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Clonal Diversity, Measured by Heterogeneity of Ig and TCR Gene Rearrangements, in Some Acute Leukaemias of Childhood is Associated With a More Aggressive Disease

T. Stankovic, J.R. Mann, P.J. Darbyshire and A.M.R. Taylor

The pattern of immune system gene rearrangements in acute leukaemias of childhood is heterogeneous. The biological significance of this heterogeneity in childhood acute leukaemia is still poorly understood. In this study, we analysed 49 children with acute leukaemia (29 B-precursor acute lymphoblastic leukaemia (ALL), 5 relapsed cALL, 6 T-ALL, 7 acute non-lymphocytic (ANLL) and 2 mixed lineage leukaemias), for the presence of different immune system gene rearrangements (Ig JH, Cκ, Cλ, TCR Jγ, Cβ, Jδ and Jα) by Southern blot hybridisation. The most prominent heterogeneity of immune system gene rearrangements was observed in the group of B-precursor ALL. The results from our study suggest that the heterogeneity of immune system gene rearrangement reflects clonal diversity in approximately one-third of patients with B-precursor ALL at presentation and in most patients in relapse. The observed association of clonal diversity with high white blood cell count, pre-B immunophenotype and age under 1 year in B-precursor ALL may have clinical significance. There was a significantly shorter disease-free survival in the group of B-precursor ALL patients with clonal diversity compared with those without clonal diversity. Clonal diversity may, therefore, be a mechanism of disease progression common to different types of aggressive B-precursor ALL.

Key words: Childhood leukaemias, immune system, gene rearrangements, clonal diversity
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INTRODUCTION

IMMUNE SYSTEM (Ig and TCR) genes rearrange during normal development of T and B lymphocytes. The complexity of these genes, which includes the existence of many V (variable), J (joining) and, for some, D (diversity) regions, allows recombination in a manner unique for each particular clone. Ig and TCR gene rearrangements appear to be good markers of clonality, as has been shown in many lymphoproliferative disorders [1–7].

The pattern of immune system gene rearrangements, however, is not always consistent with lineage commitment (B or T), the particular stage of differentiation or the presence of one leukaemic clone; rather it can be heterogeneous. Two processes may account for this heterogeneity, either clonal diversity with respect to the rearrangement of a particular immune system gene or cross lineage immune system gene rearrangements, both of which have been observed in childhood acute leukaemia.

Clonal diversity is regarded as a heterogeneous genotype in which several cell populations can be detected using immune system gene rearrangements. In some studies, for example, up to 40% of patients with B-precursor ALL (acute lymphoblastic leukaemia) showed oligoclonality with respect to the JH rearrangements [5, 7–9]. Diversity may be initiated in a tumour progenitor cell that has not previously undergone gene rearrangement and may be observed in one of the two following ways. Firstly, only a proportion of blast cells may have an immune system gene rearranged. Secondly, there may be evidence of several subpopulations of tumour cells, as shown by the presence of several rearrangements in the same Ig/TCR gene [10, 11]. Clonal diversity may also occur within a clone that has already undergone gene rearrangement as a consequence of continuing rearrangement of immune system gene(s) during the process of tumour progression. These newly derived clones show similar, related immune system gene rearrangements [12].

A variable proportion of children with acute leukaemias show heterogeneity in the form of cross lineage immune system gene rearrangements, such as rearrangements of TCR loci in B-precursor ALL, rearrangements of Ig genes in T-ALL and either Ig or TCR rearrangements in ANLL (acute non-lymphocytic leukaemia) [13–15]. Cross lineage immune system gene rearrangements in acute leukaemias are usually attributed to an immature tumour stem cell exhibiting lineage infidelity [16]. For a group of B-precursor ALL with a relatively mature immunophenotype and rearrangement of TCR genes, an alternative mechanism was proposed, in which there is involvement of a common recombinase in the relatively mature phase of B lymphoid differentiation [13].

