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A T Lymphocyte-Derived Differentiation-Inducing Factor for Myeloid Leukemia Cells: Purification and Characterization *

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Acute myeloid leukemia (AML) is characterized by an apparent maturation arrest leading to accumulation of immature cells. Such cells obviously preserve normally transient phenotypes. Recent work also indicates that leukemic transformation does not necessarily lead to an irreversible block in cell differentiation. Leukemic cell lines which grow continuously in vitro are useful systems in the search for normal regulatory molecules which can act in leukemia. The promyelocytic HL-60 line [2] is induced into granulocytes and monocytes by incubation with a variety of agents [4]. We have concentrated our work on mechanisms of action of "physiologic" inducers of differentiation in leukemia. Nitroblue tetrazolium (NBT) reduction has been used as the parameter of induced maturation in HL-60 as well as phagocytic capacity, cell surface antigen expression detected with monoclonal antibodies, nonspecific esterase, and composition of cytoplasmic granules.

Mitogen-stimulated human mononuclear blood cells release polypeptide factors which we call differentiation-inducing factors (DIF) [5, 6]. These induce HL-60 cells to mature into phagocytizing monocyte-like cells, which can reduce NBT. Gel chromatography has showed that mitogen-stimulated cells release one or two species of DIF with apparent molecular weights of 40 000 and 25 000, depending on which mitogen was used. At least the 40 000 molecular weight species of DIF was distinct from colony-stimulating factor (CSF). It was subsequently found that the T lymphocyte leukemic cell line HUT-102, established from a patient with an adult lymphoma of mature T cells and which releases the human retrovirus called human T cell leukemia virus (HTLV), is a reliable constitutive producer of DIF [8]. Large-scale production of HUT-102 supernatants can therefore be employed to obtain enough starting material for purification of DIF.

DIF has now been purified more than 10 000-fold from HUT-102 conditioned media utilizing ion exchange chromatography on DEAE–sepharose, gel filtration on Sephadex G-75, ion exchange chromatography on Mono Q, and reverse-phase chromatography utilizing the Pharmacia Pro RPC column. DIF appeared homogeneous on gel filtration with a molecular weight of approximately 60 000 while SDS–polyacrylamide gel electrophoresis revealed a somewhat lower molecular weight. However, high resolution chromatography on an ion exchange Mono Q column using the fast pressure liquid chromatography (FPLC) system, which resulted in substantial purification, also revealed considerable charge heterogeneity. Thus, DIF eluted in three separate peaks at an ionic strength of 0.05–0.11 *M* NaCl pH 8.0. Chromatofocusing on a Mono P column confirmed the charge heterogeneity of DIF, which was eluted in the pH range 5.6–5.9. Highly purified DIF eluted from the Mono Q column was also subjected to reverse-phase chromatography using the Pro RPC Hr 5/10 column with the FPLC system.

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DIF was eluted with 44% acetonitrile. Substantial purification was achieved in this step, but it has not yet been possible to elute DIF with a single protein peak. DIF is protein in nature as it is destroyed by proteases. It is relatively heat stable, resisting heating to 60 °C–70 °C. It is not inactivated by periodate oxidation and shows no affinity to lectins.

DIF acts synergistically with retinoic acid (RA) to induce maturation not only of HL-60, but also of the monoblast-like cell line U-937 (measured as percentage of cells reducing NBT) [7]. The synergistic effect indicates that DIF and RA act by different mechanisms to induce differentiation. Other inducers such as dimethylsulfoxide (DMSO), 1,25-dihydroxycholecalciferol, actinomycin D, and cAMP-inducing agents like cholera toxin and PGE_2 act additively with DIF to induce maturation of HL-60. The finding of a synergistic effect between RA and DIF prompted a study on the effects of sequential treatment with these agents. Both HL-60 and U-937 could be "primed" for differentiation by treatment for 10–20 h with low concentrations of RA followed by exposure to low concentrations of DIF. Priming with RA for the inducing effect of DIF did not depend on the normal rate of protein synthesis as it occurred even better in the presence of 1 μg/ml cycloheximide, a concentration which inhibited growth completely and protein synthesis by 86%. However, the resulting maturation obtained by addition of DIF was, as expected, inhibited by cycloheximide. Our results suggest that a decrease in synthesis of some unknown protein or proteins favors maturation of the leukemic cells.

HL-60 cells have a very high self-renewal capacity. However, some spontaneous maturation occurs toward granulocytes and monocytes, which may fit a stochastic model. If probabilities for commitment to granulocyte and monocyte are P_1 and P_2, respectively, the probability for self-renewal will be $1-(P_1+P_2)$. P_1 is increased by DMSO and RA. P_2 is increased by DIF, cAMP agents, phorbol esters, and cholecalciferol. With most inducers, probabilities for commitment are very high so that self-renewal is lost and the culture terminated. The mechanism by which DIF induces maturation into monocyte-like cells is not known. Since DIF is a polypeptide, it presumably acts through a cell surface receptor. The second messenger is not cAMP, since the latter was not increased upon addition of DIF and since adenyl cyclase inhibitors did not abolish the effect. The DIF effect was not blocked by inhibitors of calmodulin. Furthermore, the DIF effect is independent of cell proliferation since it occurred equally well in the presence of polyamine inhibitors or other agents, which blocked cell proliferation completely. It is clear that different agents can act by different mechanisms, even to induce more or less identical end stage cells. Defining the mechanisms of action of several inducers may therefore help to identify regulation of differentiation in hemopoiesis and leukemia.

The relationship, if any, of HUT-102-produced DIF to other previously described lymphokine factors is not yet established. The highly purified DIF was completely devoid of CSF activity. This does not rule out that some CSF may induce differentiation of leukemic cells as described in the mouse system [1, 3]. The physiochemical properties of DIF eliminate its identity with interleukin-1, -2, or -3. Interferon-γ did not alone induce maturation of HL-60. Furthermore, a neutralizing antibody for interferon-γ did not abolish the DIF effect on HL-60. It is concluded that DIF and interferon-γ are separate molecules.

Conclusions

DIF is a polypeptide produced by mitogen-stimulated human mononuclear blood cells and constitutively by certain T lymphocyte lines such as the HUT-102 line. It induces maturation of the HL-60 cell line into monocyte-like cells. DIF is *not* identical with CSF, interleukin-1, -2, or -3, or interferon-γ. Using supernatants from the HUT-102 line, it has been purified more than 10 000-fold and its chemical properties have been defined. Its role in hemopoiesis and leukemia now remains to be determined.

References

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