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Human Leukemic Lymphocytes – Biochemical Parameters of the Altered Differentiation Status

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A. Introduction

Deviation from normal differentiation is a general phenomenon of tumor cells. To study the misprogramming of normal gene function adequately, systems should be analyzed that are not complicated by concomitant changes in cell proliferation rates. A cell system in which the normal and the corresponding neoplastic cells do not proliferate at all is represented by normal human blood lymphocytes and lymphocytes from patients with chronic lymphocytic leukemia. Both cell types exhibit very low (^{3}H)thymidine incorporation (Wielckens et al. 1980), confirming that both types of lymphocytes are in the G_0 phase of the cell cycle. Thus, the leukemic lymphocyte is characterized exclusively by differentiation defects as represented by delayed or missing response to phytohemagglutinin and extended life span (cf. Havemann and Rubin 1968; Bremer 1978).

Altered differentiation of neoplastic cells should relate to alterations in cellular regulation. We therefore studied two parameters of posttransscriptional protein modification: the proteinphosphorylation system as represented by the protein kinase and the ADP ribosylation of nuclear proteins.

B. Protein Kinases and Regulatory Subunits

In recent years isolation of human lymphocytes has usually been performed with the aid of Ficoll/Metrizamide gradients. This procedure appears to induce marked changes of biochemical parameters. Using Percoll (Pharmacia) instead lymphocytes could be obtained with excellent preservation of the biochemical integrity (Wielckens et al. 1980). When normal human lymphocytes isolated by the Percoll-procedure were analyzed for protein kinases, about equal activities of histone kinase and casein kinase were found (Table 1). Of the total histone kinase activity, the cAMP-dependent enzyme comprised about 50% in both normal and leukemic lymphocytes as shown by immunotitration and by the use of the heat stable inhibitor. However, leukemic lymphocytes exhibited drastically reduced values of all three protein kinase activities, the most pronounced decrease (to 7% of the normal control) being observed with casein kinase.

The cAMP-dependent protein kinases in mammalian cells represent tetrameric structures composed of two catalytic and two regulatory subunits. When total regulatory subunits R were analyzed again, a reduction in leukemic lymphocytes to <20% of normal lymphocytes was found. These changes in the protein kinase system are paralleled by comparable alterations of basal cAMP levels. The data show that the functional aberrations of leukemic lymphocytes are associated with marked alterations in an important pathway of cellular regulation that uses multiple protein kinases to effect functional changes in proteins by phosphorylation.

C. ADP Ribosylation of Nuclear Proteins

Adenosine diphosphate ribosylation is a mechanism of covalent modification of nuclear proteins by enzymatic transfer of the ADP-ribose moiety from NAD, leading to the formation of mono (ADP-ribose)-protein and poly (ADP-ribose)-protein conjugates. Histones

Table 1. Protein kinases, total regulatory subunits R, and basal cAMP levels in normal and leukemic lymphocytes.[a]

Parameter	Normal	Leukemic	Leukemic / Normal
Protein Kinase (pmol incorp./min × 10^6 cells)			
"Casein kinase"	145 ±29	10 ±8	0.07
Histone kinase	174 ±29	32 ±3	0.18
– cAMP independent	87 ± 3	16 ±1	0.18
– cAMP dependent	87 ± 3	16 ±1	0.18
Total regulatory subunits R (pmol binding sites/10^6 cells)	123.3 ±40.2	23.0 ±5.7	0.19
cAMP level (pmol/10^6 cells)	6.02± 2.46	0.31±0.21	0.05

[a] For experimental details see Hilz et al. (1981, in press)