The clinical significance of different forms of immune system gene rearrangement heterogeneity in childhood acute leukaemias is still not clear and remains to be established. In our study we analysed a non-selected group of children with acute leukaemias for the presence of different rearrangements of immune system genes. The first objective was to improve the detection of clonal diversity by analysing gene rearrangements in all seven immune system gene loci (J_H, Cκ, Cλ, TCRβ, TCRγ, TCRδ and TCRα) and setting criteria for the detection of clonal diversity, including both the number of rearranged bands and their relative density. We wanted to be able to distinguish one group of patients with heterogeneity of immune system gene rearrangements within a single population of leukaemic cells from other patients in whom heterogeneity reflected clonal diversity. The second objective was to determine the clinical significance of the detected diversity in the group of patients with B-precursor ALL. We have related clonal diversity to other features at clinical presentation and compared disease-free survival in B-precursor ALL patients with or without clonal diversity.

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PATIENTS AND METHODS

Patients and cell samples

49 children with acute leukaemia (44 newly diagnosed and five relapsed patients) who presented at Birmingham Children's Hospital, U.K., were included in this study. Bone marrow (BM) or peripheral blood (PB) samples, with more than 90% of leukaemic blasts, were investigated. In 7 newly diagnosed patients a second sample was taken on the 28th day, at the end of induction treatment. In 2 of these 7 patients, samples were also taken after the end of treatment, and in 1 patient samples were collected regularly during progression of the disease. The diagnosis of leukaemia was based on standard criteria (cytomorphology, cytochemistry and immunophenotype). The mononuclear cells were isolated from bone marrow or peripheral blood using Ficoll–Hypaque density centrifugation.

Immunological marker analysis

Samples were analysed for the nuclear expression of TdT, the expression of the B-cell markers CD19 and CD10, T-cell markers CD2, CD3 and CD7, the myeloid/monocytic markers CD13, CD33 and CD15, the platelet marker GPIIIa/IIb and non-lineage-specific markers CD34 and HLA-DR. The samples were also analysed for the presence of cytoplasmic μ chains. Surface markers were visualised by the use of fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG or IgM and evaluated using either fluorescence microscopy or a FACScan flow-cytometer (Becton Dickinson).

Cytogenetic analysis

Chromosomal analysis of bone marrow samples was performed following short-term unstimulated liquid culture. Metaphases were G-banded in trypsin and stained with Leishmann's stain. 10–50 cells were analysed for each sample.

Southern blot analysis

DNA was isolated from mononuclear cells as described previously [17]. DNA (10 μg) was digested with the appropriate restriction enzymes. The restriction fragments were size-fractionated in 0.8% agarose gels, transferred to Hybond-N filters and hybridised according to Southern [18]. The configuration of Ig and TCR genes was analysed using the following probes: a 2.7-kb *Bgl*II/*Pst*I fragment specific for the J_H region (M13CTGR51A), a 0.8-kb *Hind*III/*Eco*RI fragment for the Cκ region (pRH10), an 8-kb *Eco*RI fragment for the Cλ region, a 1.5-kb *Sac*I fragment for the Jδ region (JδS16), a 5-kb *Eco*RI fragment for the Jα region (JαRR), a 0.7-kb *Hind*III/*Eco*RI fragment for the Jγ region (M13H60) and a 0.7-kb *Hind*III/*Eco*RI fragment for the Cβ region.

RESULTS

Immunophenotype

The results of the immunophenotype of 44 newly diagnosed children with acute leukaemia are presented in Table 1. Among 29 B-precursor ALL, 24 were common ALL, three were null ALL and two were pre-B-ALL: six T-ALL were positive for T cell markers (CD7, CD2). Six of the seven children with ANLL were positive for CD13 and CD33 and two of these six were also positive for GPIIIa/IIb. The 7th ANLL patient was positive for CD7 and GPIIIa/IIb.

Detection of TdT revealed positivity in 1/3 patients with null ALL, 20/24 with common ALL, 2/2 with pre-B ALL, 3/6 with T-ALL and 1/7 with ANLL (Table 1). 1 of 2 patients with mixed lineage leukaemia showed the presence of two cell populations, 1 with a common ALL immunophenotype and 1 with dual lymphoid/myeloid immunophenotype. The immunophenotype of the second mixed lineage leukaemia revealed the presence of myeloid markers only.

All 5 relapsed B-precursor ALL patients had a common ALL immunophenotype.

Southern blot analysis of immunoglobulin gene rearrangements

The results of analysis of immune system gene rearrangements in children with different types of acute leukaemia are presented in Table 2.

