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Some Aspects of Immunological and Cytochemical Markers in Leukemia

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Summary. *The value of immunological and of cytochemical markers for understanding of pathophysiology and for diagnosis in different subtypes of leukemia is discussed.*

Immunological and cytochemical markers have elucidated pathophysiological aspects of the ontogeny of lymphatic cells and improved the subclassification of leukemia and lymphoma. In this paper some more recent findings and controversial aspects will be discussed.

I. Acute Leukemia

Antisera of well-defined specificity and enzyme assays have recently become available as aids in the classification of leukemia [8, 10, 15, 23, 36]. Relevant markers are summarized in Table 1.

T-ALL is a neoplasm, in most cases probably of prothymocytes, representing approximately 30% of all cases of ALL. Phenotypically, T-ALL is characterized by T-Ag and – in about 80% of cases – by E-rosette formation. TdT is positive in about 90% of cases and is therefore valuable for differentiation from myeloid leukemia, but not from C-ALL [25]. About 90% of T-ALL give polar staining in the APh test, and most cases (about 90%) are negative for PAS. ANAE-positivity may be regarded as a valuable marker for T lymphocytes, but is inferior to APh in subclassification of ALL [20]. Special subtypes of T-ALL were detected according to functional properties of the leukemic cell population: A patient with T-ALL associated with hypogamma-globulinemia was described, in whom malignant T cells represented a clonal expansion of prosuppressor T cells that required interaction with normal T cells to express their capacity to exert a suppressor influence on B lymphocytes, in a PWM-stimulated system [6]. Ten percent to 20% of cases of T-ALL exhibit C-Ag in low concentrations, thus representing transitional forms with C-ALL [23].

C-ALL is characterized by negativity for T-Ag and E-rosette formation, but positivity for C-Ag and/or for Ia-Ag. According to immunological markers, it has recently become evident that among so-called C-ALL, cells from about 20%–30% of patients have characteristics of pre-B cells, containing intracytoplasmic but lacking surface Ig [38].

TdT is positive in most cases; Aph-positivity is less pronounced, and PAS-positive material is found more frequently than in T-ALL [1].

B-ALL constitutes about 2% of all ALL cases and is reported to involve a poor prognosis.

AML, AMoL. A specific membrane antigen can be detected on normal and malignant myeloic cells [21, 23]; this M-Ag is only weakly expressed on myeloblasts, but its intensity increases in immature granulocytic and monocytic elements. In addition, about 75% of AML and AMoL react with anti-Ia antisera.

Abbreviations used in this paper: AML, Acute myeloid leukemia; AMoL, acute monocytoid leukemia; ALL, acute lymphoid leukemia; ANAE, acid α-naphtyl acetate esterase; APh, acid phosphatase; B-ALL (-CPL, -CLL), B-lymphoid acute (prolymphocytic, chronic) leukemia; C-Ag, membrane antigen of common ALL; C-ALL, common ALL whose cells react with a specifically absorbed antiserum against ALL of non-T, non-B variety; CLL, chronic lymphoid leukemia; CPL, chronic prolymphocytic leukemia; E, rosettes with sheep erythrocytes; Ia, immune response antigen; M-Ag, myeloid antigen; POX, peroxidase; SmIG, surface membrane Ig; T-Ag, T-lymphocyte antigen; T-ALL (-CPL, -CLL), T-lymphocyte antigen-positive acute (prolymphocytic, chronic) lymphatic leukemia; TdT, terminal deoxynucleotidyl transferase; UAL, undifferentiated acute leukemia

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Table 1. Markers in leukemia

	E	T-Ag	C-Ag	SmIg	Ia	M-Ag	TdT	APh	ANAE	Nonspecific esterase	PAS (gran)	POX	Lysozyme
T-ALL	+ (−)	⊞	+ (−)	−	−	−	+ ((−))	+ ((−))[a]	+ (−)[b]	(+)	−((+))	−	−
C-ALL	−	−	⊞ (−)	−	⊞ ((−))	−	+ ((−))	− (+)	− (+)	(+)	+ (−)	−	−
B-ALL	−	−	−	⊞	+	−	− (+)			(+)		−	−
AML	−	−	−	−	+ (−)	+	− ((+))	+ (−)[d]	+ (−)[d]	(+)	− (+)	⊞	+
AMoL	−	−	−	−	+ (−)	+	− ((+))	+[d]	+[d]	⊞+	+ (−)	−/+	+
UAL	−	−	−	−	+ (−)	−	+ (−)			(+)		−	
B-CLL	−	−	−	⊞((−))	+	−	−	+ (−)[c]	+[c]	(+)	+ (−)	−	−
T-CLL	+ (−)	⊞	−	−	− (+)	−	−	+[a,c]	+ (−)[b]	(+)	− (+)	−	−
B-CPL	−	−	−	⊞	+	−	−	+ ((−))[a]	+ (−)[c]	(+)	+ (−)	−	−
T-CPL	+	⊞	−	−	− (+)	−	−	+[a]	+[b]	(+)	+ (−)	−	−
Hairy-cell leukemia	− ((+))	− ((+))	−	+ ((−))	+	−	−	⊞[c]	+[c]	(+)	− (+)	−	−

