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Immunological Diagnosis of Leukemias and Lymphomas

Edited by
Stefan Thierfelder · Hans Rodt · Eckhard Thiel

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Stefan Thierfelder · Hans Rodt · Eckhard Thiel

Immunological Diagnosis of Leukemias and Lymphomas

Preface

This two-and-a-half-day symposium has concentrated on main aspects of the rapidly expanding field of leukocyte markers in hematology. While leukemias are already being 'phenotyped' routinely in clinical centers, continued research on the developmental stage of cells and cell membranes, expanding into a malignant clone, permits new snapshots on hemopoietic differentiation. Thus the discovery of leukemia-associated antigens, which so far have not been found on subpopulations of normal cells, has greatly stimulated the discussion on 'differentiation antigens versus tumor antigens'.

The proceedings reflect the considerable success which has been achieved very recently in the classification of hemoblastomas. Consequently the number of leukemias which are unclassifiable by immunological methods have dwindled down to a small minority.

New facts give rise to new questions. By including the main points of the discussions in the proceedings, we wanted to give the reader an opportunity to get an impression of the questions and conclusions raised and drawn by the participants on the basis of new – and frequently still unpublished – data.

The editors thank both the contributors to this symposium, who made it a successful meeting and submitted their manuscripts punctually, and the publishers, who have provided a volume of high quality in good time. They are also grateful for the valuable cooperation from numerous colleages at the Institut für Hämatologie.

Munich, March 1977

S. Thierfelder
H. Rodt
E. Thiel

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The Immunological Diagnosis of Human Leukemias and Lymphomas: an Overview*

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For more than five years, a number of laboratories have been involved in studies of the membrane markers of leukemic and lymphomatous cells in order to improve the classification of human lymphoid malignancies. These immunological studies have indeed provided new informations on the nature of the proliferating cells and some insight into the pathophysiology of human leukemias and lymphomas. Since these diseases appear to represent mostly clonal proliferations of lymphoid cells with often a developmental arrest at various stages in their differentiation pathway, investigation of such patients may prove to be particularily helpful in the definition of subsets of human B and T cells with distinct markers and functions. However the reliability of the results of membrane markers studies depends upon the knowledge of a number of methodological problems and pitfalls.

I. Methodological Problems and Pitfalls

Immunoglobulins (Ig)

Surface membrane bound Ig (SmIg) detectable by immunofluorescence constitute the most reliable marker of B cells. They give evidence for the genetic commitment of the cell when truly monospecific antisera to the various Ig chains, subclasses and allotypes are used and may thus represent a clonal marker of B cell proliferations (reviewed in 1).

In order to avoid erroneous interpretations, one should be aware of a number of technical pitfalls in the study of SmIg (2). Nonspecific staining may result from the presence of damaged cells or of Ig aggregates in the reagents. The mere presence of Ig molecules at the surface of a cell does not necessarily mean that they are produced by that cell. Monocytes and other non lymphocytic cells which possess an Fc receptor may bind IgG from the serum or from the reagents. The main reasons for the detection of extrinsic Ig on lymphoid cells are the presence of labile cytophilic IgG, the attachment of immune complexes and the binding of antibodies to membrane antigens, such as antibodies directed towards leukemia associated surface antigens. It is therefore of utmost importance in the study of SmIg to use F(ab')2 reagents of proven monospecificity and to properly identify the positive cells, using for instance peroxidase staining in order to recognize monocytic cells (3). The detection of surface IgD and, for the other Ig classes, of a single class of heavy and light chains argues in favour of an actual cell product but does not rule out the possibility that one is dealing with extrinsic Ig (for instance an-

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tibodies to membrane cell antigens with restricted heterogeneity). In vitro experiments may therefore be required to ensure that surface Ig are synthesized by the cells under study (1).

The presence of intracytoplasmic Ig in a cell is of course considered as reliable evidence of its B nature. However Mason et al. (4) have recently shown that the use of the very sensitive immunoperoxidase three stage technique on fixed human leukocyte smears results in the detection of membrane bound IgM and IgD in B cells and of IgG molecules in approximately 25 % of normal lymphoid cells which represent probably Fc receptor bearing lymphoid cells with bound serum IgG. Moreover IgG was demonstrated within normal human monocytes. These findings prompt reassessment of recent reports describing the detection by this technique of Ig in lymphoma cells and Reed Sternberg cells, particularly in view of the fact that the Ig molecules found in Sternberg cells appear to be mainly IgG with both light chain types (5).