B-precursor ALL

DNA samples from 29 children with B-precursor ALL were analysed for the presence of rearranged IgH genes. The criteria for distinguishing bi and oligoclonality from monoclonality were based on the detection of an excess of bands in relation to the number of alleles present and the relative difference in density between detected bands. For example, the presence of three bands in cells with chromosomal evidence of only two alleles was considered, at least, as biclonality. Similarly, a prominent difference in density between a germline band and one or more rearranged bands was considered as evidence for the presence of two populations of cells. Rearrangement of one or both J_H alleles was found in 25/29 patients (86%). Biclonality or oligoclonality, by the criteria given above, was detected in 6/25 patients (24%) with J_H rearrangements. 9 out of 23 analysed patients showed the Cκ gene rearranged (39%). Oligoclonality was observed in a further 3 cases (in addition to the 6 cases with J_H). Rearrangements of Cλ genes were observed in 2 patients with B-precursor childhood ALL (9%).

Rearrangements of different TCR genes were detected in various proportions of patients with B-precursor ALL (Table 2). TCRγ rearrangements were observed in 18/26 patients (69%). In 9/18 patients with rearranged TCRγ genes, the rearrangement of only one allele was observed. Interestingly, in 3 of these 9 patients the rearranged band was considerably less dense compared with the germline bands, suggesting that TCRγ rearrangement was a later event in the leukaemic clone (Figures 1 and 2). The possibility that the germline bands represent the presence of a large number of normal cells can be ruled out, since hybridisation of the same filter (Figure 2) to TCRδ showed the presence of a single rearranged band with the inference that all the tumour cells were clonal with respect to this rearrangement. The presence of the single TCRδ rearrangement also excludes the possibility of incomplete digestion with *Bgl*II as the explanation for the rearranged TCRγ band. TCRCβ rearrangements were observed in 4/26 patients (15%) and TCR J δ rearrangement or deletion in 19/22 patients (86%). Biclonality was detected in a single patient with TCRδ and in a single patient with TCRβ (Table 3, patients 1–12). Rearrangement of TCRα genes was not detected in any of 20 analysed patients using the TCRJαRR probe (Table 2).

On the basis of analysis of immune system gene rearrangement in seven loci, different patterns of clonal diversity were detected in 12 patients (Table 3). 10 of the 12 patients showed subclone formation at the level of a single immune system gene locus. Of these, 6 were with respect to J_H, 2 with respect to Cκ and 1 each with respect to Jγ and Jδ. 2 patients showed subclone formation with respect to more than one gene locus; 1 patient showing biclonality with respect to both Cκ and Jγ and 1 showing simultaneous subclone formation with respect to both Jγ and Jδ. Patients with B-precursor ALL showing biclonality or oligoclonality detected either by Ig or TCR rearrangements, or in combination, were analysed in relation to different parameters at clinical presentation (Table 3). The remaining 17 patients

Table 1. *Immunophenotype of* 44 *newly diagnosed childhood ALL*

	Precursor-B ALL					
	Null ALL ($n = 3$)	Common ALL ($n = 24$)	Pre-B ALL ($n = 2$)	T-ALL ($n = 6$)	ANLL ($n = 7$)	Mixed lineage leukaemia ($n = 2$)
TdT	1/3*	20/24†	2/2	3/6	1/7	1/2
HLA-DR	3/3	24/24	2/2	1/6		2/2
CD10	—	24/24	2/2			1/2
CD19	3/3	24/24	2/2			1/2
Cyμ	—	—	2/2			
SmIg		—	—			
CD7				6/6	1/7	
CD2				6/6		
CD13					6/7	2/2
CD33					6/7	2/2
GPIIIa/IIb					3/7	

* In 1 patient the result was negative and in 1 patient the result was inconclusive. † In 4 patients the results were inconclusive.

Table 2. Distribution of Ig and TCR gene rearrangements in childhood acute leukaemias

	Rearranged genes						
Type of leukaemia	J_H	Cκ	Cλ	Cβ	Jγ	Jδ	Jα
Precursor-B ALL (newly diagnosed)	25/29 (86%) 6 biclon/oligo	9/23 (39%) 3 biclon	2/23 (9%)	4/26 (15%) 1 biclon	18/26 (69%) 3 biclon	19/22 (86%) 1 biclon	0/20 (0%)
Relapsed cALL	4/4 2 biclon	0/3	1/3	2/3 1 biclon	3/4 1 biclon	4/4	0/5
T-ALL	0/6	0/4	0/5	4/5	5/5	5/6	0/5
ANLL	0/7	0/6	0/4	1/4	0/6	2/5	0/7
Mixed lineage	2/2	0/2	0/2	0/2	0/2	0/2	0/2

biclon, biclonal; oligo, oligoclonal.

