Journal of Chromatography, 413 (1987) 242–246
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3380

Note

Purification and characterization of granulocyte-macrophage colony stimulating factor from human placenta

V. CUKROVÁ* and Z. HRKAL

Institute of Haematology and Blood Transfusion, U nemocnice 1, 128 20 Prague 2 (Czechoslovakia)

(First received July 10th, 1986; revised manuscript received August 11th, 1986)

Haemopoietic cell proliferation is completely dependent on stimulation by specific glycoprotein regulatory molecules, known as haemopoietic colony stimulating factors (CSFs) [1]. Granulocyte-macrophage, granulocyte, macrophage and multi-colony stimulating factors (GM-CSF, G-CSF, M-CSF, multi-CSF) have been purified from the media conditioned by different murine tissues and cell lines [2–6]. In spite of considerable progress, characterization of these factors at the molecular level is still incomplete. The knowledge of their biological properties (the role in survival, growth and differentiation of haemopoietic progenitors) is still exclusively based on cultivation studies [7].

Human CSFs have been less well biochemically characterized than the murine ones, owing to the great heterogeneity of most sources in CSF composition [7].

This paper reports our studies of the purification and characterization of human GM-CSF from human placental conditioned medium (HPCM). We have modified the procedure of Schlunk and Schleyer published in 1983 [8] by using chromatography on Bio-Gel P60 and fast protein liquid chromatography (FPLC) as the final purification step.

EXPERIMENTAL

HPCM preparation

The human placental tissue was cut into small pieces and, after rinsing with phosphate-buffered saline (PBS), homogenized in an electric mincer. The disrupted tissue was diluted twenty-fold with RPMI 1640 medium (prepared in our institute) containing 2.5% of human serum and incubated for seven days at 37°C

0378-4347/87/$03.50 © 1987 Elsevier Science Publishers B.V.

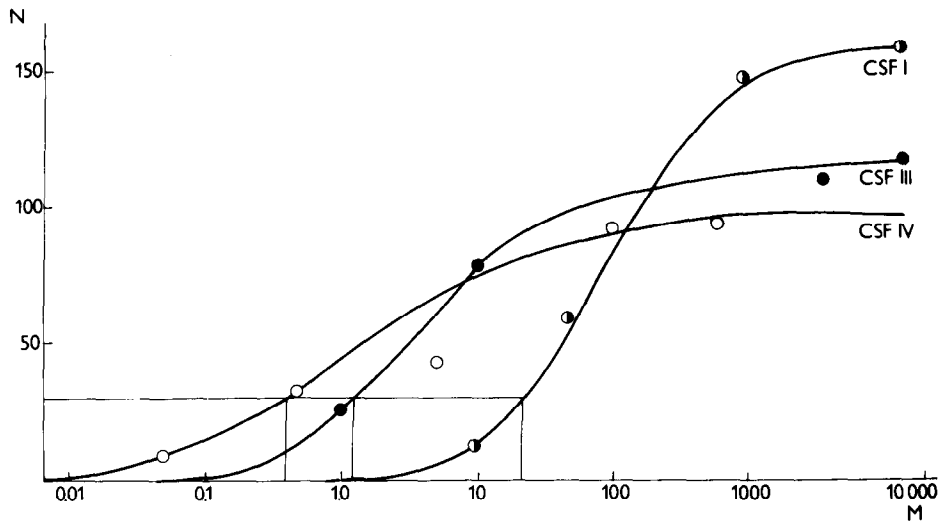

Fig. 1. Dose-response curves of CSF I, CSF III and CSF IV; N = number of colonies per dish; M = μg of protein per dish.

in 5% carbon dioxide in air. One placenta yielded 2.5 l of culture supernatant.

CSF assay

A 0.1-ml volume of HPCM or fractions after separation dialysed into PBS were added to $1.5 \cdot 10^5$ human low-density bone marrow cells from a normal donor (density 1.077 g/ml), depleted of adherent cells and resuspended in 1 ml of 0.3% agar in minimal essential medium (prepared in our institute) with 20% of human serum. The cells were cultivated in Petri dishes at 37°C in 5% carbon dioxide in air. After ten days of cultivation the colonies were counted (more than 40 cells). The tests were carried out in triplicate. For evaluation of specific activities and CSF recovery, the protein concentrations and dose–response curves of the material after the purification steps were measured. In the dose–response curves, CSF doses corresponding to the 30 colonies per dish were compared (Fig. 1). The total protein in the samples was assessed by spectrophotometric measurement at 280 nm. Both HPCM and purified materials were stored at −20°C.