Other markers

Some classical B cell markers such as the receptors for the complement components and for the Fc of IgG are not specific for B cells. The receptor for the Fc portion of IgG is present on monocytes, on the so called third population of lymphoid cells and on a subset of T cells which appear to have a suppressor activity as well as on various normal or neoplastic non lymphoid cells. Complement receptors are expressed on monocytes and on a small subpopulation of T cells, especially on thymocytes (6). Some other B cell characteristics such as the formation of rosettes with mouse erythrocytes are found only on a subpopulation of B cells. This test is positive with most chronic lymphocytic leukemia (CLL) cells but negative with most lymphomatous B cells (7).

Some other surface markers such as the receptors for the Epstein-Barr virus on B lymphocytes (8) and for the C-reactive protein (9) or the measles virus (10) on T lymphocytes are not widely used but may be of interest in the membrane phenotyping of certain human lymphoid malignancies.

Hetero-antisera specific for B or T antigens may constitute useful markers in such phenotyping. Some such antisera can be used only in cytotoxicity tests. This procedure is not satisfactory since it does not allow simultaneous checking of other markers nor direct examination of the positive cells. In addition the sensitivity of cytotoxicity tests may greatly vary between normal lymphocytes and malignant cells. Immunofluorescence is therefore by far the safer procedure but truly specific antisera at the le el of sensitivity of this method are not easy to obtain and some of them react only with subsets of B or T cells.

Problems related to the neoplastic nature of the proliferating cells

A classification which would appear relatively safe for the markers of normal B and T lymphocytes may not be valid when extrapolated to leukemic or lymphomatous cells. Neoplastic cells may undergo surface changes and express new markers or conversely lose some normal membrane properties, thus preventing their identification. Moreover leukemic cells may also express membrane antigens only at certain stages of the cell cycle and the presence of fetal antigens or tumor associated antigens may invalidate the results obtained with antisera to B or T fetal or neoplastic cells.

It should also be stressed that the leukemic or lymphomatous cells under study do not necessarily represent the "target cell" which was hit by the neoplastic event. If this target cell was an undifferentiated pluripotential stem cell, the proliferating cells could possibly express unexpected membrane phenotypes.

The need for using a panel of membrane markers is especially obvious when one studies leukemic or lymphomatous cells. It is also obvious that erroneous interpretations may result from the admixture of normal lymphoid cells in spite of careful morphological study of the individual cells with given surface markers.

II. Chronic Lymphocytic Leukemia (CLL)

1. B derived CLL

It is well established that, at least in Western countries, the vast majority of CLL represent B derived monoclonal proliferations. An apparent exception to the rule of a single SmIg product on proliferating B cells is the simultaneous presence of δ -chains and of heavy chains of another Ig class (IgM as a rule), in many patients (11 -13). However IgD and IgM molecules on the same proliferating cells of a given patient share the same light chains, idiotypic specificity (14, 15) and antibody activity (16). Both molecules therefore apparently have the same variable regions and differ only in the constant part of the heavy chains. Their simultaneous presence does thus not argue against the monoclonal nature of the proliferation.

The concept of SmIg monoclonality holds true even in those CLL cases where freshly drawn lymphocytes carry simultaneously μ, γ, $\varkappa$ and λ chains. This "mixed staining pattern" which occurred in more than 10 % of our patients represents a false polyclonal appearance since in all the cases where *in vitro* biosynthetic studies were performed, the actual cell product was a monoclonal IgM (17, 18). In addition, in some cases where the freshly drawn cells apparently bore IgG, even with a single light chain type, the lymphocytes could be shown after stripping to synthesize a monoclonal IgM (17). However CLL lymphocytic clones truly producing IgG or IgA do exist and the distribution of heavy and light chains among CLL patients roughly reflects the distribution of SmIg classes on normal blood lymphocytes (17), with a large predominance of IgM (and IgD).

In most cases of CLL without any serum monoclonal protein, the uniform and faint fluorescence pattern of all leukemic lymphocytes, with a SmIg density much lower than on normal lymphocytes, accords with the hypothesis that their development was apparently "frozen", with a block in maturation along the pathway of differentiation of the B line (17). In occasional patients with B derived CLL, SmIg are not detectable on the leukemic cells by immunofluorescence and the study of several other B and T cell markers is critical in order to distinguish such cases from T derived CLL. Conversely, in a few cases, leukemic lymphocytes may be heavily loaded with SmIg; the situation is encountered in most patients with the prolymphocytic type of CLL.

