Prognostic Significance of Lymphocyte Density Distribution Profiles in Adult Non-Hodgkin Lymphomas

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Biopsy material from 24 adults with advanced stages of non-Hodgkin lymphomas (NHL) were examined for the distribution profiles of infiltrating cells following centrifugation to equilibrium on linear density gradients. Seven of these biopsies were predominantly composed of cells with high buoyant densities and 9 further biopsies predominantly of cells with intermediate buoyant densities. Both patterns were associated with favorable histologic features and with low proliferation of lymphoma cells. Intensive chemotherapy was rarely required to achieve long-lasting disease control; in both groups, only 28% of patients died within a minimal observation period of 40 mo. In 8 biopsies, a predominance of light lymphoma cells was observed. This pattern was frequently associated with unfavorable histology and with high spontaneous tumor cell proliferation. Despite intensive polychemotherapy, rapid disease progression occurred in all cases, leading to death in 75% of the patients within a minimal observation period of 40 mo. Surface marker studies excluded the hypothesis that only the variable proportions of normal lymphocytes contaminating the lymphoma suspensions were responsible for the differences in the density distribution patterns described above; they rather suggested that these patterns reflect the individual capacity of a lymphoma to differentiate into dense tumor clones with low spontaneous proliferation.

RECENT STUDIES of normal human lymphocytes isolated from various peripheral organs suggested that the density patterns of these cells are mainly determined by the stage of their functional activation.1,2 whereas resting lymphocytes either as virgin precursor cells or as antigen-induced memory cells exhibited high densities, activated lymphoblasts proliferating in response to antigenic stimuli and mitogenic lectins or consequent to transformation with lymphotropic viruses were found to reveal light densities. Since other features of cellular activation such as blast-like morphology and increased proliferative capacity, were shown to correlate with the prognosis of NHL,3,4 we tested for the prognostic significance of the relative density distribution patterns of lymphoma cells obtained from various types of NHL.

MATERIALS AND METHODS

Patients

Biopsy specimens were studied from 27 previously untreated patients with advanced NHL. Classification of the lymphomas was based on the Kiel proposal.1 According to surface marker analyses, 3 of these biopsies were contaminated with more than 25% of nonmalignant cells and were excluded from this study. Details concerning clinical stage, histologic type, and response to primary treatment are given in Table I. Treatment of asymptomatic patients with a favorable histology and slowly progressive lymphadenopathy consisted of local intervention such as splenectomy (one case) or irradiation (three cases). Chlorambucil-prednisolone was given as the primary treatment of symptomatic patients with a favorable histology and with high spontaneous tumor proliferation. Despite intensive polychemotherapy, rapid disease progression occurred in all cases, leading to death in 75% of the patients within a minimal observation period of 40 mo. Surface marker studies excluded the hypothesis that only the variable proportions of normal lymphocytes contaminating the lymphoma suspensions were responsible for the differences in the density distribution patterns described above; they rather suggested that these patterns reflect the individual capacity of a lymphoma to differentiate into dense tumor clones with low spontaneous proliferation.

Linear Density Gradient Separation

Polyscrose-metrizoat linear gradients were established according to the method of Loos and Roos5 using the modifications previously described. Biopsy material from 24 adults with advanced stages of non-Hodgkin lymphomas (NHL) were examined for the distribution profiles of infiltrating cells following centrifugation to equilibrium on linear density gradients. Seven of these biopsies were predominantly composed of cells with high buoyant densities and 9 further biopsies predominantly of cells with intermediate buoyant densities. Both patterns were associated with favorable histologic features and with low proliferation of lymphoma cells. Intensive chemotherapy was rarely required to achieve long-lasting disease control; in both groups, only 28% of patients died within a minimal observation period of 40 mo. In 8 biopsies, a predominance of light lymphoma cells was observed. This pattern was frequently associated with unfavorable histology and with high spontaneous tumor cell proliferation. Despite intensive polychemotherapy, rapid disease progression occurred in all cases, leading to death in 75% of the patients within a minimal observation period of 40 mo. Surface marker studies excluded the hypothesis that only the variable proportions of normal lymphocytes contaminating the lymphoma suspensions were responsible for the differences in the density distribution patterns described above; they rather suggested that these patterns reflect the individual capacity of a lymphoma to differentiate into dense tumor clones with low spontaneous proliferation.

Blood, Vol. 60, No. 6 (December), 1982

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RESULTS

Density distribution profiles were evaluated on single cell suspensions of 27 NHL biopsies by means of equilibrium centrifugation on linear gradients. Three of these specimens derived from neoplastic B-cell proliferations were contaminated with substantial proportions of nonmalignant T cells and were excluded from this series. All three biopsies were derived from centroblastic-centrocytic lymphomas and were predominantly composed of dense cells. The distribution profiles of the remaining 24 lymphomas are demonstrated in Fig. 1. According to the predominant cell density class, these profiles were grouped into one of the three following density patterns: predominance of dense lymphoma cells (group A), predominance of lymphoma cells with intermediate density (group B) and predominance of light lymphoma cells (group C). All lymphomas in group A exhibited a favorable histology, low spontaneous proliferation and long-lasting response to local irradiation, splenectomy, or monochemotherapy (Table 1). Lymphomas in group B, with one exception, revealed a favorable histology; they had, on average, a low proliferative capacity, and long-lasting disease control was achieved in most cases by local irradiation or monochemotherapy (Table 1). In contrast, half the lymphomas in group C exhibited unfavorable histologic features, all showed high numbers of S-phase cells, and despite intensive polychemotherapy, a long-lasting remission was only achieved in 1 of 8 cases (Table 1). Interestingly, the 4 lymphomas of this group with favorable histology all exhibited morphological features associated with a less benign
DENSITY DISTRIBUTION PROFILES OF NHL

Fig. 1. Density distribution profiles of NHL cells. Histology: CLL—21. 28. 33. 45. 78. 83. 91. 120. Immunocytoma—22. 74. 80. 82. 106. Centroblastic-centrocytic—3. Centrocytic—37. Mycosis fungoides—26. 71. 78. 105. Lymphoblastic lymphoma—1. 51. Immunoblastic lymphoma—46. 59. 81.

