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STUDIES ON THE TISSUE SPECIFICITY OF COLONY STIMULATING FACTOR

A. Rabbani*, M. Razmara, and B. Goliaei,

Institute of Biochemistry and Biophysics, University of Tehran, P.O. Box 13145-1384, Tehran, Islamic Republic of Iran

Abstract

In this study granulocyte-macrophage colony stimulating factor (CSF) was prepared from rabbit and guinea pig lung tissues. The biological activity of these CSFs was measured on semisolid agar culture and compared with those of mouse and rat. Weight of animals, incubation time and cross-reactivity of CSFs with different bone marrow sources were also investigated. The results showed that both rabbit and guinea pig lung tissues produced and released CSF in the conditioned medium. The CSFs were active on ammonium chloride treated bone marrow cells from balb/c mouse and on their own ammonium chloride treated bone marrow cells but could not stimulate the progenitor cells from other sources.

Introduction

Regulation of blood cell formation and differentiation is a complex process in which all these cells are produced as a result of the balance between self-renewal and commitment of hemopoietic stem cells. Recent progress in our understanding of hemopoiesis has clearly indicated that the different cell lines are derived from a common precursor pool and that the control of their production is partially regulated by chemically similar stimulatory molecules [1,2]. Colony stimulating factors (CSFs) are a group of glycoprotein growth factors which regulate the survival, proliferation and differentiation of hemopoietic progenitor cells [3,4]. CSF activity has been reported in a wide variety of tissues and body fluids [5,6]. They have also been found in different cell types [7]. In the mouse, at least four major and distinct CSFs have been characterized as interacting to control the formation and function of granulocytes and macrophages. They are granulocyte CSF (G-CSF), macrophage CSF (M-CSF), granulocyte-macrophage CSF (Gm-CSF) and multi-CSF [1,8]. Lung tissue has been demonstrated as a rich source of CSF and it contains mainly GM-CSF and some G-CSF [1, 6]. CSF from rat lung has also been reported [9]. Therefore, it was of interest to look at these proteins in other mammals to find out indications about their specificity.

In this study the production of CSF in guinea pig and rabbit lung tissues in comparison to mouse and rat CSFs and also cross-reactivity of CSFs with different sources of bone marrow cells was investigated.

Keywords: Colony-stimulating-factor, Bone marrow cells; Hemopoiesis

Materials and Methods

Animals: Mice and rats were obtained from the Razi Institute (Hesarak) and the rabbits and guinea pigs were from the Pasteur Institute. Balb/c mice weighing 30-40g of either sex, male Wistar albino rats weighing 150-250g and male rabbits and guinea pigs of different weights were used for the preparation of conditioned medium. The weight of animals for the biological assays were; balb/c 18-22g, rat 50-70g, guinea pig 100-150g and rabbit 400-500g.

Materials: Dulbecco's modified eagle medium (DMEM) was obtained from Gibco. It was subsequently supplemented with antibiotics (200 U/ml penicillin and 200 mg/ml streptomycin) and 30 mg/ml of asparagine pH= 7.4. The medium was filtered through a millipore filter (0.45 μm) and refrigerated until use. Fetal calf serum was prepared in our laboratory. Bovine serum albumin was from Sigma.

Lung conditioned medium: Conditioned medium from each animal lung tissue was prepared separately as described before [9]. Animals were anesthetized by ether and the lungs were removed from the chest cavity in sterile conditions. The lungs were washed with normal saline, minced and incubated in 5 ml of DMEM for 48 hours at 37°C in a fully humidified atmosphere of 5% CO_2 in air. After centrifugation for 30 min at 2000g, the clear supernatants thus obtained were heated at 56°C for 30 minutes, centrifuged at 2000g and dialysed against two changes of distilled water for 48 hours. The dialysates were centrifuged as above and to supernatants, polyethylene glycol was added at a final concentration of 1% before they were sterilized by filtration through 0.45 uμ membrane filter (Millipore).

Bone marrow treatments: Bone marrow cells from different animals were suspended in DMEM and centrifuged at 1500g for 10 min (4°C). Supernatants were discarded and to each pellet the desired volume of 0.85% sterile ammonium chloride (10 ml of 0.17M tris, 90 ml of 0.16M ammonium chloride pH adjusted to 7.2 with 2.5% ammonia) was added and left on ice for 20 min. The cells were centrifuged as above and the final cells washed three times with DMEM. The viability and intactness of the cells were measured using trypan blue dye. Biological assay: Samples were assayed by semi-solid agar culture technique as described by Metcalf [1]. Bone marrow cells (10^5 cells) were plated into 35mm plastic petri-dishes (NUNC) containing 1 ml of DMEM supplemented with 0.3% agar, 20% fetal calf serum and 0.1 ml of conditioned medium. The plates were incubated as above for seven days. Colonies with greater than 50 cells were scored using a dissecting microscope.