In most patients with CLL and a serum monoclonal Ig, the very same Ig chains (17) with similar idiotypic determinants (5, 19) are found on the leukemic lymphocytes and in the serum monoclonal Ig. These findings led to postulate that these cases usually represent a B cell proliferation with some degree of persistent maturation of the neoplastic clone into plasma cells, a situation intermediate between that of common CLL with a complete block in the maturation process and Waldenström's macroglobulinemia with uninterrupted maturation of the proliferating clone up to the IgM secreting cell (20).

The proliferating lymphocytes of B derived CLL display several unusual membrane characteristics which may be related to their leukemic nature since they are not found on most normal circulating B lymphocytes. CLL cells possess a complement receptor for C3d (i.e. inactivator-cleaved C3b) but lack the receptor for C3b (21, 22). Leukemic lymphocytes from patients with B derived CLL have a receptor for *Helix pomatia* A hemagglutinin, whereas in normal peripheral blood Helix pomatia hemagglutinin binds mainly to the T lymphocytes (23). Recent experiments have also indicated that most lymphocytes of B derived CLL patients form rosettes with IgM coated bovine ery-

throcytes (24). In contrast to the findings for the Fcμ receptor present on a subpopulation of normal T lymphocytes (25), the IgM receptor of the CLL cells is demonstrable prior to overnight incubation.

2. Double monoclonal proliferation and supervening acute malignancies

Double lymphoid proliferations with separate cell populations featured by distinct SmIg were found in several patients whose clinical presentation was most often similar to CLL (17, 26). Various patterns were encountered with regard to intracytoplasmic Ig staining and serum monoclonal Ig reflecting persistent maturation of the corresponding clone: both cell populations, only one (usually producing IgG or IgA) or none may pursue some maturation into plasma cells secreting their monoclonal Ig in the serum. In some patients the SmIg of the two clones differed in both heavy and light chains. In other instances, both proliferating populations shared the same light chain type which suggests, especially when λ light chains were found, that they may have originated from a common clone. In one such CLL patient (17) the findings were compatible with the hypothesis of an original IgM λ clone shifting to IgGλ producing and secreting cells with a transitional population of cells producing the two classes of heavy chains. Such a switch mechanism involving a transition from IgG to IgA synthesis at the B lymphocyte level was recently documented in another CLL patient (26) with a dual population of lymphocytes bearing either IgGκ or IgAκ associated with a single serum monoclonal IgGκ protein whose idiotypic determinants were detected on the surface of both the IgG and IgA bearing cell populations.

During the course of B CLL, a change in the character of the disease to a more severe proliferation may occur. Examples of this situation are the rare acute blastic crisis and the so called "reticulum cell" sarcoma supervening on CLL. Since the component cells are totally different in morphology from the chronic-phase lymphocytes, it has been suggested that in such cases the "new" acute disease is distinct and unrelated to the original chronic leukemia. The results of the study of membrane markers in such cases do not support this view. They showed that the super-imposed blastic or lymphomatous cells were B cells synthesizing the same Ig chains (with the same antibody specificity) as the lymphocytes of the previous chronic phase, indicating that the two cell populations were derived from the same clone (27, 28).

3. T derived CLL

T derived CLL is probably less rare than previously considered since we have recently diagnosed 18 consecutive cases, 11 of which were already reported (29). It is of interest that close to half of the CLL cases reported from Japan (where CLL is quite uncommon) were T derived (30). Most of our patients exhibited unusual clinical and hematological features: massive splenic enlargement in half of the cases, moderate but definite blood and marrow invasion by the leukemic lymphocytes. The cells had always a high content of lysozomal enzymes (ß glucuronidase and acid phosphatase) and, in 11 cases peculiar cytoplasmic azurophilic granules were present. In view of the characteristic skin involvement of the Sezary syndrome and of mycosis fungoides which were proven to be T cell derived proliferations (31, 32), it is of interest that eight of our eighteen patients had skin lesions (without any cytological or histological features of the Sezary syndrome) suggesting a preferential homing of T lymphocytes to the skin. Further work is necessary to see if T CLL carries a different prognosis from B CLL and responds differently to treatment.

