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Glucocorticoid-Induced Lysis of Various Subsets of Acute Lymphoblastic Leukemia

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A. Introduction

Glucocorticoids are regularly employed in the therapeutic regimes of hematologic malignancies. In attempting to understand partially the therapeutic effect of these hormones, the in vitro cortisol-induced lysis of leukemic cells was studied. In previous studies [2], we have shown that the viability of malignant cells from chronic and acute myeloid leukemias was not affected by 20 h incubation with 10^{-5} *M* cortisol. Chronic lymphatic leukemic cells, however, were readily lysed by cortisol. The cells of acute lymphoblastic leukemia (ALL) patients were divided into two groups; those that were resistant to lysis and those that were sensitive. The purpose of the present study was to correlate sensitivity to cortisol-induced lysis to the phenotype of the ALL cells as defined by monoclonal antibodies and rosetting capacity.

B. Materials and Methods

Thirty-nine patients with ALL were studied. These included new cases at presentation and relapse patients. Most cells were isolated from bone marrow samples, but in several cases in which there were greater than 80% blast cells in the peripheral blood, blood lymphocytes were analyzed.

I. Cell Sensitivity to Glucocorticoids

Briefly, aliquots of 0.2 ml cells (10^6cells/ml) were incubated in flat bottom microwells (Cooke) for 20 h at 37 °C in a humidified CO_2-air (5% : 95%) atmosphere with 10^{-5} *M* cortisol (Ikapharm, Israel). Since a variable proportion of the cells incubated with the glucocorticoids was lysed within 20 h, the amount of cells lysed was assessed by determining the concentration of the remaining viable cells (Trypan-blue exclusion test) in a hemocytometer with a magnification of 400. Their percentage (% lysis) was calculated according to the formula: $(a-b)/a \times 100$, where "a" is the concentration of viable cells in the wells containing medium without steroids and "b" equals the concentration of viable cells in wells containing the drug.

II. Phenotyping of the ALL Cells

The ALL cells were phenotyped using a variety of monoclonal antibodies (see list below) in an indirect immunofluorescent assay and E-rosettes. If the cells typed "common" ALL, they were then examined for the presence of intracytoplasmic IgM (cold acetone-fixed cytospin preparations stained with rabbit antihuman IgM-FITC) to determine whether the cells were of the pre-B phenotype. All cells were also tested with an affinity purified antiterminal deoxynucleotidyl transferase.

Monoclonal antibodies:
J-5 (anti-common ALL [4])
DA-2 (anti-HLA-DR [1])
S33 (anti T cell; produced by Peter Beverly, ICRF, London)
WT-1 (anti T cell [5]).

Cells reacting with either or both S33 and WT-1 are referred to as T^+ cells. Cells that react with DA-2 are noted Ia^+ and those reacting with J-5 are noted $J\text{-}5^+$. Greater than 20% staining is considered positive for the particular antigen.

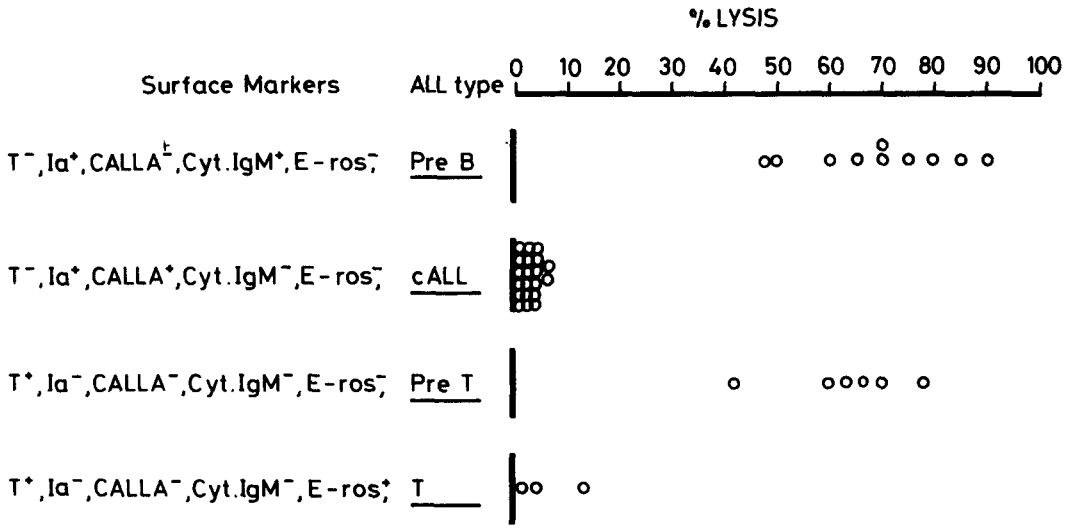

Fig. 1. Cortisol-induced lysis of leukemic cells of various ALL types

C. Results and Discussion

The leukemic cells from 22 patients with common ALL ($J\text{-}5^+$, Ia^+, T^-, E^-) were found to be resistant to lysis following incubation with 10^{-5} *M* cortisol. In contrast, the cells from ten patients with pre-B leukemia ($J\text{-}5^+$ Ia^+ $cIgM^+$ T^- E^-) were readily lysed by cortisol (Fig. 1). Cells from five patients with early or pre-T leukemia ($J\text{-}5^-$ Ia^- T^+ E^-) were similarly sensitive to cortisol-induced lysis, whereas cells from three patients with a more mature T-cell phenotype ($J\text{-}5^-$ Ia^- T^+ E^+) were found to be resistant.

Normal cell populations have been shown to differ in their steroid sensitivity [2]. The human prothymocyte subpopulation was easily lysed by cortisol whereas the thymocytes and peripheral blood lymphocytes were resistant. Interestingly, immunologically activated T-lymphocytes were also found to be sensitive to steroid-induced lysis [3]. Normal bone marrow and polymorphonuclear cells were completely resistant. The steroid sensitivity of the T-ALL cells parallels that of their normal counterparts according to their differentation state. The sensitivity of the pre-B ALL versus the resistance of the cALL cells may likewise be attributed to a differentiation state related phenomena.

The in vivo relavence of this in vitro assay is currently under study. It is suggested that use of this assay may allow for a more efficient use of the steroids in the treatment of leukemic malignancies.

References

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