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Note

Isolation of CSF-1 from large volumes of human urine by hydrophobic interaction chromatography

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Colony stimulating factors (CSF) are haemopoietic growth factors that act on undifferentiated bone marrow cells to produce granulocytes, monocytes and macrophages[1]. CSF-1 is a lineage-specific subclass responsible for monocyte/macrophage production. It is a glycoprotein that has been isolated from human urine[2] and from serum-free conditioned medium of human and mouse cell lines[3,4]. It is assayed by the *in vitro* formation of macroscopic colonies from bone marrow cells in semi-solid agar culture[5] or by radioimmunoassay[6].

This paper describes a procedure involving Carbowax concentration followed by column chromatography on Phenyl-Sepharose CL-4B, to partially purify CSF-1 from large volumes of human urine. Its advantage over previous methods is that it is a simple and rapid procedure for obtaining material of relatively high specific activity, *viz.*, a 75-fold purification with 74% recovery of biological activity.

EXPERIMENTAL

A 40.2-l volume of human urine was concentrated to 2 l in dialysis tubing by Carbowax 20M (Union Carbide). Sodium chloride was added until the conductivity was $3 \cdot 10^5$ μmho, equivalent to 4 *M* sodium chloride. A 500-ml volume was applied at any one time to a 22 × 5 cm I.D. column of Phenyl-Sepharose CL-4B (Pharmacia, Uppsala, Sweden), previously equilibrated with phosphate-buffered saline (PBS) (pH 7.2) containing 4 *M* sodium chloride. The effluent was monitored at 280 nm with a UV-1 single-path monitor (Pharmacia) at a.u.f.s. 20. The column was washed with two column volumes of the same buffer and eluted stepwise with decreasing concentrations of sodium chloride in PBS, at a linear flow rate of 15 cm h^{-1}. This was followed by 4 *M* guanidine hydrochloride in 10 m*M* sodium hydroxide and 20% ethanediol (pH 11) to elute firmly bound material. The chaotropic agent was removed on a 38 × 5 cm desalting column of Ultrogel AcA 202 (LKB, Bromma, Sweden) before assay for CSF-1 activity. The Phenyl-Sepharose was regenerated by washing with two column volumes of ethanol.

CSF-1 was assayed in semi-solid agar culture using mouse bone marrow cells as the target cells, as previously described[7]. A unit of CSF-1 was defined as the amount giving rise to one colony under the standardized conditions of 50,000 bone marrow cells in 1.1-ml cultures. It was determined from the linear portion of the

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titration curve. A stable urinary preparation was defined in this manner and was used as a reference in all experiments.

Protein was determined by the Coomassie Blue dye-binding assay of Bradford[8].

RESULTS

The behaviour of CSF-1 on Phenyl-Sepharose CL-4B is shown in Fig. 1. Most of the material absorbing at 280 nm passed through the column when the sample was loaded and washed with starting buffer. No CSF-1 activity was detected in the effluent, provided that the column load did not exceed the equivalent of 25 ml of native urine per millilitre of gel. Lowering the sodium chloride concentration caused CSF-1 to be eluted, the optimal concentration being 1 *M*. The high-activity fractions were pooled. CSF-1 interacted weakly with the gel matrix and was easily eluted. No activity was found in fractions showing strong hydrophobic interaction. Table I summarizes the results. There was a 75-fold purification with respect to protein and the resulting material had a relatively high specific activity of $6.4 \cdot 10^4$ units/mg protein.