A panel of surface markers was used to establisch the T cell nature of the leukemic cells from these patients. In some cases only a small percentage of the leukemic

lymphocytes were able to form E rosettes, a finding similar to that previously recorded for Sezary cells (31). The use of different antisera against T cell antigens showed striking variation from patient to patient. Whereas normal blood T lymphocytes and the cells from seven patients reacted equally well with antisera to peripheral T cells and to fetal thymocytes, leukemic cells from the other patients reacted strongly with only one of these two antisera (more frequently with the antiserum to thymocytes). An antiserum to human brain which reacts only with a subset of peripheral T cells (36) stained most or all leukemic lymphocytes in six cases but gave negative results in the five other cases studied. These differences provide evidence for the homogeneity of this population of leukemic T lymphocytes (29) for which no monoclonal marker is yet available. The few caryotypic studies performed in such cases (33, 34, 36) and the preliminary results of the functional study of these leukemic cells are in accordance with this hypothesis. It should be noted that terminal desoxynucleotidyl transferase was not found at significant levels in these T derived leukemic cells in the few cases where it was determined.

4. CLL with unusual surface marker phenotypes

It is not always possible to infer the cellular origin of CLL lymphocytes if a limited number of membrane markers is used. In three reports the leukemic cells bound sheep erythrocytes and also bore a receptor for complement (35, 36) or for the Fc fragment of IgG (37). Since a low percentage of normal peripheral blood T lymphocytes have these properties, these leukemic cells should not necessarily be considered as lymphocytes with dual B and T receptors and they might represent a malignant expansion of a minor subset of normal T lymphocytes.

The lymphocytes from five of our patients exhibited monotypic SmIg and also formed spontaneous rosettes with sheep erythrocytes. In one case the leukemic cells proved to be B cells with SmIg M possessing antibody activity against the Forssman antigen (38). In two other cases the SmIg were exogeneous and the T cell origin of the leukemic cells was confirmed by the reactivity with an antiserum against human T cells. In the two last cases however the SmIg appeared to be actual cell products and the leukemic cells could represent a malignant clone expressing an aberrant cell surface phenotype. Another case (39) was described in which the leukemic cells synthesized SmIg, bound sheep erythrocytes, bore a receptor for complement and were killed by heteroantisera to T cells. Although the percentage of cells positive for these markers varied during the course of the disease, an overlap between several markers for B and T cells was found on a large number of proliferating cells.

III. Non Hodgkin's Lymphomas

The immunologic studies have provided a definite confirmation of the validity of some pathologic and clinical statements in several groups of lymphomas such as well differentiated diffuse lymphocytic lymphomas, nodular lymphomas and Burkitt lymphomas (which are all monoclonal B cell malignancies).

The close relationship of "well differentiated" diffuse lymphocytic lymphoma to chronic lymphocytic leukemia was confirmed since the study of membrane phenotype gave identical results in both groups of patients. In those cases without any serum monoclonal Ig, SmIg belong mostly to the IgM class and give a uniform and faint fluorescence pattern on all the proliferating lymphocytes (40).

The results of immunologic studies in "nodular" lymphomas have supported the views of Lennert and of Lukes and Collins who proposed, on the basis of morphologic studies, that these lymphomas arise from follicular center cells. Practically all cases thus

far studied in various laboratories, regardless of the cytologic subtype and of the degree of nodularity, have been proven to be B derived malignancies with usually a high density of Ig molecules bound to the membrane of the lymphomatous cells. These cells bear also C3 receptors similarly to the predominant cell of the lymphoid follicle (41).

The neoplastic cells from African cases of Burkitt's lymphoma were evaluated for surface markers and glucose-6-phosphate dehydrogenase and this disease was shown to be a monoclonal B cell proliferation (42). Similar surface characteristics were found on Burkitt's tumor cells in French and American patients whose disease presented as lymphoma (43) or as acute leukemia (44). Although the neoplastic cells of both endemic and non endemic cases are cytologically and immunologically indistinguishable, we don't know if we are dealing with a single disease caused by the same agent. It should also be stressed that, although Burkitt's lymphoma is defined by precise morphological and immunological characteristics, its course varies considerably from patient to patient with prolonged survival and probable cure following chemotherapy in some cases and a fulminating course and inefficient treatment in others. This great variability in evolution outlines the limitations of the prognostic value of surface markers and histology in non Hodgkin's lymphoma.

