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A Model Scheme of Hematopoietic Cell Differentiation Based on Multiple Marker Analysis of Leukemia–Lymphomas: T Cell Lineage

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A. Introduction

The analysis of human leukocyte differentiation antigens has been greatly facilitated by studies of leukemia and lymphoma cells [1]. Because of the two characteristics common to both leukemia and lymphoma, i.e., single clonal cell population of respective hematopoietic cell type and arrested differentiation profile in each case, studies of multiple marker analysis and in vitro induced cellular differentiation system by a variety of chemical and natural inducers have continued to provide steady progress in this area of knowledge. Furthermore, recent advances in molecular biology relative to cytogenetic nonrandom abnormalities in the hematopoietic malignancies provide further insight into pathophysiology of these diseases [2].

We have been primarily interested in characterizing both permanent leukemia –lymphoma cell lines and fresh uncultured leukemia–lymphoma cells by means of multiple marker analysis. At present, we have a total of 85 proven human leukemia –lymphoma cell lines and a couple dozen of their clonal sublines and mutants in the laboratory. These lines include T cell, B cell, lymphoid precursor, myelomonocyte, erythroid, and histiocytic lineages. Over 675 cases of patients with various types of hematopoietic malignancies have also been studied in the laboratory. Despite a continuous hope of finding a "tumor-specific" marker, all of the markers thus far studied were of normal gene products, except cytogenetic abnormalities [3]. Consequently, we have been able to propose a hypothetical differentiation scheme of human hematopoietic cells which are assumed to be reflected by the marker profiles of these tumor cells [4].

In our earlier studies [5], we were able to divide such a model scheme of T cell differentiation into five stages of maturation. The present report summarizes the results of 30 permanent T cell leukemia–lymphoma cell lines and 58 cases of patients with T cell malignancies.

B. Material and Methods

I. Cell Lines and Fresh Leukemia–Lymphomas

A total of 85 factor-independent leukemia –lymphoma cell lines were maintained in RPMI medium 1640 supplemented with 5%–10% heat-inactivated fetal calf serum at 37 °C. Details of each cell line establishment and characterization have previously been reported [4]. Mononuclear cells were prepared by Hypaque–Ficoll gradient centrifugation for the fresh leukemia–lymphoma study.

II. Multiple Marker Analysis

Multiple marker analysis of leukemia –lymphoma has been developed in our

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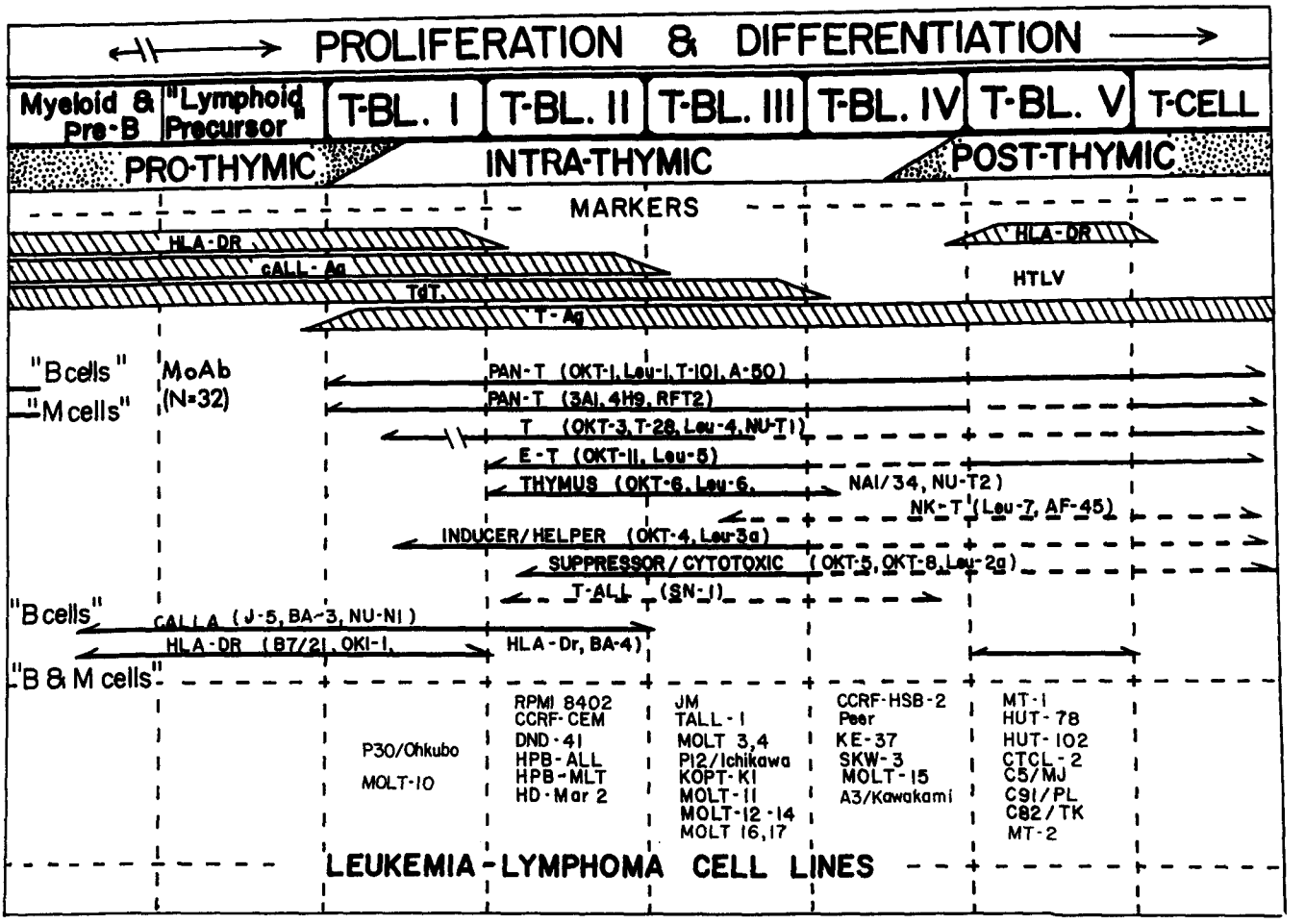

