Prognostic factors in acute lymphoid leukaemia of childhood. II. Cell surface markers

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Monoclonal sera have been used to determine the surface phaenotype of leukaemic cells during the last three years. Bone-marrow specimens of 57 children with recently diagnosed acute lymphoid leukaemia were examined; four cases were classified as T-cell leukaemia, 2 cases as B-cell leukaemia, in 37 cases cALLa was positive and fourteen children were classified as O-cell type, based on the absence of markers. Analysis of symptom-free survival revealed a very poor prognosis in B-cell leukaemia; there was no significant difference between the remaining groups. Within the cALLa positive cases L_1 exhibited a markedly more favourable prognosis than L_2.

Recognition of the membrane properties of the two principal cell types, T and B has opened a new approach to refined classification of lymphoid leukaemia. A great deal of lectins, viral and bacterial antigens have been found to bind differently to various types of leukaemic cells; thereby they can be used for characterisation of cell populations [6]. Detection of the common acute lymphoid leukaemia antigen (cALLa) had a large impact on the classification of childhood leukaemia since this is the antigen of differentiation, indicating the degree of maturation at which differentiation of the leukaemic lymphocytes has been arrested or at which malignant proliferation has started [7, 9]. Studies on the cell surface properties not only entail better biological characterisation of the blast cells but also are of prognostic value. As early as in the late seventies were published the first observations indicating that T and B cells carry a poor prognosis and that cases with cells exhibiting cALLa positivity have a better chance for survival [3, 4, 19]. The initially used methods using E rosette formation, demonstration of the presence of immunoglobulins at the cell surface or heterologous anti-cALLa sera have been gradually replaced by monoclonal antigens. The great advantage of the latter is their high-grade specificity and availability in nearly unlimited quantities.

Techniques using monoclonal antigens have led to exact typing of leukaemic cells and thereby to modernization of diagnostics. In this paper we report on our preliminary results.

MATERIAL AND METHODS

Patients. Data of 57 patients diagnosed since 1981 were examined (Table I). There were 35 boys and 22 girls, the mean of the groups lay between 3 and 6 years. According to the surface properties the patients were classified into four groups: the lymphoblasts of the bone-marrow or peripheral blood reacted either with T-cell antisera or B-cell antisera or antisera against the common ALL antigen or with none of these sera; the latter cells were termed O-cells.

The initial clinical and laboratory findings of the four groups were compared by histograms constructed as described ear-lier. There were no significant intergroup differences in leukocyte count and rate of hepatosplenomegaly, phenomena reflecting initial blast mass. Mediastinal lymph-node enlargement was encountered more frequently in cases affected by T-cells than in the remaining groups.

Therapy was uniform, corresponding to the recommendations of the National Working Group for Therapy of Childhood Leukaemia. Morphological classification was carried out according to the FAB principles. The patients' remission time was compared by the log-rank test [16].

Cell surface marker tests. Blast cells were obtained from the bone-marrow aspirate

TABLE I

Initial clinical data of ALL patients

Number of cases	cALLa positive 37	O-cell 14	T-cell 4	B-cell 2	P
Initial leukocyte count, higher than					
30/lower than 30 G/l	29/8	10/4	3/1	1/1	0.8
Hepatomegaly	4	3	0	1	0.2
Splenomegaly	10	4	1	1	0.9
Mediastinal tumour	1	2	2	0	0.01
Hb over 6.2/below 6.2 mmol/l	31/6	13/1	4/0	2/0	0.6
Median age, years	4	4	3	6	
Boys/girls	20/17	9/5	4/0	2/0	

TABLE II

Monoclonal antibody (MoAB) panel used for phenotyping leukaemic cells

MoAB	Specificity	Reference
J5	common ALL antigen	21
VIL—A1	common ALL antigen	11
VID—1	Type Ia	15
VIB—C5	B-cells and precursors, cALL cells, neuroblastoma cells	12
VIB—E3		
VIP—1	OKT—9 equivalent transferrine receptor	12
VIP—2b	OKT—10 equivalent activated T-cells, blast cells, etc.	12
Leu—1	pan-T-cells	14
VT—12		
Leu—2a	T-suppressor cells	14
VIT—8		
Leu—3a	T-helper cells	14
VIT—4		

<center>TABLE III</center>

<center>MoAB labelling pattern of the principal ALL phenotypes</center>

MoEA	Type of leukaemia			
	cALL	"O"-cell	T-cell	B-cell
J5	+	—	—	±
VIL—A1				
VIB—C5	+	—	—	+
VIB—E3				
HLA—Dr	+	±	±	+
VID—1				
VIP—1	—	—	—	—
VIP—2b	+	±	±	—
VIT—6	—	—	±	—
VIT—12	—	—	+	—
Leu—1				
Leu—3a	+	—	±	—
VIT—4				
Leu—2a	—	—	±	—
VIT—8				
TdT	+	±	±	—
SMIg	—	—	—	+
ER	—	—	+	—