( ) In 10% to less than 50% of cases
(( )) In less than 10% of cases
⊞, Most important marker in certain type of leukemia
[a] Granular and preferentially paranuclear staining
[b] Dot-like
[c] Dispersed
[d] Diffuse (according to references indicated in text)

Activity of TdT can only be detected in very few cases. Lysozyme may be regarded as an equally reliable enzyme marker for delimitation of AML and histiocytic malignancies from ALL [28]. In the first place, of course, POX must be mentioned for diagnosis of AML, and nonspecific esterase (which can be inhibited by fluoride) for diagnosis of AMoL and histiocytic malignancies [31].

Unclassifiable Leukemias. About 7% of leukemias are unclassifiable with the panel of markers indicated in Table 1, or express only Ia-like antigens [23]. It is

probable that these cases represent a heterogeneous group possibly including some megakaryoblastic leukemias, which might be diagnosed if tests for platelet-specific POX were included [5].

Blastic Crisis of CML. In 10%–20% of cases, blasts in blastic crisis show similarities with lymphoblasts – according to morphology, cytochemistry (granular positivity of PAS in some cases), and membrane markers (C–Ag in most cases, T-Ag in some exceptions) [22, review: 32]. Among 72 cases with blast crisis, 24 were TdT-positive, but only 10 were morphologically lymphoblastic [25]. In another series of patients [22], correlation of C-Ag- and of TdT-positivity was fairly good. The value of C-Ag-positivity in prediction of response to vinca alkaloids and prednisone is established. And, with reservation, TdT holds true as a marker for the lymphoblastic variant of blastic crisis in CML; and its validity for prognosis and prediction of response to vinca alkaloids and prednisone still remains to be established.

Conclusions

Immunological marker investigations have allowed *subclassification* of a variety of ALL – an advance that was made a decade ago by introduction of enzyme markers in AML. In the subclassification of ALL, immunological markers, especially membrane antigens, are superior to cytochemical tests. When specific antisera or fresh leukemic cells are not available, a combination of the APh and PAS reactions is of some value in distinguishing T-ALL from C-ALL. TdT is useful for distinction of ALL from AML. Cytochemical reactions are most helpful to differentiate AML from ALL and to identify subgroups in AML.

What kind of significance may we anticipate – besides a better understanding of the pathophysiological background? *Diagnosis* of leukemia itself may be made possible under certain conditions: Questionable blasts in bone marrow, exudates, of cerebrospinalfluid may be recognized as leukemic on the grounds of their specific markers; remaining leukemic blasts in partial remission or in early relapse may be identified by their specific pattern of immunological or cytochemical markers; certain subtypes of AML (e.g., very immature myeloblastic or microgranular promyelocytic leukemia) can be recognized only by cytochemical tests (especially POX) because the promyelocyte granules may be very few or may be smaller than can be recognized in conventionally stained imprints [13].

Natural history and *prognosis* are different in C-ALL and in T-ALL [4, 30]. Therefore, different *treatment* protocols for both subtypes of ALL have been proposed by some groups to make the appropriate treatment possible and to prevent overtreatment. Some had hopes of the treatment of ALL resistant to conventional therapy by *transfusion* of *autologous bone marrow* obtained during remission and pretreated with specific anti-T, or anti-C antisera. A prerequisite for these therapeutic measures is, of course, the exact classification of the leukemic cells. The pathophysiological or prognostic significance of the *remaining 'normal' lymphocytes* in blood and bone marrow of patients suffering from leukemia is open to discussion. The exact enumeration of these normal cells is made possible by immunological marker tests; a fast and easy quantification is made possible by staining for ANAE, which stains peripheral T lymphocytes [14, 20].

II. CLL, CPL and Hairy-cell Leukemia

In chronic lymphoid malignancies, immunological and cytochemical markers proved especially useful for subclassification and made pathophysiological insights possible (Table 1).

B cell. About 95% of cases of CLL examined for lymphocyte surface markers have been reported to be monoclonal proliferations of B lymphocytes. In B-CLL, absolute numbers of T cells are generally normal or elevated [7]. SmIg of B-CLL lymphocytes is IgM in most cases, sometimes together with IgD; its concentration on single CLL cells is usually lower than in normal B lymphocytes. When microphotometric quantitative immunoautoradiography is applied [33], SmIg levels in the single CLL cells are of striking uniformity in each patient investigated. The discovery of a group of alloantigens that are primarily expressed on B cells and bear a strong similarity to the Ia-Ag of the mouse has provided a new tool for the analysis of the various lymphocyte subpopulations. When applied in B-CLL, Ia-Ag are readily detected in leukemic cells [24]; and most of the null cells, i.e., cells unclassifiable by conventional marker analysis and claimed to be elevated in B-CLL [11], may be recognized as belonging to the leukemic cell clone. B-CLL cells exhibit a noncharacteristic, finely granular, and dispersed staining pattern when cytochemical reactions for ANAE and for APh are applied.

