear cells [21] as well as from unstimulated human malignant cell lines [22,23]. Since all these growth factors have been operationally defined as anything that enhances the formation of CFU-e colonies in culture, it is not clear whether the CFU-e promoting activities from various sources are different in nature or not. IGF I on the other hand, is a mitogen of which the chemical structure has been established in great detail [10]. Therefore, IGF I is the first clearly defined mitogen that stimulates the late stages of erythroid differentiation independently of Ep.

Table 1

Effect of Ep on the growth of erythroid colonies from fetal mouse livers in the presence of IGF I

Ep \ IGF I	0	10 ng/ml (1.3×10^{-9} M)	100 ng/ml (1.3×10^{-8} M)
0	2 (13)	24 ± 3 (5)	52 ± 2 (5)
5 mU/ml (1.8×10^{-12} M)	25 ± 4 (13)	42 ± 1 (4)	not determined
10 mU/ml (3.6×10^{-12} M)	42 ± 2 (13)	not determined	73 ± 4 (4)
120 mU/ml (4.3×10^{-11} M)	98 ± 2 (13)	not determined	105 ± 4 (4)

The number of erythroid colonies is expressed as a percentage of the maximum response to Ep (2530 ± 120 colonies/10^5 cells, mean ± SEM, n = 13) in the same experiment. Numbers in parenthesis indicate the number of experiments

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