A new factor capable of inducing differentiation of human leukemic cell line U-937 into macrophages was found and partially purified from the conditioned medium of U-937 previously treated with 12-O-tetradecanoylphorbol 13-acetate. The purification procedure included ultrafiltration, DEAE-Sephacel and butyl-Toyopearl column chromatography. The purified factor gave a major band of protein with a molecular weight of 67,000 daltons which coincided with the biological activity of differentiation-inducing factor, and it was not adsorbed on a concanavalin A column. These results suggest that this factor is distinct from other differentiation-inducing factors.

Key words: Macrophage differentiation-inducing factor — Human monocytic cell line — 12-O-Tetradecanoylphorbol 13-acetate

U-937, a human monocytic cell line derived from a histiocytic leukemia, is known to be induced to differentiate into macrophages by 12-O-tetradecanoylphorbol 13-acetate (TPA), metabolites of vitamin D and interferon alpha, beta and gamma. The differentiation-inducing factors (DIFs) produced by organisms have been detected as agents that induce differentiation of a mouse myeloid leukemia cell line, M1, and have been found in culture fluids of various cells and body fluids. DIFs inducing differentiation of the human promyelocytic cell line, HL-60, have been reported, but they have little or no effect on U-937 cells.

When U-937 cells were seeded at $5 \times 10^4$ cells/ml in 10 ml of RPMI 1640 with 10% fetal calf serum and grown in the presence of $1.6 \times 10^{-7} M$ TPA for 24 hr at 37°C, the cells ceased to proliferate and started differentiating into macrophages as described. After incubation, the medium was discarded and the differentiated cells were washed twice and incubated in a serum-free medium (DM160, Kyokuto, Tokyo) at 37°C for 4 days. The medium was collected every day and used as conditioned medium (CM). CM was capable of inducing the differentiation of proliferating U-937 cells (Fig. 1A and B), indicating that it may contain DIFs. The CM was concentrated at 0°C from 11 liters to about 50 ml by ultrafiltration and the DIF was partially purified by column chromatography on DEAE-Sephacel and butyl-Toyopearl, followed by high-performance liquid chromatography (HPLC) (TSK G-2000SW). The final preparation amounted to about 400 μg of protein. Even if the CM had been contaminated by TPA, the amount of it in the purified factor obtained after ultrafiltration and column chromatography would be negligible. A routine assay of DIF was conducted by observation of U-937 adhesion after overnight incubation of the cells with CM or chromatographic fractions. The biological activity was confirmed by both morphological and cytochemical methods after incubation with each fraction for 3 days (Fig. 1 C and D). The DIF caused U-937 cells to undergo a marked change in appearance. The cell adhered to the substratum and became flat; the cytoplasm became more vacuolated and the membrane, more villous. The differentiated cells showed greater NBT (Fig. 1D) and more intense nonspecific esterase staining. The DIF activity in the conditioned medium increased after washing out TPA, reaching a plateau at 24 hr (Fig. 2). DIF production continued at a maximum level for 3 days, followed by a gradual decrease (Fig. 2).
The partially purified DIF was analyzed by SDS-polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue (Fig. 3A). The major protein band had an apparent molecular weight of 67 kD (Fig. 3A). The DIF activity was found to coincide with 67 kD protein on HPLC on a gel filtration column (Fig. 3B) and was confirmed by both morphological and cytochemical methods (Fig. 1C and D). This factor was found to have no affinity for either concanavalin A-Sepharose or Affi-Gel Blue.

The data presented above show for the first time that TPA-treated U-937 cells produce a DIF capable of inducing the differentiation of U-937 cells, an effect which is not induced by other DIFs reported previously. In the human bone marrow cell culture, this conditioned medium appeared not to exhibit any colony-stimulating activity. These data indicate that this factor is distinct from all factors so far reported to regulate the differentiation of leukemic cell lines such as CSFs, INFs and DIFs. Therefore, we designated this factor as macrophage differentiation-stimulating factor (MDF).

The human monomyelocytic cell line HL-60 and mouse myeloid leukemic cell line M1 are also susceptible to this conditioned medium and were found to be differentiated into macrophages (to be published elsewhere). The MDF may be useful for the suppression of development of myeloid leukemia and its treatment. The results of our
preliminary experiments indicate that the purified MDF has activity for stimulating the differentiation of monocytes and/or activating human peripheral blood macrophages. Thus, this factor may function in the normal regulatory system of macrophage maturation and/or stimulation in vivo. Although its physiological role remains to be clarified, it is interesting that differentiated U-937 produces a factor to induce U-937 differentiation, triggering a cascade of differentiation. Since the production of this factor is transient (Fig. 2), triggering of the cascade may also be transient.

This work was supported in part by a Grant-in-Aid for Scientific Research from Fujita-Gakuen Health University.

(Received Nov. 7, 1986/Accepted Jan. 21, 1987)

REFERENCES