Most cases of diffuse poorly differentiated lymphocytic lymphomas in adults are B cell malignancies (28, 45-47). This appears to be always the case when these diffuse lymphomas are morphologically recognized as beeing of follicular center cell origin on the basis of the presence of cleaved cells (48). In roughly 10 % of the patients, the neoplastic cells had T surface characteristics and in a similar percentage of cases no membrane markers were detectable.

Immunologic studies have helped to define a subgroup of childhood lymphoma which was previously included in the diffuse poorly differentiated lymphocytic lymphomas and which is often denominated now "childhood lymphoblastic lymphoma" (49, 50). The proliferating cells may have a "convoluted" appearance (51) but this feature is not constant (49). The presence of an anterior mediastinal mass in many of these children has indicated clinically a possible thymic origin for these tumors. The presence of T lymphocytic markers on the neoplastic cells has been demonstrated in many cases (6, 50-54). Complement receptors have been reported on these neoplastic T lymphoid cells (as on the thymic cells) by several groups (6, 50, 54). Cytochemical studies indicate a strong positivity of the proliferating cells for acid phosphatase (6). Leukemia develops rapidly in many of these patients and these childhood lymphoblastic lymphomas share many morphologic, cytochemical and clinical features with the T derived subgroup of acute lymphoblastic leukemia (ALL) (see next section). On the basis of both clinical and immunologic data, these two diseases are closely related and may represent different manifestations of the same neoplastic process.

Diffuse large cell lymphomas ("histiocytic" lymphomas, "reticulum cell" sarcomas) appear immunologically to represent a heterogeneous group of neoplasms (55). Only occasional cases may be truly related to the monocytic series. The study of membrane markers has provided suggestive evidence in favour of this hypothesis in a few patients, including a single case in our series of 17 large cell lymphomas. In one instance *in vitro* culture of such lymphomatous cells has proven that they were able to synthesize lysozyme (56). Fifty to sixty per cent of large cell lymphomas appear to originate from B cells. In our series, 5 of the 8 cases that were of monoclonal B cell origin arose in patients previously affected with chronic lymphocytic leukemia, Waldenström's macroglobulinemia, α chain disease or follicular lymphoma, which are lymphoproliferative diseases of well documented B cell origin. Our results strongly suggest that, in such patients, the supervening large cell lymphoma does not represent the emergence of a second malignant clone but is clearly related to the original B cell proliferation (55). T cell derived large cell lymphomas appear to be unfrequent since they account for less than 10 % of the cases in our study as well as in the literature. In more than 30 % of the patients (six cases in our series of 17 patients), the large lymphomatous cells are devoid of the usual

membrane markers of mature B or T lymphocytes. In our hands, the use of specific hetero-antisera to T or B cells did not give further evidence for the cellular origin in these unclassified cases.

In lymphomas with a mixture of small and large cells (mixed lymphocytic-histiocytic type of Rappaport's classification), both cell types share the same surface markers or both lack B and T membrane markers.

There are some indications that those patients with diffuse lymphomas whose malignant cells demonstrate B membrane markers may survive longer than the other patients (see paper by Kersey et al. in this Symposium). The study of many more patients treated in an identical manner within the various morphological subgroups is warranted in order to evaluate the possible prognostic significance of the membrane markers. This evaluation is of special importance for the large cell lymphomas where long survivals are obtained in 25-30 % of the cases after intensive chemotherapy. Further studies should indicate whether or not the lymphomas of these patients belong to a given immunological subgroup.

Any future immunologic classification of the non Hodgkin's lymphomas should of course be combined with the data provided by the morphological studies and there is an urgent need for a workable, reproducible and scientifically accurate pathologic classification.

IV. Acute Lymphoblastic Leukemias (ALL)

1. Immunological classification

A heterogeneous pattern has emerged from the study of the membrane properties of blast cells from patients with ALL which allowed their classification into three subgroups according to the phenotype for the main markers of B and T cells.

Acute leukemias with B cell markers constitute a minor subgroup of ALL. These B derived ALL cells produce monotypic SmIg and thus appear to be of a monoclonal origin. In our experience most B cell acute leukemias belonged to two specific entities characterized by unusual clinical or cytological features as outlined above: blast crisis supervening in CLL (27) and acute leukemias featured by Burkitt's tumor cells (44). Within "common" ALL, the incidence of B-derived proliferations is roughly 2 %. It is of interest that in three of our four patients, the cells had some unusual cytological features suggestive of poorly differentiated lymphocytic lymphoma, a finding which suggests that many of these patients may in fact be affected with a lymphoma with leukemic presentation (57).