Isolation procedure

Human placental conditioned medium (2.5 l) was concentrated four-fold with an Amicon ultrafiltration device and a Diaflo YM-10 membrane, and dialysed to 0.02 *M* ammonium acetate (pH 6.4) containing 0.05 g/l polyethylene glycol (PEG). After dialysis the solution was adjusted to pH 4.0 with acetic acid and the precipitated material was removed by centrifugation at 40 000 *g* for 30 min (CSF I). The supernatant was applied to a column of Whatman CM 52 cellulose (column size 30 × 2.6 cm I.D.), equilibrated with 0.05 *M* ammonium acetate (pH 4.0) containing 0.2 g/l sodium nitride and 0.05 g/l PEG, and eluted with a stepwise gradient of 0.05 *M* ammonium acetate pH 4.0, pH 5.0 (CSF IIA) and pH 6.0

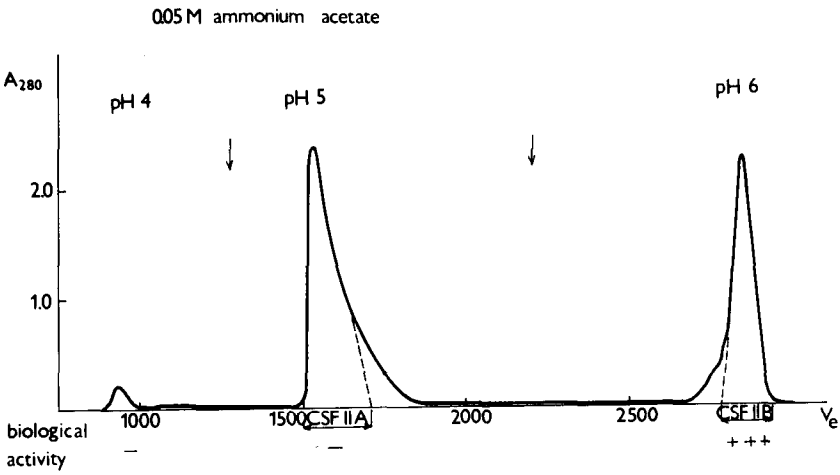

Fig. 2. Ion-exchange chromatography of CSF I on a carboxymethylcellulose CM 52 column (33×2.6 cm I.D.). Flow-rate, 60 ml/h; five fractions per hour; A_{280} = absorbance at 280 nm; V_e = elution volume (ml).

(CSF IIB) at a flow-rate of 60 ml/h. The protein fraction eluted at pH 6.0 was concentrated by ultrafiltration to a volume of 7.5 ml and dialysed to 0.01 *M* phosphate buffer pH 7.4 (0.2 g/l sodium nitride, 0.05 g/l PEG). The fraction was chromatographed on the Bio-Gel P60 column (90×1.6 cm I.D.) at a flow-rate of 10 ml/h, equilibrated with the same buffer. The fractions exhibiting the CSF activity were pooled (CSF III) and further chromatographed with a Pharmacia FPLC system on Mono Q 5/5 column. The protein fraction in 20 m*M* Tris–HCl (pH 7.4) was developed with a linear gradient of the same buffer containing 1 *M* sodium chloride (CSF IV).

RESULTS AND DISCUSSION

Chromatography of CSF I on carboxymethylcellulose CM 52 is shown in Fig. 2. The CSF activity is contained in the fractions eluted at pH 6.0 (CSF IIB). Fractions of CSF IIA did not contain any biological activity at all, which is in contrast to the results obtained by Schlunk and Schleyer [8]. The proteins of the pooled fractions CSF IIB were concentrated and subjected to gel permeation chromatography on Bio-Gel P 60 (Fig. 3). The CSF activity was contained in the fractions with a relative molecular mass M_r centered at 22 000 (fractions of CSF III), which is a similar result to that of Schlunk and Schleyer [8]. Because of its low molecular weight, CSF III approximates the type II of placental CSF characterized by Wu and Yunis [9]. CSF III is of 17.7 times higher purity than the starting material CSF I (Table I). The final purification was achieved by FPLC on a Mono Q anion-exchange column. Part of the material did not bind to the column under the experimental conditions used (20 m*M* Tris–HCl, pH 7.4), but this did not contain any CSF activity. The bound material resolved into about a dozen peaks, all containing CSF activity (Fig. 4) with the principal activity at an ionic strength of 0.22 (fraction CSF IV). The data on CSF activity throughout the purification procedure are summarized in Table I. The specific activity of CSF