(in three cases from peripheral blood) obtained at the first examination, the blasts were separated by Ficoll gradient centrifugation. The serum panel used for determination is shown in Table II. The cells were treated first with the corresponding monoclonal antigen, this was followed by the $Fab_{(2)}$ fragment of fluorescein-labelled anti-mouse Ig serum of rabbits. The test was evaluated by fluorescent microscopy, 200 cells were examined in each specimen.

Evaluation, phenotype groups. T-cell leukaemia was diagnosed if more than 50% of the blast cells were positive for E-rosette formation and could be labelled with monoclonal anti-T sera. For B-cells, the criteria were demonstration of surface immunoglobulin (Sm Ig) or labelling with anti-B-cell sera. In the cALLa positive group the cells were positive for Ia(HLA-DR) and dTt in addition to positivity with the specific anti-cALLa serum. Cells showing no binding with cALLa antiserum were termed O-cells. In some cases there was only Ia labelling, in these patients acute myeloid leukaemia was excluded by cytochem-

istry and absence of positive reactions with antimyeloid serum.

RESULTS

Out of the 57 patients 37 had "simple" cALLa positive leukaemia, there were four cases with T-cell, 2 cases with B-cell and 14 with O-cell leukaemia (Table I). The groups did not differ in respect of initial peripheral leukocyte count, the rate of hepatosplenomegaly was nearly identical in the four groups. Mediastinal tumour was present in 2 out of 4 patients with T-cell leukaemia; this was in spite of the small number of cases, significantly higher than in the three other groups (p = 0.01). There was no difference in initial haemoglobin levels, age and sex-ratio among the groups.
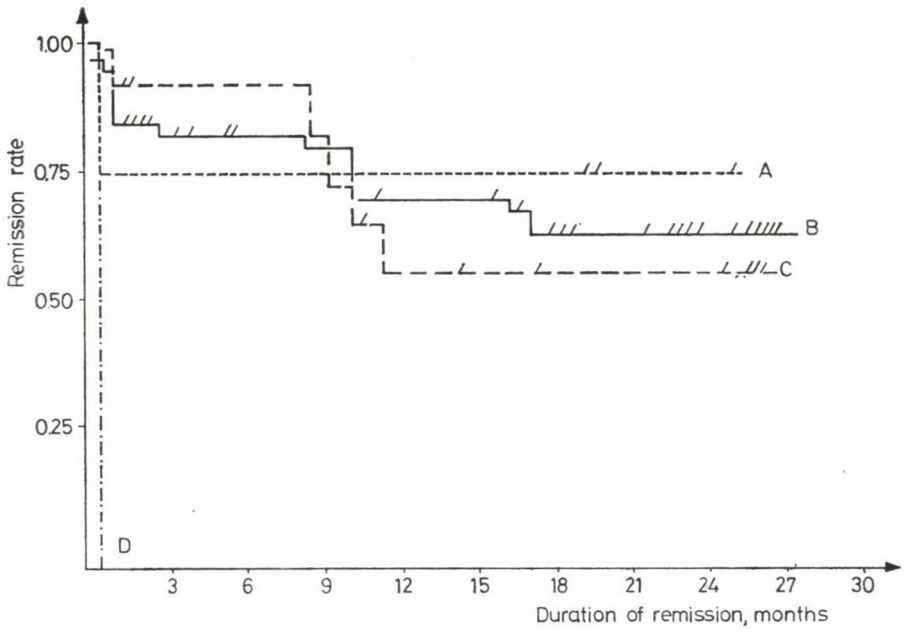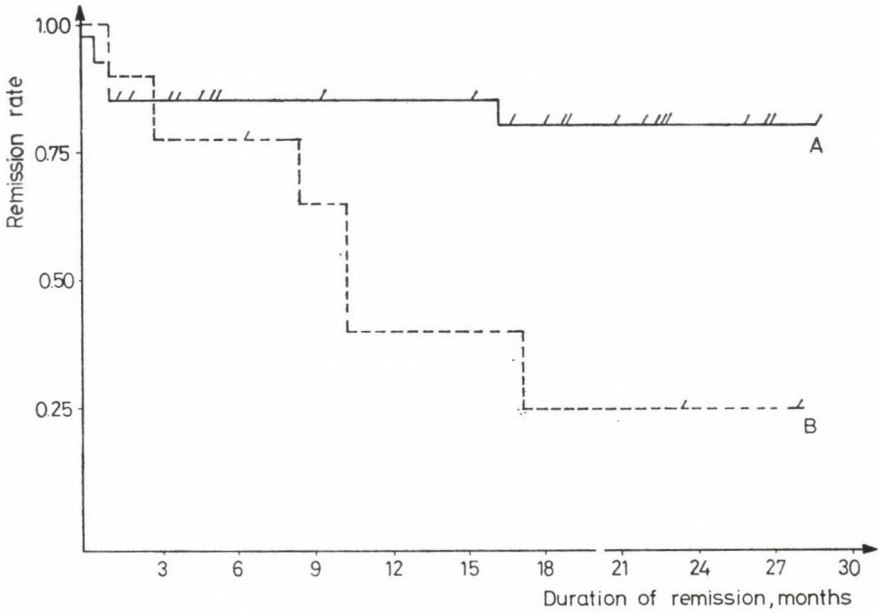
Fɪɢ. 1.

