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Multimarker Analysis of Childhood Acute Lymphoblastic Leukemia (ALL): Heterogeneity of Cellular Phenotypes and Clinical Relevance of Immunological Defined ALL Subclasses *

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A. Introduction

The comparative study of normal and leukemic cells by means of immunological phenotyping has provided insights into the origin of the malignant cell clones and the putative "target" cells of malignant transformation. The similarity found between normal and leukemic cell phenotypes suggests that leukemic blasts represent lymphoid cells which are arrested at a given stage along the individual differentiation pathway [4].

In the majority of childhood ALL (ca. 70%) the blasts exhibit the composite cellular phenotype of normal lymphoid bone marrow precursor cells: they are positive for the "common" ALL antigen (CALLA) and the HLA-DR/Ia antigen complex and express the nuclear enzyme terminal transferase (TdT) [5]. In approximately 20% of "common" ALL cases the cells additionally show small amounts of cytoplasmic mu chains [13], thus reflecting the phenotype of normal bone marrow pre-B cells [7]. Therefore during childhood this cell type probably represents a main candidate for leukemic transformation. Other childhood ALL subclasses are T-ALL (ca. 20%) and the rare B-ALL (1%–2%). In 10%–20% of ALL the origin of the blasts is unknown (U-ALL). It has been documented in several studies that the biological heterogeneity of leukemic cells is linked to a remarkable clinical heterogeneity. Thus T-ALL is usually associated with a higher initial blast count than "common" ALL. A possible explanation for the latter finding could be a different site of proliferation of malignant clonogenic cells (bone marrow in "common" ALL versus thymus in T-ALL). Prognostically the immunological phenotype is not an independent parameter since it is linked to other important clinical features (e.g., tumor load). However, the enormous importance of leukemic cell phenotyping for our understanding of the biology of malignant cells, for the improvement of diagnosis, and for a better definition of patients with high-, standard-, or low-risk disease is undoubted.

In the following we report on our experiences with leukemic cell phenotyping. The main aims of this study were (a) to investigate the heterogeneity of ALL subclasses with special emphasis on the U-ALL subclass and (b) to analyze the prognostic value of ALL subtyping.

B. Materials and Methods

Leukemic cells were derived from heparinized bone marrow and blood samples of children with previously untreated ALL. All patients have been treated in a cooperative study (COALL 80) according to the Hamburger protocol ALL V/79, which is a less aggressive modification of the West Berlin Study Programm BFM 76/79 [14]. In all cases diagnosis had been confirmed by conventional morphological and cytochemical methods. Immunological multimarker analyses was performed using the following reagents: an extensively absorbed rabbit antiserum against non-B –

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non-T ALL cells was used for the detection of the "common" ALL antigen. Later in the study this serum was replaced by monoclonal antibodies J-5 [11] and VIL-A 1 [10]. A cytotoxic heteroantiserum against T-cell antigen (anti-HUTLA) was prepared in rabbits which were immunized with thymic cells from children undergoing cardiac surgery. After absorption with normal and leukemic B cells this antiserum exclusively reacted with thymocytes and T-cell leukemias. Membrane immunoglobulin (Ig) was detected with commercial FITC-labelled polyvalent $F(ab)_2$ rabbit anti-human Ig antiserum.

For TdT detection an affinity-purified rabbit anti-calf TdT antiserum was used ([2]; BRL, United Kingdom). Monoclonal antibody DA2 recognizes a framework structure of the HLA-DR/Ia antigen complex [3] whereas monoclonal antibody Y 29/55 reacts with an epitope restricted to cells of B lineage [6].

Monoclonal antibodies BA1 [1] and BA2 [8] detected determinants highly specific for cells of lymphoid origin. Furthermore mononclonal antibodies from the OKT series (ORTHO, New Jersey) namely OKT3, OKT4, OKT6, and OKT8, were used. The E rosette assay was performed with neuraminidase-treated sheep red blood cells according to standard procedures. Binding of peanut (*Arachis hypogaea*) lectin was studied using TRITC-labelled affinity-purified peanut agglutinin (MILES) [12].

C. Results and Discussion

I. Heterogeneity of Phenotypes

In a prospective clinical study between 1979 and 1981 the blast from 54 children with previously untreated ALL were classified by immunological marker analysis. Table 1 shows the marker profile of the four ALL subclasses detected in this study. In 34 cases the blasts showed the typical phenotype of "common" ALL cells, i.e., CALLA+Ia+TdT+. In three cases a considerable number of cells (over 30%) were positive for B-cell specific markers: Ig or Y29/55. These findings indicate a partial differentiation of the blasts along the B-cell differentiation pathway and can be explained by the fact that ca. 20% of "common" ALL are pre-B cell leukemias [13]. The absence of T-cell markers in "common" ALL and recent results from Ig gene rearrangement studies [9] in ALL cells may suggest that a higher proportion of "common" ALL is derived from transformed pre-B cells. In our study ten cases could be identified as T-cell malignancies (seven T-ALL, three T-NHL). In every case the blasts were HUTLA positive and HLA-DR/Ia negative, whereas only one case was CALLA positive. Using the E rosette assay, anti-TdT, and the monoclonal antibodies of the OKT series a remarkable heterogeneity within this ALL subclass was detectable; e.g., in five of ten cases the T blasts did not bind sheep red blood cells; one of seven cases was TdT negative, and only three of eight reacted with OKT antibodies. The one patient with B-ALL showed blasts with monotypic surface Ig composed of mu and kappa chains.