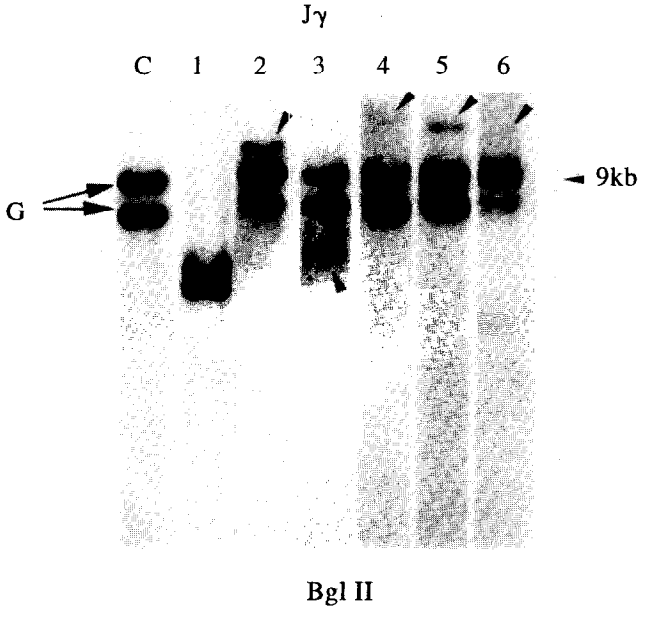

Figure 1. Differences in TCRγ gene rearrangements between childhood B-precursor ALL and T-ALL following digestion of DNA with *Bgl*II. Lane C shows germline bands for TCRγ. Lane 1—patient with T-ALL showing both TCRγ alleles rearranged in all blast cells. Lanes 2–6—patients with B-precursor ALL showing only a minor population of leukaemic blasts with TCR γ rearrangements.

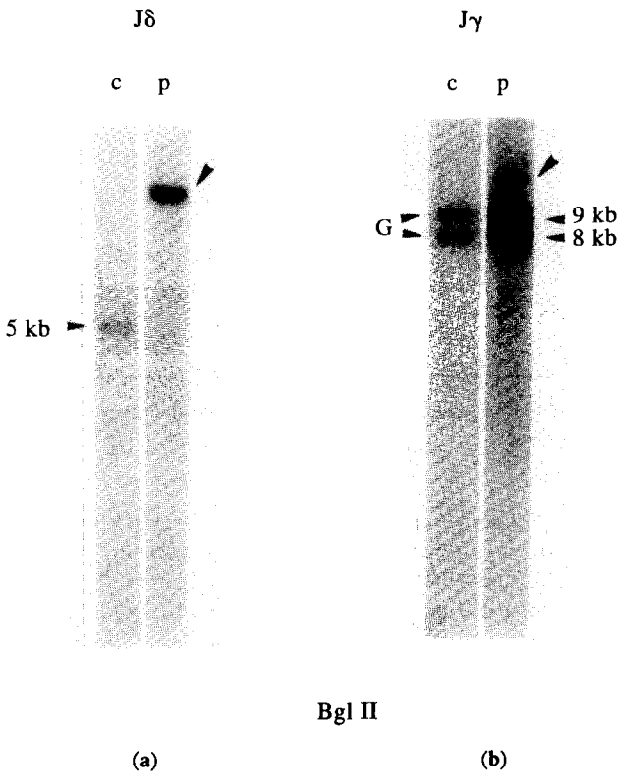

Figure 2. Comparison of clones with TCRδ and TCRγ rearrangements in the same patient with B-precursor ALL, following digestion of DNA with *Bgl*II. (a) A TCRδ rearrangement present in all the blast cells. (b) A TCRγ rearrangement present in only a proportion of blast cells. Lane C in both figures shows germline bands.

with B-precursor ALL, with or without TCR cross lineage rearrangements but with no evidence of clonal diversity, were related to parameters at presentation as a separate group (Table 3, patients 13–29).