Fɪɢ. 2.

Analysis of the symptom-free survival rate showed that continuous remission could be achieved in three out of four patients afflicted by T-cell leukaemia while both children with B-cell leukaemia died within a few months after onset of the disease. The remission curves of the cALLa positive and O-cell group showed an intermediate position and did not attain 50% at the time of compilation of the manuscript (Figure 1). The significant difference between the two groups was due exclusively to the loss of the two B-cell patients. Within the cALLa positive group the effect of cell morphology on survival rate was also examined; a much better symptom-free survival rate was encountered with type L_1 than with L_2 (Figure 2).

DISCUSSION

We have used monoclonal antibodies for diagnostic purposes since 1981. At the time of diagnosis, 37 patients out of 57 had "simple" cALLa positive leukaemia, i.e. this developmental antigen was present on the surface of the blast cells. Their proportion within the whole material was lower than 70%, the mean percentage published in the literature [8, 9, 13]. At the same time, the proportion of O-cell leukaemia cases (14 per 57) in our material was higher than expected on the basis of the published 10—15%. The discrepancy may be ascribed to the small number of patients. It may also be suspected that in the first cases poor binding of the

applied antibodies resulted in false-negative results with cALLa. Examination of a large series will help us to clarify the real situation.

The prognostic value of surface marker tests was examined by their effect on the symptom-free survival rate (Figure 1). Quite surprisingly, the highest curve was found in T-cell leukaemia, where only one out of four children relapsed during follow-up. There is some controversy in the literature about the predicting role of the T-cell property. While some authors judge it unequivocally to predict a poor prognosis [4, 19], others and ourselves think that after correction for initial leukocyte counts and introduction of appropriate intensive treatment its ominous character disappears [8, 17]. Another unexpected finding was the comparatively good remission curve of cases with O-cell leukaemia, attaining that of cALLa positive cases.

False-negative cases, present spuriously in the O-cell group, might be one explanation. Both patients with B-cell leukaemia were lost soon after onset, within two and three months; the leukaemic process advanced inexorably. Now we already realise that our treatment, corresponding to methods recommended internationally in those days, was not capable of stopping this particularly rapid and aggressive type of leukaemia. It seems that new protocols, based on entirely different principles, will bring about dramatic improvement in the prognosis of this type of hitherto extremely sombre outlook.

2

Since the advent of marker tests several authors have questioned the prognostic value of morphological features. We have therefore attempted to find out whether a difference could be found between the remission curves of L_1 and L_2 patients within the cALLa positive group. Patients with L_1-leukaemia exhibited a significantly better survival rate. This has once again confirmed our earlier statement on the importance of morphological types [10]. We shall settle the problem by multivariance analysis of all imaginable prognostic factors in a large series.

In addition to their diagnostic value, monoclonal antibodies play an increasing role in the treatment of leukaemia. At present they are principally used in the preparative actions before autologous and allogeneic bone-marrow transplantation. While in autologous transplantation, removal of residual leukaemic cells is aimed at, in the case of allogeneic transplantation elimination of the host's T-cells is desirable in order to prevent graft versus host reactions. In fact, there have been attempts to utilise mono-clonal antibodies in vivo. Unfortunately, the initial results are not too promising, since their effect is weak and transient. One of the reasons of this failure is the fact that these antibodies have no or only weak activating effect on the human complement system, thereby the desired blast-killing is not achieved. Another problem is antigen modulation, i.e. the antigen disappears from or is modulated on, the surface of surviving cells treated with the antibody, and this prevents further binding of antibodies to the cell surface. The use of new-type, non-modulating antibodies or their coupling with toxins may open new prospects to leukaemia therapy of this kind.

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