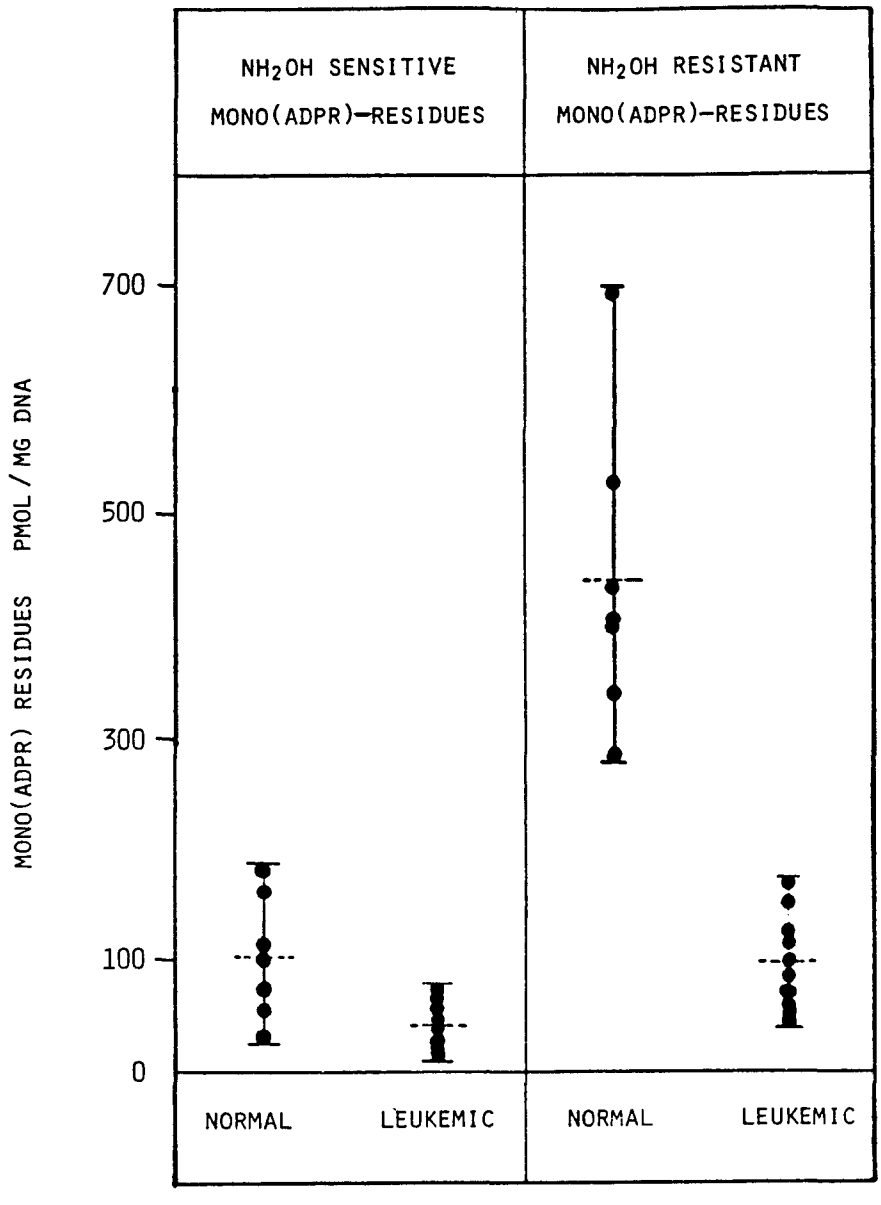

Fig. 1. Levels of hydroxylamine (NH_2OH) sensitive and resistant mono(ADPR)-in normal and leukemic lymphocytes. Taken from Hilz et al. (1980). For experimental details see Wielckens et al (1980)

and nonhistone proteins can serve as acceptors. Recent data suggest that poly ADP ribosylation of protein is involved in DNA repair processes while modification by mono (ADP-ribose)-residues of nuclear proteins appears to be involved in the maintenance of cellular differentiation (Juarez-Salinas et al. 1979; Hilz et al. 1980). Therefore, quantitation of mono (ADP-ribose)-protein conjugates was of special interest. Using a sensitive radioimmunological procedure, two types of mono(ADP-ribose) protein conjugates could be determined that differ in their sensitivity towards neutral NH_2OH. From analyses in the cell cycle (Wielckens et al. 1979), it had become evident that the two subfractions of the mono(ADP-ribose)-conjugates are independently synthesized and therefore may serve independent functions in the chromatin. Determination of these conjugates in differentiating Dictyostelium discoideum (Bredehorst et al. 1980) and during liver development (Hilz et al. 1980) had indicated that it is primarily the subfraction of the NH_2OH resistant mono-(ADP-ribose)-conjugates that correlated with the degree of (normal) differentiation.