From an analysis of 12 patients with different patterns of clonal diversity, some associations with clinical features at presentation were noted. First, the 2 patients with pre-B immunophenotype, known to have a less favourable prognosis, showed clonal diversity (biclonality with J_H and Cκ, respectively). Second, the majority of patients (9/12) from this group had a high WBC above 20×10^9/l (mean WBC 57×10^9/l). The 4 patients (including patient 7) with the highest WBC (> 100×10^9/l) belonged to this group. Third, all 3 patients aged 1 year or under (patients 4, 6 and 9) showed oligoclonality with respect to at least one immune system gene locus (Table 3).

Structural chromosomal abnormalities were detected in 4 patients with clonal diversity of immune system gene rearrangements but none of the observed abnormalities was known to be associated with a poor prognosis. Male and female patients were equally distributed in the group of B-precursor ALL showing clonal diversity (7 males and 5 females) (Table 3).

B-precursor ALL patients with no evidence of clonal diversity were also analysed in relation to parameters at presentation (Table 3). In 14/17 patients from this group cross lineage rearrangements within the TCR loci were detected, but there was no evidence of the presence of more than one population of

Table 3. Clinical characteristics and Southern blot analyses of B-precursor childhood ALL with or without clonal diversity

Patient No.	Age/Sex	WBC × 10^9/l	Type of ALL other than cALL	Duration of remission (months)	J_H	Cκ	Jγ	Cβ	Jδ
1	2/M	5.2		40+	B	G	G	ND	R
2	2/F	32		20	O	G	G	G	G
3	10/M	7.1		52+	B	G	G	G	R
4	1/F	144	Pre-B	44+	B	ND	G	G	G
5	4/F	26		0	R	B	B	G	R
6	1/M	25		42	R	B	R	G	ND
7	5/F	94	Pre-B	55+	R	B	R	G	R
8	3/M	116		24	R	G	B	G	G
9	1/M	138		45	R	G	B	G	B
10	4/M	51		26	B	G	G	G	R
11	10/F	7.1	Null ALL	15	B	ND	R	R	ND
12	2/M	39		5	R	R	R	B	R
13	6/F	3.2		56+	R	R	R	G	ND
14	2/M	7.5		47+	R	ND	ND	G	ND
15	5/M	40		32+	R	G	G	G	R
16	8/F	5.2		53+	G	G	G	ND	ND
17	2/M	44		29	R	R	R	G	R
18	8/F	7.7		54+	R	G	R	G	ND
19	7/M	10	Null ALL	35+	G	G	R	R	R
20	12/M	1.6	Null ALL	52+	G	ND	ND	G	ND
21	5/M	13		29	G	G	R	G	R
22	4/M	3.1		32+	R	G	R	G	R
23	3/M	32		29+	R	R	R	R	R
24	2/M	34		29	R	R	R	G	R
25	4/M	36		56+	R	G	R	G	R
26	4/M	16		0	R	R	G	G	R
27	2/M	8.6		54+	R	ND	R	G	R
28	3/M	4.0		51+	R	ND	R	ND	R
29	4/F	5.5		49+	R	G	ND	G	R

M, male; F, female; R, rearranged; O, oligoclonal rearrangement; B, biclonal rearrangement; G, germline; ND, not done; +, still in remission.

leukaemic blasts. These patients had considerably lower WBC in comparison with patients showing clonal diversity (mean of 16 × 10^9/l); only 5/17 (Table 3) had WBC above 20 × 10^9/l. The ages of patients from this group varied between 2 and 12 years, with no patients under the age of 1 year. Karyotypes were not remarkable. There was no striking association of features reflecting poor prognosis in this group. An excess of male patients (13/17), however, was noted.

Disease-free survival

In the group of B precursor ALL with clonal diversity 7 of the 12 patients relapsed and in the group of B precursor ALL without diversity only 3 of 17 patients relapsed (Table 3). Figure 3 shows Kaplan–Meier plots of the relationship between disease-free survival and clonality in patients with B-precursor ALL. 4-year survival in the group with clonal diversity was 27% compared with 74% in the group without clonal diversity. A log rank test showed a significant difference between these two plots (P = 0.010).