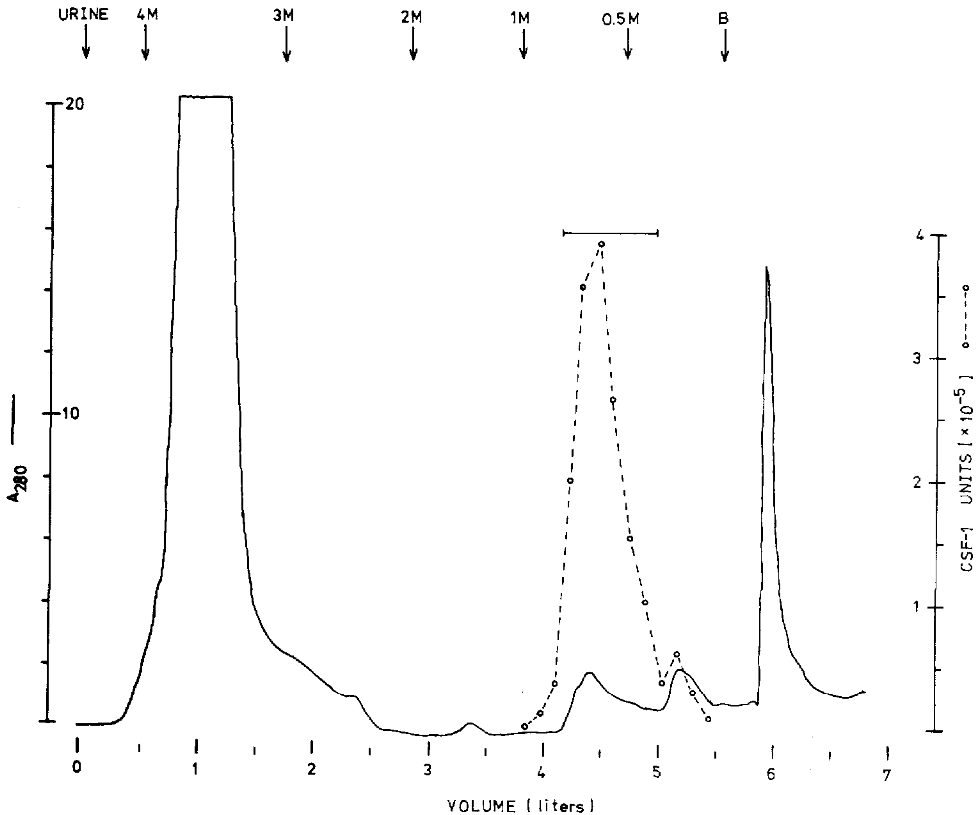

Fig. 1. Hydrophobic chromatography of concentrated human urine on Phenyl-Sepharose CL-4B, pre-equilibrated with 4 *M* sodium chloride in PBS. Stepwise elution with decreasing sodium chloride concentrations in PBS is indicated by the arrows. B indicates the changeover to 4 *M* guanidine · HCl in 10 m*M* sodium hydroxide containing 20% ethanediol. The absorbance at 280 nm was monitored and CSF-1 activity is indicated by the dotted line.

TABLE I

PARTIAL PURIFICATION OF URINARY CSF-1 ON PHENYL-SEPHAROSE CL-4B

Sample	*Protein (mg)*	*Specific activity (units/mg)*	*Purification*	*Recovery (%)*
Urine (40.2 l)	8040	850	1-fold	100
Pooled 1 *M* NaCl fractions	78.9	63,900	75-fold	74

DISCUSSION

The hydrophobic interaction of CSF-1 with Phenyl-Sepharose offers an excellent preparative purification step. At high salt concentrations (4 *M* sodium chloride) it binds reversibly to the gel matrix, thus eliminating the majority of urinary components. On lowering the salt concentration to 1 *M*, CSF-1 is eluted and separated from components that bind firmly to the gel and require chaotropic agents for their elution. The latter include other haemopoietic factors such as human erythropoietin[9] or murine interleukin-2[10]. Murine CSF-1 apparently behaves in a similar manner and can be eluted with 0.8 *M* ammonium sulphate[10].

The proposed procedure offers distinct advantages over previous techniques, where at least three chromatographic steps were required[2,11]. It is rapid and requires fewer manipulations to attain the same degree of purification with good recovery of biological activity. The resulting material had a specific activity similar to that obtainable with conditioned meedium of cell lines cultured under serum-free conditions[3,4]. However, culture conditions contribute to the heterogeneity of CSF-1. Urinary CSF-1, on the other hand, is apparently more homogeneous[2], which may be an advantage over other sources for its purification to homogeneity.

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REFERENCES

1 E. R. Stanley, in W. E. Stewart and J. W. Hadden (Editors), *The Lymphokines*, Humana, NJ, 1981, p. 102.
2 E. R. Stanley and L. J. Guilbert, in R. van Furth (Editor), *Mononuclear Phagocytes — Functional Aspects, Part 1*, Martinus Nijhoff, The Hague, 1980, p. 415.
3 M. C. Wu, J. K. Cini and A. A. Yunis, *J. Biol. Chem.*, 254 (1979) 6226.
4 E. R. Stanley and P. M. Heard, *J. Biol. Chem.*, 252 (1977) 4305.
5 T. R. Bradley and D. Metcalf, *Aust. J. Exp. Biol. Med. Sci.*, 44 (1966) 287.
6 S. K. Das, E. R. Stanley, L. J. Guilbert and L. W. Forman. *Blood*, 58 (1981) 630.
7 P. V. Byrne, W. Heit, H. Heit and B. Kubanek, *J. Lab. Clin. Med.*, 96 (1980) 903.
8 M. M. Bradford, *Anal. Biochem.*, 72 (1976) 248.
9 S. Lee-Huang, *Blood*, 56 (1980) 620.
10 M. L. Hilfiker, R. N. Moore and J. J. Farrar, *J. Immunol.*, 127 (1981) 1983.
11 H. Laukel, W. D. Gassel, H. M. Dosch, W. schmidt and K. Havemann, *J. Cell. Physicol.*, 94 (1978) 21.