T-CLL. In a small minority of cases CLL is characterized by the presence of T-lymphocyte, but

lack of B-lymphocyte markers. According to morphology and function of the leukemic T lymphocytes, at least two subtypes of T-CLL must definitely be distinguished.

In the *first subtype* of T-CLL, the malignant cells are similar to normal blood lymphocytes in their morphology, cytochemistry, and membrane markers [17]. Quantitative immunoautoradiography of single cells shows that the concentration of T-Ag of the single leukemic cells is lower, but more constant than in normal T lymphocytes, suggesting a clonal origin [34]. Findings on PAS and APh staining are not specific, but all cases investigated so far with ANAE cytochemistry could be delimitated from B-CLL on the grounds of distinct 'dot-like' ANAE-positivity [20].

The *second subtype* of T-CLL, in addition to membrane markers, as indicated above, is characterized by a strong receptor for Fc-IgG. The leukemic cells are larger than normal T lymphocytes of the blood. They are rich in small azurophilic granules, which, in electron microscopy, show dense central cores. Interestingly, the cells cannot be stimulated in vitro by PHA (unpublished observation). Granules showing strong APh- and ANAE-positivity are distributed around the nucleus. With these features, lymphocytes of this subtype of CLL might correspond to a physiological subgroup of lymphocytes acting as suppressor cells in B-lymphocyte differentiation. Cases of T-CLL presumably identical with this second subtype described above were published by Catovsky [8].

Finally, a peculiar chronic lymphoproliferative disorder will be mentioned, marked by E-rosette formation, by an avid receptor for Fc-IgG, and, especially, by cytoplasmic inclusion bodies consisting of *parallel tubular arrays* [27]. The physiological counterpart of this presumably 'leukemic' lymphatic cell was defined according to its peculiar morphology [16] and its extraordinarily strong receptor for Fc-IgG [19], but its functional properties remain open to discussion [2].

CPL. The term CPL designates a lymphoproliferative disorder closely related to CLL [12], and should not be confused with a subtype of ALL designated with the same term by Mathé [26]. Leukemic cells in CPL may be of B- or T-cell nature according to membrane markers. When quantitative immunoautoradiography was applied [35], the expression of T antigen on the single leukemic cells of individual patients tested appeared rather uniform, its concentration lying between that in T-CLL and that in T-ALL. CPL cells of B and of T type are marked by a high content of APh distributed in a granular and preferentially paranuclear pattern.

Hairy-cell Leukemia. The abnormal cells in hairy-cell leukemia are characterized by their peculiar morphology and, in the majority of cases, by ribosome-lamella complexes. The B-cell nature of hairy cells has been indicated by the fact that they have SmIg that is produced by the cells and clearly reflects a monoclonal expansion [29]. Hairy cells also possess receptors for Fc-IgG. Recently a patient was described in whom hairy cells formed E-rosettes; in addition, they possessed T-Ag, SmIg, and Ia-like antigen [9]. Therefore, this case seemed to be an example of malignancy with features of both T and B lymphocytes. In the diagnosis of hairy-cell leukemia cytochemistry may be useful: The abnormal cells have been shown to have tartrate-resistant APh and to lack lysozyme in most cases. ANAE is distributed perinuclearly in a very typical granular pattern [37].

Conclusions

In chronic lymphoid malignancies *subclassification* into B and T-subtypes may be obtained by means of membrane markers and cytochemistry. Especially in CLL, ANAE cytochemistry – besides the membrane marker tests discussed above – is extremely reliable [20]. And in hairy-cell leukemia staining for APh and ANAE is of more diagnostic significance than marker tests. Obviously, in disease entities allowing no differentiation between normal and malignant cells on the basis of morphology alone – as in CLL – the demonstration of monoclonality by investigation of SmIg and of B- or T-lymphocyte nature by ANAE staining can be of great importance for early *diagnosis* and for diagnosis in body fluids or lymph node cell suspensions. The value of exact subclassification and early diagnosis with regard to *prognosis* and natural history will have to be established, because of the rarity of T subtypes. Direct *therapeutic* implications are scarcely given: Treatment of T-cell malignancies with specific antiglobulin must be regarded as an experimental measure [3]. Finally, by means of membrane marker tests, but especially ANAE cytochemistry, the portion of *normal T lymphocytes* in B-CLL, in CPL, and in hairy-cell leukemia may easily be quantitated in blood, bone marrow, and cell suspensions of lymph nodes.

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