Nine patients had blasts which were unclassifiable with the marker panel used in this study. However, as is shown in Table 1, the so called acute "unclassifiable" leukemias U-ALL do not represent a homogeneous subclass. The exact delineation of U-ALL cells is of utmost clinical importance in that some U-ALL may be of myeloic origin and need a different treatment. For this reason we used among other markers the monoclonal antibodies BA1 and BA2 and a plant lectin – the peanut agglutinin – in an attempt to further dissect this subclass.The results are shown in Table 2. Per definitionem all cases are negative for B- and T-cell markers and CALLA.

The following different U-ALL phenotypes could be identified: (1) Ia−TdT−; (2) Ia+TdT−; (3) Ia+TdT−BA1−BA2+; (4) Ia+BA1+BA2+; (5) Ia+TdT−BA1+BA2+; and (6) Ia−TdT−BA1−BA2−. Two out of five cases were positive for peanut lectin receptors. One of these cases (F.H.) was negative for all other markers. Of special value in the subclassification of U-ALL are the antibodies BA1 and BA2 in that these antibodies recognize determinants which are highly restricted to lymphoid lineage cells. However, the many questions concerning the origin and clinical features of U-ALL subclasses can only be answered in a larger study.

n (%)	"Common" ALL	T-ALL/NHL	B-ALL	U-ALL
	34 (62.9)	10 (18.5)	1 (1.9)	9 (16.7)
Anti-CALLA	34/34	1/10	0/1	0/9
Anti-TdT	20/21	6/7	0/1	3/9
Anti-Ia	34/34	0/10	1/1	5/9
Anti-Ig	2/34	0/10	1/1	0/9
Y29/55	1/13	0/8	1/1	0/7
Anti-HuTLA	0/34	10/10	0/1	0/9
SRBC	0/34	5/10	0/1	0/5
OKT3	0/21	0/7	0/1	0/5
OKT4	0/21	3/8	n.t.	0/5
OKT6	0/21	3/8	n.t.	0/5
OKT8	0/21	3/8	n.t.	0/5

Table 1. Reactivity of ALL blasts of 54 children with different markers

II. All Subclasses and Response to Treatment

Table 3 shows the clinical data in relation to the immunological ALL subclasses of the 54 children included in the prospective study. The most striking differences between the different subclasses are (a) a higher mean age in U-ALL and T-ALL/NHL, (b) a predominance of males in T-ALL/NHL and U-ALL, (c) a higher mean platelet count in T-ALL/NHL, and (d) a lower mean WBC in "common" ALL. The two patients with mediastinal mass both had T-NHL. High-risk and low-risk patients were defined on the basis of initial WBC (high-risk WBC over 25×10^9/liter). Children with high-risk disease received a reinforced reinduction therapy. As shown in Table 4 relapses occurred in all ALL subclasses. With respect to the patient fractions in first CCR a life table analysis revealed no significant difference between the only immunologically defined ALL subclasses. A remarkablely worse outcome showed patients with T-NHL but not those with T-ALL. These data confirm the finding of the BFM study group that with intensified treatment programs the T-ALL shows no worse prognosis than non-T ALL. However, on the basis of the immunological subtype and initial WBC count we were

Table 2. Reactivity of U-ALL cells with a selected marker panel

Patient	Age (years)	Sex	CALLA	SmIg	HuTLA	Ia	TdT	BA1	BA2	PNA
1. S.H.	16	m	–	–	–					
2. E.R.	13	m	–	–	–					
3. G.A.	8	m	–	–	–					
4. G.C.	10	m	–	–	–					
5. H.K.	18	m	–	–	–	+				
6. R.M.	10	f	–	–	–	+		–		
7. B.S.	11	f	–	–	–	+	+			–
8. C.C.	1.5	f	–	–	–	+	+			
9. S.P.	19	f	–	–	–	+	+			
10. H.R.	11	f	–	–	–	–				
11. V.G.	8	m	–	–	–	+	–			(+)
12. H.M.	1	m	–	–	–	+	+	–	+	–
13. C.G.	12	m	–	–	–	+	–	+	+	–
14. R.R.	9	m	–	–	–	+		+	+	
15. H.F.	7	m	–	–	–	–	–	–	–	+
16. Y.B.	12	m	–	–	–	+				

n	"Common" ALL	T-ALL/ NHL	B-ALL	U-ALL
	34	10	1	9
Age (mean)	4.5	6	11	9
< 2 years	1	1		1
> 10 years	4	4		4
Sex m/f	16/18	9/1	0/1	6/3
Platelet count (mean) ($\times 10^9$/l)	32	111	134	60
HK (1/1)	21	26.5	38	28
WBC ($\times 10^9$/l) (mean)	5.9	10.2	5.8	10.2
<25	29	6	1	5
>25	5	4		4
CNS involvement at diagnosis	2	2	0	0
Mediastinal mass at diagnosis	0	2	0	0

