Haematology and Blood Transfusion Vol. 28
Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler

A Model Scheme for Human Hematopoietic Cell Differentiation as Determined by Multiple Markers of Leukemia-Lymphomas*

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A. Introduction

The analysis of leukocyte differentiation has been greatly enhanced by developments in three areas of methodology: Firstly by the establishment of relatively stable permanent hematopoietic cell lines of a variety of origin, secondly by the development of numerous specific polyclonal and monoclonal heterologous antibodies to various leukocyte antigens, and thirdly by the introduction of many functional assays of both hematopoietic progenitor cells and mature leukocyte subsets aided by various new cell culture and cell separation procedures.

We have been primarily interested in characterizing both permanent leukemia-lymphoma cells and fresh uncultured leukemia-lymphoma cells. At the present, we have a total of 74 proven human leukemia-lymphoma cell lines in the laboratory. These lines include T-cell, B-cell, lymphoid precursor, myelomonocyte, erythroid, and histiocytic lineages [6, 7]. The advantages of using leukemia-lymphoma are twofold: (1) individual leukemia-lymphoma presents an expanded monoclonal population and (2) the marker profile reflects an arrested stage of various point of hematopoietic cell differentiation [4, 5]. Furthermore, all antigens found in the leukemia-lymphoma cells are not tumor specific, but these antigens appear to be the normal gene products. In contrast, the normal hematopoietic cell populations are extremely heterogeneous, with a polyclonal population. For this reason, studies on markers of normal immature cells often lead to equivocal findings.

The present report is limited to some aspects of T-cell leukemia-lymphomas.

B. Materials and Methods

I. Cell Lines and Fresh Leukemia-Lymphomas

A total of 74 factor-independent leukemia-lymphoma lines were maintained in RPMI 1640 medium supplemented with 5%–10% heat-inactivated fetal calf serum. Details of each cell line establishment and characterization have previously been described [5–7]. Mononuclear cells were prepared by the Hypaque-Ficoll gradient centrifugation for the fresh leukemia-lymphoma study.

II. Multiple Marker Analysis

Multiple marker analysis has been developed in our laboratory [6]. The method includes rosette assay, immunofluorescence assay, enzyme assay, cytochemical assay, and cytogenetic assay. In this study, in addition to the polyclonal rabbit antisera to T-cell antigens, Ia-like antigens, common ALL antigens, myelomonocytic antigens, terminal transferase antigens, EB virus antigens, and immunoglobulin chains, a large battery of murine monoclonal hybridoma antibodies were also used for the immunofluorescence test. Cytogenetic analysis was kindly performed by Dr. A. Sandberg and his associates of our Institute, using various banding methods.

* Supported by USPHS Grants CA-14413 and AI-08899

C. Results and Discussion

The present report is limited to some findings associated with 22 T-cell leukemia-lymphoma cell lines and 24 cases of fresh T-cell leukemia-lymphomas.

I. Identification of the Marker Profile for the Earliest T-cell Differentiation

Two of the 22 T-cell leukemia-lymphoma cell lines (P30/Okubo and MOLT-10) and 3 of the 24 fresh T-cell leukemia-lymphomas were found to exhibit a new type of marker profile. The markers included T-cell antigen (T-Ag), common ALL antigen (cALL), terminal transferase (TdT), and Ia-like antigen (Ia). Definitive B-cell marker (immunoglobulins) and myelomonocyte antigens (MAg) were not detected by these leukemic cells, and thus it was concluded that the marker profile is of T cells.

The thymus antigen as defined by the monoclonal antibodies (OKT-6, Leu-6, and NA1/34) was detected in half the T-cell leukemia lines and in two-thirds of the fresh T-cell leukemias of this phenotype, respectively. The findings suggest that the expression of the thymus antigen is within this early stage of T-cell differentiation. Other antigen systems, such as Inducer/Helper (OKT-4, Leu-3A), Suppressor/Cytotoxic (OKT-8, Leu-2A) are also expressed at such an early stage of the T-cell differentiation. As already described [7], the present finding contradicts in part with the model of human T-cell differentiation scheme reported by Reinherz et al. [8].

II. Five Stages of the T-cell Differentiation

It is conceivable that the phenotype of such earliest T-cell differentiation is based on the malignant T-cell leukemias and hence might be an aberrant expression of the antigen profile, not reflecting its normal counterpart. From studies in the past, however, it is still strongly suggested that an extremely small number of normal T cells may express such a phenotype during or before their differentiation in the thymus. This possibility is further strengthened by the fact that the lymphoid precursor with the phenotype of Ia^+, $cALL^+$, and TdT^+ is assumed to be the cells one step earlier than both T-cell and B-cell lineages.