Fig. 2. Density distribution profiles of normal T or B lymphocytes from various sources.

Fig. 3. Density distribution profiles of human hemopoietic cell lines. Lane 1—Burkitt lymphoma lines. lane 2—nonmalignant, lymphoblastoid cell lines derived from normal donors. lane 3—leukaemia lines. lane 4—myeloma line.

NORMAL LYMPHOCYTES ON THEIR DIFFERENTIATION PATHWAY FROM A VIRGIN PRECURSOR CELL TO A RESTING MEMORY CELL UNDERGO CHARACTERISTIC DENSITY CHANGES: RESTING LYMPHOCYTES AT THE STAGE OF VIRGIN PRECURSORS OR AT THE STAGE OF SENSITIZED MEMORY CELLS EXHIBIT HIGH DENSITIES; \(^\text{1,2}\) ANTIGENICALLY ACTIVATED EFFECTOR CELLS, IN CONTRAST, WERE FOUND TO BE RATHER LIGHT. \(^\text{1}\) WE HAVE USED ANALYSES OF THE DENSITY DISTRIBUTION PROFILES OF NHL BIOPSY TO OBTAIN INFORMATION ABOUT THE ACTIVATION STAGE OF NEOPLASTIC LYMPHOCYTES NOT DIRECTLY PROVIDED BY MORPHOLOGICAL CRITERIA. RESULTS WERE COMPARED WITH

DISCUSSION

Disease course: both CLL cases (nos. 28 and 33 in Fig. 1) showed formation of pseudofollicles; the immunocytoma (no. 82 in Fig. 1) revealed marked pleomorphism; the fourth case (no. 37) was derived from a centrocytic lymphoma.

MORPHOLOGY, IN VITRO PROLIFERATIVE CAPACITY, AND CLINICAL COURSE OF THE MALIGNANT DISEASE.

AS ALREADY POINTED OUT IN A PREVIOUS PUBLICATION, \(^\text{3}\) DENSITY PATTERNS OF MALIGNANT LYMPHOCYTES FAILED TO DISCLOSE ANY SPECIFICITY FOR MALIGNANT CELLS. THEY RATHER SUGGESTED SIMILARITIES OF THE DENSITY DISTRIBUTION BETWEEN LYMPHOMA CELLS AND CERTAIN NORMAL LYMPHOCYTES: DENSITY PATTERNS OF GROUP A LYMPHOMAS REMEM-
bled those of normal T cells in lymph nodes, tonsils, and spleen. An example is given in Fig. 2. Interestingly, 3 of these 7 biopsies are derived from well differentiated neoplastic T-cell proliferations (nos. 71, 78, and 105 in Fig. 1). On average, 17% of normal T cells were found to contaminate the other 4 non-T-cell-derived lymphoma suspensions of this group (nos. 21, 80, 106, and 120 in Fig. 1). Assuming that these normal cells exhibit a distribution pattern comparable to that of normal lymph node T cells, less than one-third of the cells from the dense peak of these lymphomas might be of non-neoplastic origin. This would contribute to, but not sufficiently explain, the predominance of dense cells in these suspensions. The density patterns of group B lymphomas resembled those of normal B cells isolated from lymph nodes, tonsils, or spleen. A typical distribution profile of surface Ig-positive cells is shown in Fig. 2. Seven of these 9 biopsies were obtained from neoplastic B-cell proliferations, and the proportion of contaminating T-lymphocytes was rather small in all cases. Density patterns of group C lymphomas clearly resembled those of antigen- or lectin-activated B or T cells1 3,8 or those of established hemopoietic cell lines. Examples for the distribution profiles of the latter are demonstrated in Fig. 3.

From these data it appeared that the capacity of individual lymphomas to form clones with intermediate or high density is frequently associated with a favorable histology, low proliferative capacity, and slow disease progression. This impression is further supported by the retrospective analysis of the survival rates. As shown in Fig. 4, more than two-thirds of lymphoma patients exhibiting the density patterns of groups A and B are still alive after a minimum observation period of 40 mo. In contrast, lymphomas arrested in their differentiation at the density level of activated lymphocytes only rarely showed favorable histologic features and were mostly characterized by rapid in vitro proliferation and accelerated course of the disease. As demonstrated in Fig. 4, more than two-thirds of these patients died within 40 mo.

In conclusion, our results obtained from a still small group of NHL patients suggested that the capacity of individual tumors to form clones with intermediate or high densities is associated with favorable histology with lower spontaneous proliferation and long life expectancy. In contrast, lymphomas showing restriction of tumor cell distribution to the light density region of the gradients were characterized by unfavorable histology, rapid proliferation, and aggressive course of the disease. Surface marker studies further revealed that the capacity to form denser lymphoma cells is neither caused by the derivation of neoplastic clones from the B- or T-cell lineage nor by the proportion of normal lymphocytes contaminating these suspensions. We thus conclude that analysis of density distribution profiles of NHL enables us to define a group of neoplastic proliferations exhibiting features of activated lymphocytes and unfavorable prognosis.

ACKNOWLEDGMENT

The authors are indebted to Karin Krenkl and Anne M. Födinger for their excellent technical assistance and to Janet Gschnitzer for secretarial help.

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