Protein assay: The content of proteins in each sample was determined by the Hartree method [10] using bovine serum albumin as a standard.

Cross-reactivity: The experiment was essentially as described in the biological assay section. CSF obtained from the lung tissue of each animal was reacted with the bone marrow cells of other animals indicated in this paper.

Results and Discussion:

Conditioned medium was prepared from rabbit and guinea pig lung tissues and their ability in producing colonies from mouse and their own marrow cells was analysed on semi-solid agar cultures. The results showed that CSF from both cases produced only few colonies when cultured on mouse marrow cells but no colonies were observed when they were added to their own normal marrow cell cultures. However, under the same conditions, mouse and rat conditioned medium (0.1 ml) produced 100-120 colonies on mouse marrow cells.

Ammonium chloride at low concentrations is usually used for lysis and removal of red cells [11]. Therefore, experiments were designed in such a way that marrow cells were first treated with ammonium chloride before plating on agar cultures. Table 1 shows the effect of

Table1: The effect of ammonium chloride treatment on the CSF induction colonies by mouse marrow cells.

Source of CSFs (lung tissue)	No. of colonies/ml	
	Normal marrow cells	ammonium chloride treated cells
Guinea pig	8	30
Rabbit	3	8.5
Mouse	92	140
Rat	83	160

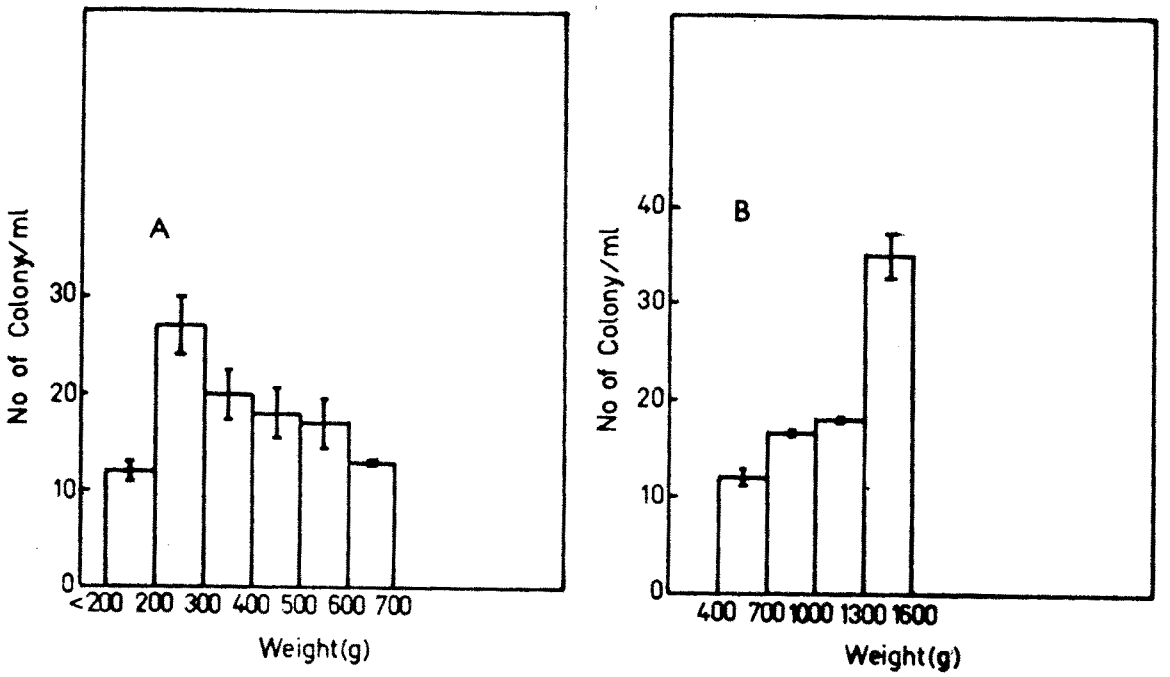

Figure 1: The effect of weight of animals on CSF production and colony formation on their own marrow cells. A; guinea pig, B; rabbit. Means ± SEM of at least three experients each in duplicate.