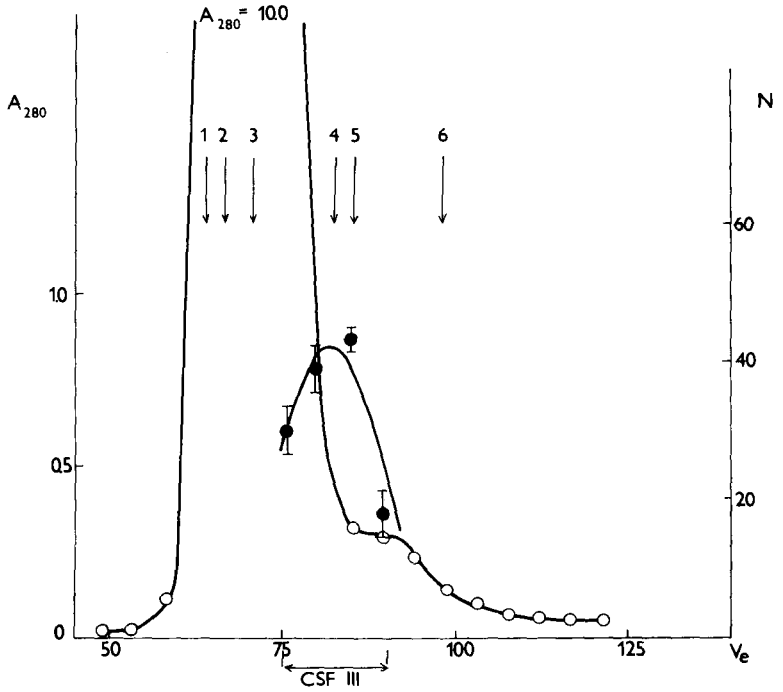

Fig. 3. Gel chromatography of CSF IIB on a Bio-Gel P60 column (90×1.6 cm I.D.). Flow-rate, 9 ml/h; two fractions per hour; N = number of colonies per dish (●); A_{280} = absorbance at 280 nm (○); V_e = elution volume (ml). Arrows indicate molecular weight standards (in M_r): 1 = albumin, 67 000; 2 = hemoglobin, 64 500; 3 = ovalbumin, 43 000; 4 = chymotrypsin A, 22 000; 5 = myoglobin, 17 200; 6 = cytochrome *c*, 12 400.

IV was higher by a factor of 57.5 than that of the CSF I. The plateaux of the dose–response curves of CSF III and CSF IV were lower than in CSF I, suggesting a loss of biological activity during the purification procedure (Fig. 1). This could be accompanied by a removal of synergistically interacting factors [8].

TABLE I

PURIFICATION OF CSF FROM HUMAN PLACENTAL CONDITIONED MEDIUM

N.D. = not determined.

		Total protein (mg)	Specific activity (rel. units)	CSF recovery (%)
	CSF I	8360	1	100
Carboxymethylcellulose CM 52	↓			
	CSF IIB	664	N.D.	N.D.
Bio-Gel P 60	↓			
	CSF III	145.2	17.7	31
Ion-exchange FPLC	↓			
	CSF IV	14.08	57.5	9.7

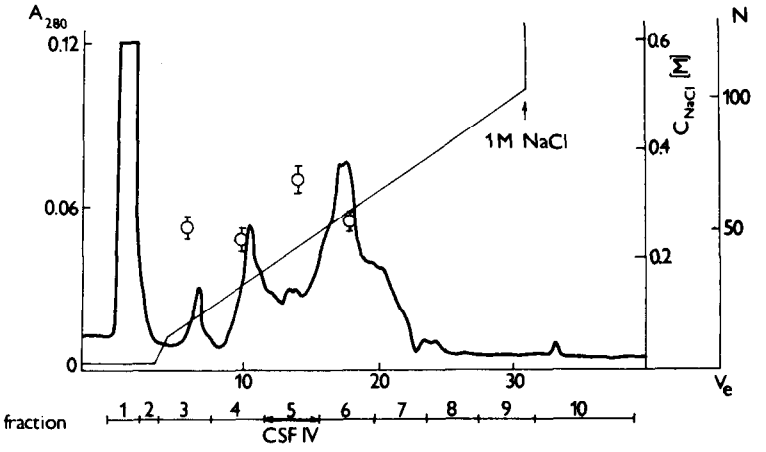

Fig. 4. Fast protein liquid chromatography: flow-rate, 1 ml/min; column, Mono Q 5/5; N = number of colonies per dish (○); A_{280} = absorbance at 280 nm (unbroken line); V_e = elution volume (ml).

In conclusion, the purified stimulating factor is a glycoprotein consisting of heterogeneous moiety of molecules with different electric charge, with an average M_r of ca. 22 000. It stimulates the growth of mixed colonies composed of granulocytes and monocyte-macrophages. The final specific activity of purified material exceeds that of the starting material ca. 60-fold.

REFERENCES

1 D. Metcalf, Hemopoietic Colonies, Springer Verlag, Berlin, 1977.
2 A.W. Burges, J. Camakaris and D. Metcalf, J. Biol. Chem., 252 (1977) 1998.
3 E.R. Stanley and B.M. Heard, J. Biol. Chem., 252 (1977) 4305.
4 A. Waheed and R.K. Shaduck, J. Lab. Clin. Med., 94 (1979) 180.
5 N.A. Nicola, D. Metcalf, M. Matsumoto and G.R. Johnson, J. Biol. Chem., 258 (1983) 5290.
6 J.N. Ihle, J. Keller, L. Henderson, F. Klein and E.W. Palaszynski, J. Immunol., 129 (1982) 2431.
7 N.A. Nicola and M. Vadas, Immunol. Today, 5 (1984) 76.
8 T. Schlunk and M. Schleyer, Blut, 47 (1983) 211.
9 M.C. Wu and A.A. Yunis, J. Clin. Invest., 65 (1980) 772.