When normal and leukemic lymphocytes were analyzed for the extent of protein modification by mono(ADP-ribose)-residues, a marked reduction was noticed in the chronic

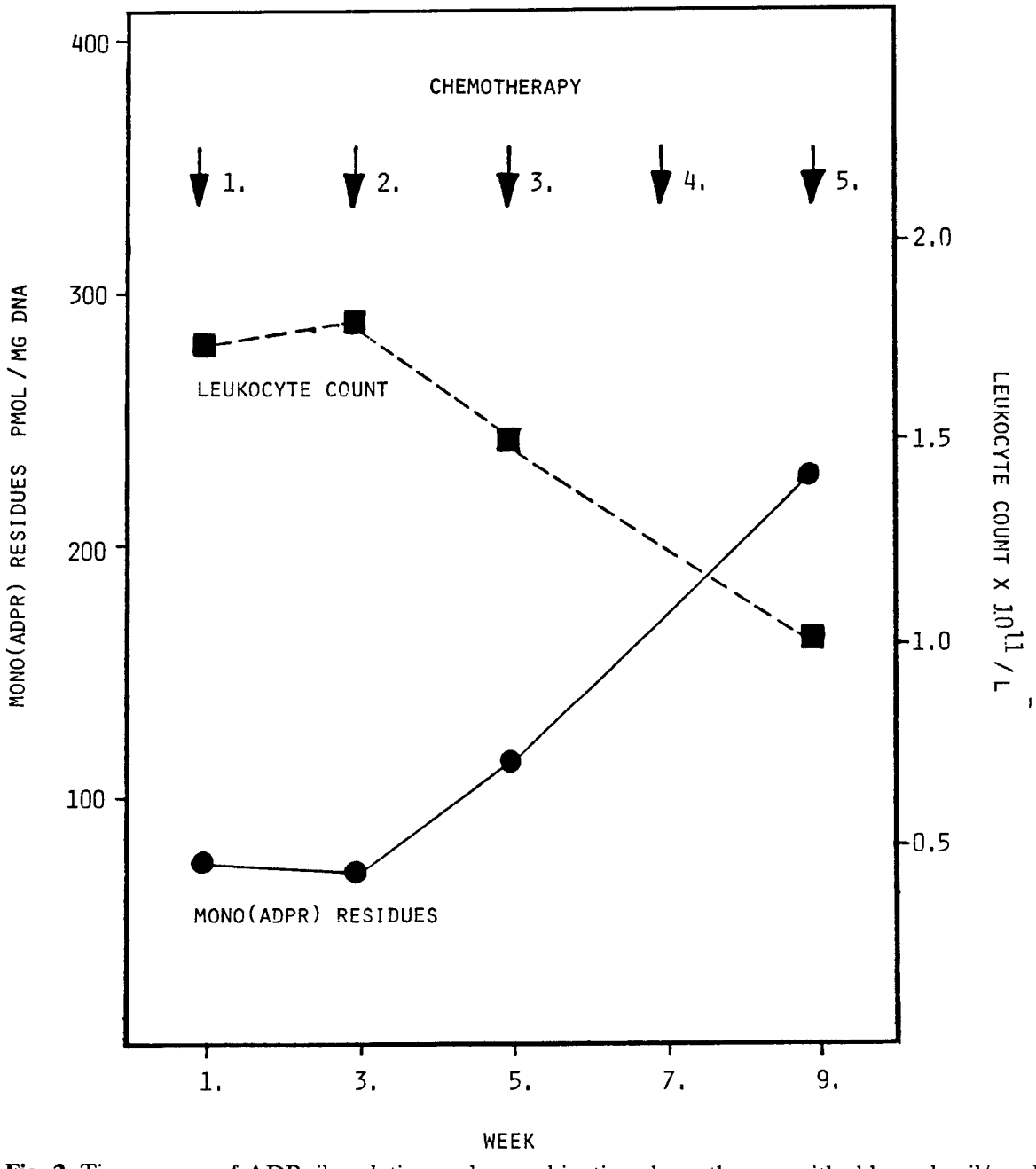

Fig. 2. Time course of ADP ribosylation under combination chemotherapy with chlorambucil/prednisone. Data from one patient with CLL. For experimental details see Wielckens et al. (1980)

lymphocytic leukemia (CLL) cells. This low level of mono(ADP-ribose)-protein conjugates, however, retained the NH_2OH sensitive subfraction only to a limited degree, the main loss being due to a pronounced diminution of the NH_2OH resistant conjugates. (Fig. 1). In all patients so far tested the values of this subfraction were well below the normal range.

The low degree of ADP ribosylation found in leukemic lymphocytes is not the consequence of chemotherapy, because patients with and without chemotherapeutic treatment were studied. Moreover, in a case of effective treatment, a progressive decrease in leukocytes was associated with increasing levels of ADP-ribose residues (Fig. 2).

The marked alterations of the ADP ribosylation status in lymphocytes from patients with CLL is characteristic for that disease and not the result of a shift to a lymphocyte population rich in B-type cells: Isolated B-lymphocytes from normal donors exhibited the same degree of mono(ADP)-ribosylation as total blood lymphocytes with their preponderance of T lymphocytes (Wielckens et al. 1980).

D. Conclusions

Marked reductions in various protein kinases, including the cAMP-dependent enzyme and its regulatory subunits, represent a for-reaching restriction of the specific functions of CLL lymphocytes compared to normal blood lymphocytes. This expression of dedifferentiation may be the consequence