Fig. 1. T cell differentiation model. *Hatched horizontal bars* represent HLA-DR, cALL-Ag, TdT, and T-Ag detected by rabbit polyclonal antibody reagents. *Full horizontal line* represents antigen expression detected by MoAb. Portion of *broken line* in the *full line* indicates that the antigen may not be detectable on all cells within respective differentiation stages. "B cells" and "M cells" denote certain B cell and myelomonocytic cell subsets which are cross-reactive with respective MoAb

laboratory [1]. The analysis includes rosette assay, immunofluorescence assay, enzyme assay, cytochemical assay, cytogenetic assay, and certain functional assays. In the present report, in addition to the polyclonal rabbit antisera to pan-T cell antigens, HLA-DR antigens, common ALL-associated antigens, pan-myelomonocytic antigens, terminal deoxynucleotidyl transferase antigens, EB virus antigens, HTLV antigens, and immunoglobulin chains, a large battery of murine monoclonal hybridoma antibodies were also used for the immunofluorescence assay.

C. Results and Discussion

I. Five Stages of T Cell Differentiation

It is conceivable that the marker profiles of malignant T cells may be an aberrant expression of these markers, not reflecting their normal counterparts. However, this possibility has never been proved with unequivocal experimental evidence. On the contrary, these marker studies of leukemia–lymphomas have been taken as a mirror image of what normal hematopoietic differentiation should be.

Figure 1 illustrates the five stages of our T cell differentiation model. The 30 T cell leukemia–lymphoma cell lines are assigned into respective T blast stages according to individual marker profiles. The possible relationship of the T cell differentiation steps involved in the thymic environment is shown at the top of the figure. Four conventional markers as determined by polyclonal antibodies (HLA-DR, cALL-Ag, TdT-Ag, and pan-T-Ag) are the principal marker combination by which the staging