Figure 1 illustrates the five stages of our T-cell differentiation model. Twenty-two

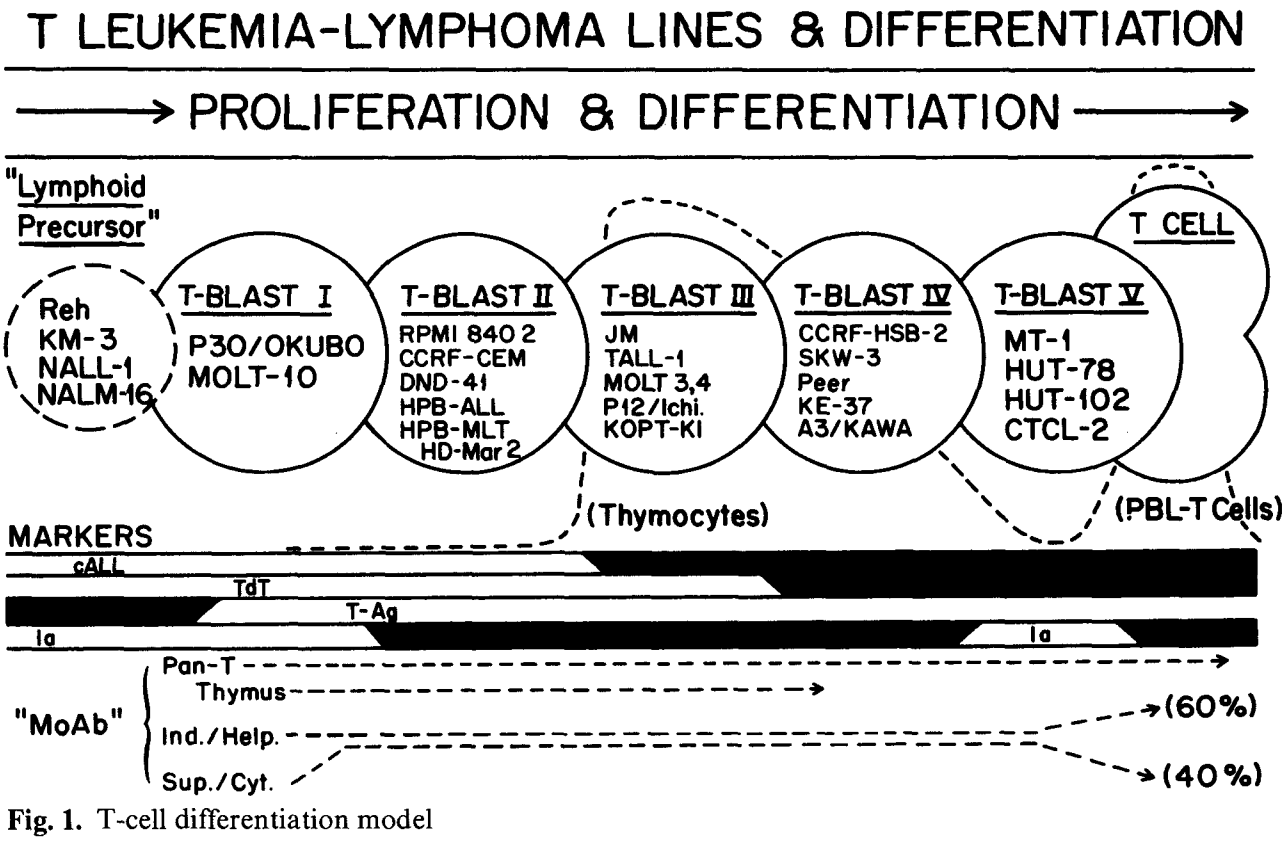

Fig. 1. T-cell differentiation model

T-cell leukemia-lymphoma cell lines are assigned into respective T-blast stages according to individual marker profiles. Four non-T, non-B common ALL cell lines (Reh, KM-3, NALL-1, and NALM-16) are also shown in the "lymphoid precursor" compartment in Fig. 1. Data from analysis with murine monoclonal antibodies (MoAb) are very complicated, and in fact some may not be associated with differentiation as such.

III. Antigens Associated with "Natural Killer Cells" and "Human T-cell Leukemia Virus" (NK- or HTLV-Related Antigens)

Using the monoclonal antibody to an antigen associated with the "natural killer" subpopulation (Leu-7: [1]), three T-cell leukemia cell lines (CCRF-HSB-2, JM, and P12/Ichikawa) among a total of 22 T-cell leukemia-lymphoma lines tested were found to express this "NK" antigen. Interestingly, another monoclonal antibody (AF-45), which had been raised against human prostate cancer cells, reacted only with those three T-cell leukemia lines (CCRF-HSB-2, JM, and P12/Ichikawa). As shown in Fig. 1, while CCRF-HSB-2 is represented in the stage of "T-Blast IV", both JM and P12/Ichikawa are represented in the stage of "T-Blast III". Significance of the finding, however, remains to be determined.

Gallo and his associates [3] have isolated a novel C-type retrovirus from some of the cutaneous T-cell lymphomas. They subsequently developed a monoclonal hybridoma antibody (RG-p19) to a 19,000 mol. wt. viral protein [9]. By the RG-p19 antibody, three T-cell leukemia-lymphoma cell lines (CTCL-2, HUT-102, and MT-1) were found to be positive for the HTLV p19 antigen. All three T-cell lines which had been derived from the Sezary syndrome, mycosis fungoides, and Japanese adult T-cell leukemia, respectively, are represented in the stage of "T-Blast V" (mature T-cells), as shown in Fig. 1. Thus it is of significance that among other possibilities the HTLV infects and manifests itself only in the mature human T cells with the subset phenotype of Inducer/Helper (data not shown).

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