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Molecular Properties of a Factor Inducing Differentiation in Murine Myelomonocytic Leukemic Cells*

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A. Introduction

The possibility of therapeutic manipulation of normal regulatory molecules in leukemia has recently gained interest with the demonstration, both in vivo and in vitro, that terminal differentiation and leukemic stem cell suppression can be induced in several mouse myeloid leukemic cell lines by normal tissue products [2–4, 8, 9].

Although a known regulator of normal hemopoietic cell growth, granulocyte-macrophage colony-stimulating factor (GM-CSF) can induce some differentiation in WEHI-3B and M1 leukemic cells [6, 8], another closely related regulator, macrophage-CSF, is devoid of such activity [1, 7]. Moreover, it is becoming clear that the potent differentiation-inducing activity of conditioned media from some cell lines, normal murine tissues, and sera from endotoxin-injected mice cannot be accounted for by their content of GM-CSF [1, 5, 7, 12].

In this report we show that a potent differentiation-inducing activity (DF) in mouse lung-conditioned medium (MLCM) can be completely separated from GM-CSF by chemical fractionation. DF has been highly purified and throughout all fractionation procedures remained associated with a granulocyte-CSF activity.

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B. Results

I. Molecular Properties of the Differentiation Factor

The apparent molecular weight of the differentiation factor (DF) in mouse lung-conditioned medium was determined on native gel filtration, gel filtration in 6*M* guanidine hydrochloride, and in 1*M* acetic acid. All methods gave apparent molecular weights for DF between 21,000 and 26,000. A similar value of 21,000 was obtained for DF by sodium dodecyl sulfate polyacrylamide gel electrophoresis (10%–15% linear gradient in acrylamide). DF showed considerable charge heterogeneity when chromatographed on a high-performance packing using the chromato-focusing technique (mono P column, Pharmacia). Although this technique is capable of separating proteins with isoelectric point (IEP) differences of less than 0.2, the DF activity was spread over a pH unit (IEP 4.8–5.8). Rechromatography of selected fractions of DF on the same column established that this was a true charge heterogeneity and not due to buffer interactions. Moreover, chromatography of DF on a high-performance ion-exchange packing (mono Q column, Pharmacia) revealed the same degree of charge heterogeneity.

II. Separation of the Differentiation Factor from the Granulocyte-Macrophage Colony-Stimulating Factor

In addition to charge separation, several other preparative techniques could be used to separate DF from GM-CSF. Although

Step	Ratio of CSF/DF activities
Mouse lung conditioned medium	3.40
DF fraction after salting out	0.87
DF fraction after phenyl-Sepharose	0.43
DF fraction after Biogel P-60	0.38
DF fraction after reverse-phase HPLC	0.40

Table 1. Dissociation of DF and GM-CSF activities during the purification of DF

there was partial separation of DF and GM-CSF using chromatography on concanavalin A-Sepharose (DF failed to bind while GM-CSF showed both binding and nonbinding components), this step proved to be of little use because of the low overall purification of DF. As shown in Table 1, there was a very good separation of DF from GM-CSF using salting out chromatography. When this was followed in sequence by chromatography on phenyl-Sepharose the remaining GM-CSF was completely separated from the DF, with a large number of fractions separating the two activities. Nevertheless, there was a biochemically distinct form of CSF that co-chromatographed with the DF. In all subsequent purification steps and analyses the DF activity remained associated with this CSF activity with no evidence of any separation, and the ratio of the two activities remained unchanged. The CSF activity associated with the DF stimulated only a small number of normal bone marrow colonies (5–20 per 75,000 cells) even at plateau doses (compared to 100–150 for GM-CSF) and, in contrast to GM-CSF, stimulated predominantly granulocytic colonies to develop. It is, therefore, designated granulocyte-CSF (G-CSF).

The current purification scheme involves salting out chromatography and phenyl-Sepharose chromatography as the first two steps because these completely separate GM-CSF from DF and result in a good overall purification (nearly 40-fold) with nearly quantitative yield. This is followed by chromatography on Biogel P-60 in 1*M* acetic acid and then reverse-phase high-performance liquid chromatography (HPLC) using a phenyl-silica based column and an acetonitrile gradient. In this step the DF/G-CSF activity was associated with a small single peak of protein and was over 18,000-fold purified relative to the starting mouse lung conditioned medium. It was half-maximally active in both the in vitro WEHI-3B differentiation and normal bone marrow colony formation assays at 10^{-11} -10^{-12}*M*, paralleling the specific activity of purified GM-CSF and M-CSF. However, stringent criteria of purity on this purified preparation of DF/G-CSF have not yet been performed.

C. Conclusions

The factor in mouse lung conditioned medium inducing differentiation of WEHI-

	DF/G-CSF	GM-CSF
Ammonium sulfate elution concentration in salting out	1.4 *M*	2.4 – 1.6 *M*
Ammonium sulfate elution concentration on phenyl-Sepharose	0	0.75 – 0.5 *M*
Binding to concanavalin A-Sepharose	–	+/–
Isoelectric point	4.8 – 5.8	4.0 – 5.0
Acetonitrile elution concentration on phenyl-silica HPLC	51%	43%
Molecular weight	21,000 – 26,000	23,000

Table 2. Different molecular properties of differentiation factor and granulocyte-macrophage colony-stimulating factor

3B murine myelomonocytic leukemic cells is different from GM- and M-CSF. This has been clearly demonstrated for M-CSF, since antibodies to M-CSF completely failed to inhibit DF activity [1, 7]. In this report it was shown that GM-CSF can be completely separated biochemically from DF and a summary of their different molecular properties is given in Table 2. Although DF appeared as a single peak of activity through most of the purification steps it did display charge heterogeneity, but the reasons for this are not yet clear. DF was highly purified by the fractionation steps described and, throughout, remained associated with a G-CSF activity. Its role in normal hemopoiesis appears to be to stimulate a small subset of granulocyte colony-forming cells. The availability of purified DF/G-CSF should allow the delineation of the mechanism of action of this molecule, the relationship of the leukemic cells to the normal cell subsets, and exploration of the usefulness of such factors in leukemic cell control.

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