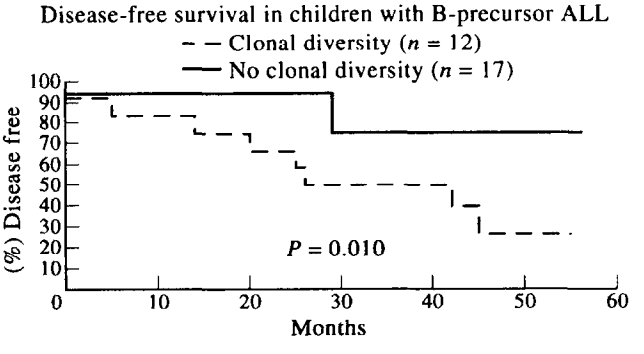

Figure 3. Disease-free survival in children with B-precursor ALL. The solid line represents a group of patients without evidence of clonal diversity and the broken line a group of patients with clonal diversity. 4-year survival is 74% in the first group and 27% in the second group, P = 0.010.

Relapsed common ALL (cALL)

2 out of 4 analysed children with cALL who relapsed showed a biclonal pattern of J_H rearrangements and 2 patients showed a monoclonal pattern (Table 2).

4 children with relapsed cALL showed rearrangements within TCR genes (4/4 within Jδ, 3/4 within Jγ region and 2 within Cβ). Biclonality with respect to TCR gene rearrangements was observed in 2 patients in addition to biclonality shown in patients by J_H. Thus, 4 of 5 children with relapsed cALL showed evidence of clonal diversity. Interestingly, one of these patients, with biclonality detected by both Cβ and Jδ rearrangements, had several relapses.

T-ALL

In the group of 6 patients with T-ALL, no Ig gene rearrangements were detected (Table 2). 4 of 5 analysed patients showed Cβ rearrangements, 5/5 analysed showed Jγ rearrangements and 5/6 patients showed Jδ rearrangement. Of the 5 patients with both Jδ and Jγ rearrangements, all had both Jγ alleles rearranged (Figure 1). TCRα rearrangements were not detected in 6 children with T-ALL (Table 2). There was no evidence for the presence of clonal diversity with respect to any of the rearranged TCR genes.

ANLL

Rearrangements of immunoglobulin genes were not detected in this group of patients (Table 2). 1 patient showed rearrangement of Cβ genes and 2 patients rearrangement of Jδ, without evidence for the presence of more than one population of cells.

Mixed lineage acute leukaemia

Rearrangement of the J_H locus was detected in both patients with mixed lineage leukaemia. In 1 patient two bands were detected, one germline and one rearranged, the densities of which corresponded to the proportion of myeloid and lymphoid cells, respectively, observed by morphological analysis (two-thirds lymphoid population, one-third myeloid population) (Figure 4, lane 1). In the second case of mixed lineage leukaemia, two rearranged bands of different density were observed using the J_H probe, suggesting the presence of two populations of cells both with J_H genes rearranged (Figure 4, lane 2). Two populations of malignant cells with different immunophenotypes were detected, one positive for lymphoid markers only and the other positive for both lymphoid and myeloid markers. The immunophenotype of the second case was therefore consistent with the molecular observation. Cκ, Cλ and TCR genes retained germline configurations at presentation in both of these patients.

Assessment of immune system gene rearrangements during the course of disease

Reassessment of J_H rearrangements was performed in 7 newly diagnosed patients (6 with common ALL and 1 with mixed lineage leukaemia) after the end of induction treatment. Southern blot analysis showed the reappearance of the germline band without residual rearranged bands in 6 patients. Residual disease was detected in 1 patient using the J_H probe. In this patient, the same two rearranged bands as at presentation but of decreased density, plus a germline band were observed. The same patient was followed during the progression of disease and two additional rearranged bands with the J_H probe were observed (see [11]). As a consequence of clonal evolution, a minor population of cells showing rearrangement within Cβ locus was detected in the terminal phase of disease in this patient.

In 2 patients, without evidence of clonal diversity at presentation, both TCRγ and TCRδ rearrangements were reassessed, in addition to J_H, after the end of induction treatment. The reassessment by Southern blot hybridisation showed a synchronous disappearance of IgH, TCRγ and TCRδ rearranged bands (Figure 5). These 2 patients were also analysed at the end of treatment and did not show any residual IgH, TCRγ and TCRδ rearranged clones on Southern blot filters.