CSFs on the number of colonies produced in the cultures with treated and untreated marrow cells. In all samples, 0.1 ml of CSF was used as a stimulator. In this case 30 and 8 colonies with more than 50 cells were counted for guinea pig and rabbit CSFs respectively. Since ammonium chloride in the absence of CSFs had no stimulatory effect on the cells, therefore, in all experiments described treated marrow cells were used. Fig 1 illustrates the weight of animals whose lungs could produce sufficient amounts of CSF in the medium. As seen, guinea pigs at a weight of 200-300g (1A) and rabbits with a weight of more than 1.3kg (1B) produced the highest level of CSF. Desired incubation time necessary for the maximum CSF production and release was also measured by culturing lung tissues under standard conditions and analysing CSF activity on agar cultures at different time intervals. The results are given in Fig 2. Mouse lung conditioned medium was also prepared in the same way and used as a control. It is shown that 24-48 hours was sufficient for the production and release of CSFs from the lung. This was similar to the incubation time of mouse lung conditioned medium.

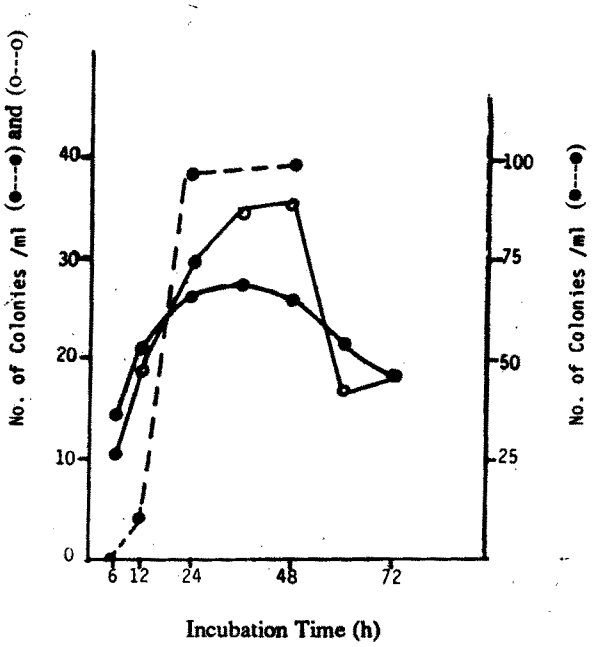

Figure 2: Number of colonies produced from the incubation of lung tissue from mouse (●---●) guinea pig (●—●) and rabbit (O---O) at different time intervals on their own marrow cells

Cross-reactivity experiments were performed under identical conditions. Conditioned media from rabbit, guinea pig, rat and mouse lung tissues were prepared and after 48 hours of incubation and biochemical processing, their ability to produce colonies from bone marrow cells of other sources was investigated. Table 2 represents the results thus obtained. It is seen that CSF from different sources were active on their own marrow cells but the number of colonies produced by rabbit and guinea pig CSFs was considerably lower than those of mouse and rat conditioned media. The reason for this is still unclear. Rabbit and guinea pig CSFs were able to produce colonies from balb/c marrow cells but not from rat or from each other. On the other hand, CSF from balb/c lung tissue was active on its own and on rat marrow cells but not on rabbit and guinea pig cells. CSF obtained from rat lung was only active on balb/c marrow cells and its own progenitor cells produced only a few colonies.

From the results presented above it is concluded that the weight of animals plays an important role in the CSF production and release. Comparative studies of incubation time indicated that the mechanism and regulation of CSF synthesis is similar in lung tissues from various sources. However, the action of CSFs on the progenitor cells from various animals was different. It has been reported that although GM-CSF from mouse and human lungs show 60% structural homology, mouse lung conditioned medium is incapable to proliferate and differentiate stem cells from human marrow cells but the reverse is perfomed [12,13]. On the other hand, preliminary work done on the effect of CSF from guinea pig serum on the mouse marrow cells did not show any detectable colonies or clusters on agar cultures [14] while lung CSF prepared in this study produced few colonies.

As in other cellular systems, CSFs almost certainly function via cell surface receptors. Because of the very low number of GM-CSF receptors, their characterization and distribution on the progenitor cells is poorly understood. Whether the difference observed in the effect of lung CSFs on different marrow cells corresponds to protein chain specificity or to other factors demands further investigation.

Table 2: Number of colonies produced from the cross-reactivity of CSFs with different bone marrow cells. Results are means ± SEM of three experiments each in duplicate.

Normal CSFs	Bone marrow sources			
	Balb/c	Rat	Guinea pig	Rabbit
Balb/c	140 ±7.1	20 ±2.3	0	0
Rat	160 ±3.3	9 ±1.02	0	0
Guinea pig	30 ±4.5	0	27 ±2.4	0
Rabbit	8.5 ±3.02	0	0	35 ±4.5

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