Table 3. Clinical details of immunologically defined ALL subclasses

ALL subclass	Relapse frequency (cumulative proportion in first CCR)		
	High-risk	Low-risk	Total
"Common" ALL	2/ 5 (0.6)	1/29 (0.97)	3/34 (0.91)
T-ALL	0/ 4 (1.0)	1/ 3 (0.67)	1/ 7 (0.86)
T-NHL			2/ 3 (0.33)
B-ALL		1/ 1 (0.0)	1/ 1 (0.0)
U-ALL	1/ 4 (0.75)	1/ 5 (0.80)	2/ 9 (0.78)
	3/13 (0.77)	4/38 (0.89)	9/54 (0.84)

Table 4. Immunologically defined ALL subtypes and response to treatment. Modified from Winkler et al. [15]

able to define a patient fraction with a cumulative proportion in first complete clinical remission CCR of 0.97: these patients had "common" ALL and less than 25×10^9 WBC/liter. The cumulative proportion in first CCR of patients with "common" ALL and over 25×10^9 WBC/liter, T-ALL/NHL, B-ALL, or U-ALL taken as one group is, at 0.68, significantly lower ($P < 0.05$). From the results of this study it is concluded that for further approaches to individualized therapy programs (i.e., reduction of ineffective toxicity) immunological phenotyping is a powerful tool for the identification of patients with high- and low-risk disease.

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References

1. Abramson CS, Kersey JH, LeBien TW (1981) A monoclonal antibody (BA-1) reactive with cells of human B lymphocyte lineage. J Immunol 126:83–88
2. Bollum FJ (1975) Antibody to terminal deoxynucleotidyl transferase. Proc Natl Acad Sci USA 72:4119–4122
3. Brodsky FM, Parham P, Barnstable CJ, Crumpton MJ, Bodmer MF (1979) Hybrid myeloma monoclonal antibodies against MHL products. Immunol Rev 47:3–61
4. Greaves MF, Janossy G (1978) Patterns of gene expression and cellular origin of human leukemias. Biochim Biophys Acta 516: 193–230
5. Greaves MF, Janossy G, Francis GE, Minowada J (1978) Membrane phenotypes of human leukemic cells and leukemic cell lines. Clinical correlations and biological implications. In: Differentiation of normal and neoplastic hematopoietic cells. Cold Spring Harbor Laboratories, pp 823–841
6. Gudat FG, Forster HK, Girard MF, Albrecht R, Ludwig C, Obrecht JP (1981) Recognition of leukemic B-lymphoma cells by monoclonal anti-Y29/55. In: Knapp W (ed) Leukemia markers. Academic Press, pp 109–112
7. Janossy G, Bollum FJ, Bradstock KF, Mc Michael A, Rapson N, Greaves MF (1979) Terminal transferase positive human bone marrow cell exhibits the antigenic phenotype of common acute lymphoblastic leukemia (ALL). J Immunol 123:1525–1528
8. Kersey JH, LeBien WT, Abramson CS, Newman R, Sutherland R, Greaves MF (1981) p 24: a human leukemia-associated and lymphohemopietic progenitor cell surface structure identified with monoclonal antibody. J Exp Med 153:726–731
9. Korsmeyer SJ, Hieter PA, Ravetch JV, Poplack DG, Leder P, Waldmann TA (1981) Patterns of immunoglobulin gene arrangement in human lymphocytic leukemias. In: Knapp W (ed) Leukemia markers. Academic Press, pp 85–97
10. Liszka K, Majdic O, Bettelheim P, Knapp W (1981) A monoclonal antibody (VIL-A1) reactive with common acute lymphatic leukemia (CALL) cells. In: Knapp W (ed) Leukemia markers. Academic Press, pp 61–64
11. Ritz J, Pesando JM, Notis-McConarty J, Lazarus H, Schlossman SF (1980) A monoclonal antibody to human acute lymphoblastic leukemia antigen. Nature 283:583
12. Thoene I (1982) Correspondence between the distribution of peanut agglutinin receptors and terminal transferase expression of human normal and leukemic cells (Abstract). J Clin Chem Clin Biochem 20:133
13. Vogler LB, Christ WM, Bockman DE, Pearl AR, Lewton AR, Cooper MD (1978) Pre-B cell leukemia: a new phenotype of childhood lymphoblastic leukemia. N Engl J Med 298:872–878
14. Winkler K, Matzke E, Caspers S, Erttmann R, Grosch-Wörner I, Kabisch H, Müller J, Landbeck G (1981) Vorstellung der kooperativen Studie COALL-80 zur Behandlung der akuten lymphoblastischen Leukämie im Kindesalter. Klin Päd 193:41–45
15. Winkler K, Beron G, Thoene I, Jürgens H, Goebel U, Gutjahr P, Spaar HJ, Drescher J, Thomas P, Landbeck G (to be published) Therapie der ALL im Kindesalter