In our series of unselected and untreated patients (adults and children) with common ALL, the blast cells showed T cell surface features in 27 % of the cases (58, 59). This incidence is somewhat higher than that reported by some other laboratories (60-63). We have concluded to the T cell origin of ALL from the results of the E rosette formation and from the reactivity of the blastic cells with an antiserum to peripheral T cells (57). Whereas in 80 % of the cases, both tests yielded positive results, 20 % of the patients with T-derived ALL were classified as such mostly on the basis of the presence of T cell antigens since less than 10 % of the blast cells formed E rosettes. In 85 % of our cases of T-derived ALL, most blast cells exhibited a strong acid phosphatase positivity (59), a finding in accordance with the report of Catovsky et al. (64).

Several characteristics of the T-derived ALL cells are much more similar to those of normal thymocytes than to those of peripheral T lymphocytes. The formation of E rosettes occurs at both 4^0 C and 37^0C whereas the more mature peripheral blood T cells fail to form rosettes at 37^0C (65). These blast cells show reactivity with some antisera to thymocytes which have been previously absorbed by peripheral T cells (66). They also

contain a precipitating antigen which is found in thymocytes and not in peripheral T cells (67). Some antisera to T-derived ALL cells enable to demonstrate some antigenic determinants shared by these cells and thymocytes which are absent on peripheral T cells (68). Finally these T-derived blast cells are found to contain terminal deoxynucleotidyl transferase, an enzyme normally found in high quantities in thymocytes but lost as the cell further matures to peripheral T lymphocytes (69).

The third subgroup which includes 70 % of the ALL cases in our series is defined by the absence of detectable B or T cell markers on the blast cells (including absence of reactivity with our hetero-antisera to peripheral T cells and to thymocytes). It should however be noted that in two patients 30 % of the blast cells showed positivity for μ and λ immunoglobulin chains. As outlined in Table I, these "non-T-non-B" ALL cells

	Incidence	Monoclonal SmIg and other B markers	Antigens of peripheral T cells and of thymocytes	"Null" ALL specific antigen (s)*	"CLL-thymus" antigen (s)*	"Ia-type" antigen (s)*	N-acetyl hexosaminidase	Terminal deoxynucleotidyl transferase
"Burkitt"	1 %	+	–	–		+		
B common ALL	2 %	+	–	–		+	–	–
T (Thy)-derived ALL	27 %	–	+	–	–	–	–	+
"Non-T-non-B" ALL	70 %	–	–**	+***	+****	+	+	+

* See text for explanation
** Reactivity with some antisera to thymocytes in a fair percentage of cases
*** Positive in 69 % of the cases
**** Positive in 90 % of the cases.

Table I: Schematic pattern of the main markers of the blast cells in the various immunological subgroups of human acute lymphoblastic leukemias

are characterized by several positive findings which enable to distinguish them from T-derived ALL cells : the presence of membranebound Ia type of antigens revealed by alloantisera or heteroantisera (68, 70, 71), the detection of N-acetyl-hexosaminidase (72) and the presence of at least two distinct kinds of leukemia-associated antigens (59, 73). The leukemia specific antigen revealed by rabbit homologous antisera and described by Greaves (73) is found on the blast cells of 69 % of the patients with "non-B non-T" ALL (74). The antigen shared by CLL cells and thymocytes is found on the blast cells from 90 % of these patients (59). The cytological appearance of the blast cells does not differentiate "non-T non-B" ALL from T-derived ALL. The size of the blast cells, the chromatin pattern, the appearance of the nucleoli or the presence of cytoplasmic granules were not discriminating (59, 75). A strong positivity for acid phosphatase with numerous granules in the Golgi area of the majority of blast cells was found in less than 10 % of cases of "non-T non-B" ALL. In those cases where the blast cells are negative for all B and T markers and negative for the leukemia-associated antigens mentioned above, peroxidase staining should be carefully checked since a low percentage of blasts may show positivity and some of these undifferentiated leukemias may be myeloblastic despite the lymphoblastlike cytological appearance of the cells (59).

The question of the true cellular origin of "non-T non-B" ALL remains presently unsettled. Several findings have led to the suggestion that it may derive from a bone marrow immature thymocyte precursor. Terminal deoxynucleotidyl transferase is present in the blast cells of most cases of "non-B non-T" ALL (but this enzyme appears to be