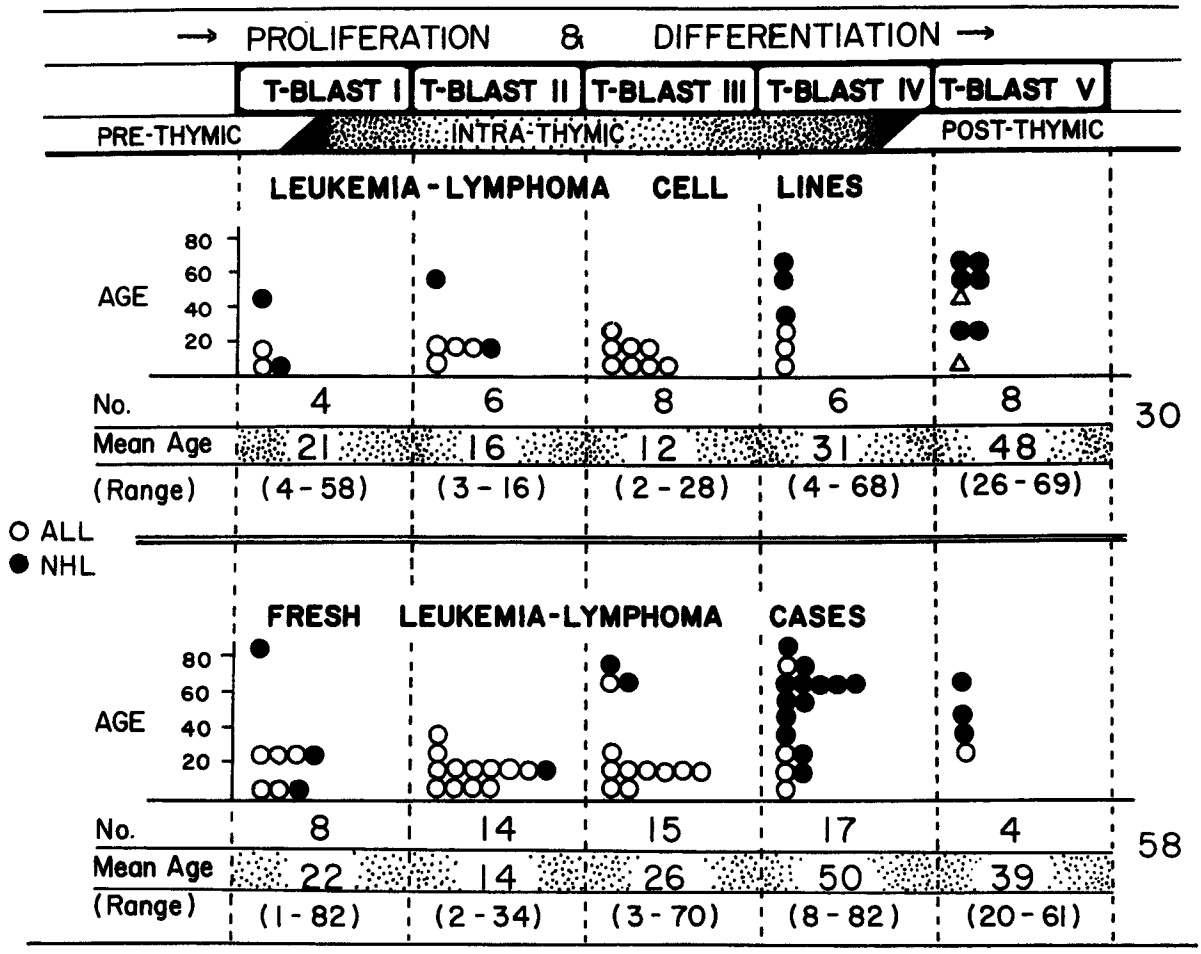

Fig. 2. Correlation among T cell malignancies of ALL or NHL manifestation, stage of differentiation arrest in T cell tumors, and patient's age

was made [5]. A total of 32 murine monoclonal antibodies (MoAb) is utilized and their expression along the T cell differentiation is also illustrated in the Fig. 1. It should be noted that no single MoAb among them fulfills the requirement of "pan-T" specificity in the strict sense. Those which are reported as pan-T MoAb are found to react with certain B cells ("B cells"), myelomonocytic cells ("M cells"), or both. Caution in the use of MoAb and choice of MoAb panel for a particular purpose is necessary. Furthermore, evidence for wider cross-reactivity of some MoAb has been documented. Nevertheless, the exquisite specificity and unlimited availability of those MoAb would enhance the importance and significance of multiple marker analysis for leukemia–lymphoma diagnosis and for basic hematology –immunology research [6–10].

II. Differentiation Arrest of T Cell Tumor Cells and Ages of Patients

As summarized in Fig. 2, there appears to be a significant correlation between the age of individual patients with T cell malignancy and the stage of differentiation arrest of malignant T cell tumor cells. Reviewing a total of 33 T cell lines for their donors' ages, it became apparent that a majority of T cell lines in T blast I, II, and III stages were derived from the patients in a younger age bracket as opposed to those of T cell lines in T blast IV and V which were mainly derived from older patients. This correlation is also related to conventional diagnosis between ALL and non-Hodgkin's lymphoma (NHL). The upper portion of Fig. 2 illustrates these observations. Two T cell lines (MT-2 and C82/TK) are excluded from the analyses for mean age and age range owing to the fact that they are in vitro-transformed normal T cells by HTLV (human T cell leukemia–lymphoma virus) (marked by triangles in Fig. 2).

Because of the tissue culture environment as a limiting as well as a selection factor, analysis was extended to fresh uncultured T cell leukemia–lymphomas. As illustrated to the lower portion of Fig. 2, a total of 58 cases of patients with T-cell tumor demonstrate essentially the same features. Neither the reasons for such a discrete differentiation arrest of T cell tumor cells relative to the host patient's age nor for the form of clinical manifestation of T cell tumor (ALL versus NHL) are known at the present time. The present study nonetheless may provide further insight into the pathophysiology of hematopoietic malignancy.

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