of a modified chromatin function as indicated by the altered extent of nuclear protein modification by mono(ADP-ribose)-residues. Since CLL cells represent a G_0 type tumor cell, the alterations in nuclear mono (ADP)-ribosylation appear to be characteristic of tumors independently of their growth rate. Therefore, quantitation of the NH_2OH resistant protein conjugates may become a general tool to determine the degree of differentiation in tumor cells.

Acknowledgments

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References

Bredehorst R, Klapproth K, Hilz H, Scheidegger C, Gerisch G (1980) Protein-bound mono(ADP-ribose) residues in differentiating cells of dictyostelium discoideum. Cell Differ 9:95–103 – Bremer K (1978) Cellular renewal kinetics of malignant non-Hodgkin's lymphomas. Recent Results Cancer Res 65:5–11 – Havemann K, Rubin A (1968) The delayed response of chromic lymphocytic leukemia lymphocytes to phytohemagglutinin in vitro. Proc Soc exp Biol Med 127:668–671 – Hilz H, Adamietz P, Bredehorst R, Wielckens K (1980) Covalent modification of nuclear proteins by ADP-ribosylation: Marked differences in normal and tumor tissues. In: Wrba H, Letnansky K (eds) Kugler, Amsterdam, pp 409–414 – Hilz H, Schwoch G, Weber W, Gartemann A, Wielckens K (1981) Eur J Biochem (in press) – Juraz-Salinas H, Sims JL, Jacobson MK (1979) Poly (ADP-ribose) in carcinogen treated cells. Nature 282:740–742 – Wielckens K, Sachsenmaier W, Hilz H (1979) Protein-bound mono(ADP-ribose) levels during the cell cycle of the slime mold physarum polycephalum. Hoppe-Seylers Z Physiol Chem 360:39–43 – Wielckens K, Garbrecht M, Kittler M, Hilz H (1980) ADP-ribosylation of nuclear proteins in normal lymphocytes and low-grade non-Hodgkin lymphoma cells. Eur J Biochem 104:279–287