DISCUSSION

Immune system gene rearrangements were assessed in 49 patients consecutively diagnosed with childhood acute leu-

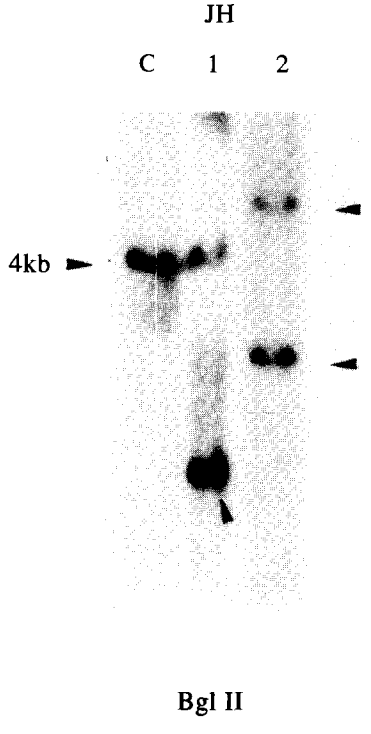

Figure 4. IgJ_H rearrangement in hybrid leukaemia. Lane C shows a germline band of 4 kb for J_H following digestion with *Bgl*II. Lane 1—a hybrid leukaemia, from the first patient, with the minority of blast cells retaining the germline configuration of J_H genes and the majority of cells showing a rearrangement of J_H. Lane 2—a hybrid leukaemia, from a second patient, with two populations of blast cells as shown by the different densities of the J_H rearranged bands.

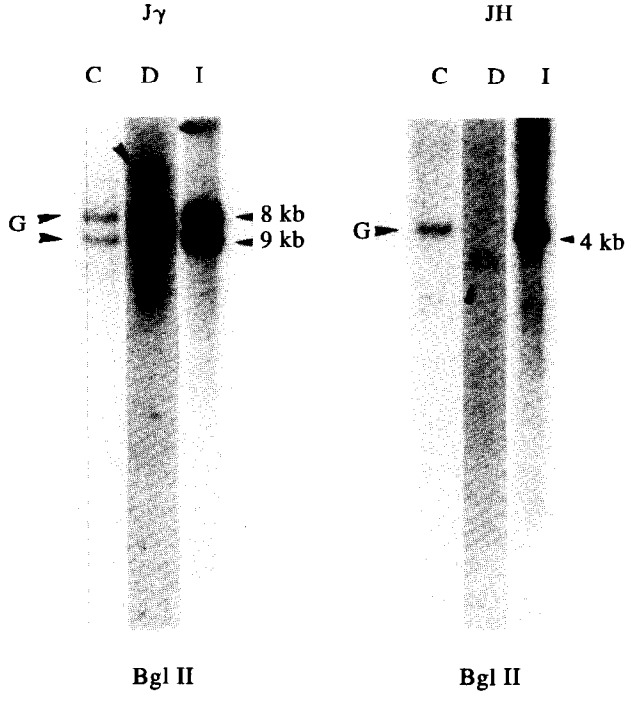

Figure 5. Synchronous disappearance of TCRγ and IgH rearranged clones in the same patient, after induction treatment for B-precursor ALL. Lane C shows germline bands for TCRγ and IgH, following *Bgl*II digestion of DNA. Lane D shows rearrangements (arrowed) at the time of diagnosis of ALL. Lane I shows disappearance of rearranged bands at the end of induction treatment.

kaemia. Analysis revealed heterogeneity of Ig and TCR gene rearrangements in all subgroups of childhood acute leukaemias. The most prominent heterogeneity of immune system gene rearrangements was observed in the group of childhood B-precursor ALL. The frequency of detected gene rearrangements in analysed patients with B-precursor ALL (86% J_H, 39% Cκ, 9% Cλ, 15% Cβ, 69% Jγ, 86% Jδ and 0% Jα) is in general agreement with other published results [1–4].

We analysed patients with B-precursor ALL and different patterns of immune system gene rearrangements in relation to prognostic parameters at clinical presentation: age, sex, WBC, immunophenotype and cytogenetic analysis. The comparison between two groups of patients, with and without evidence of clonal diversity, showed a remarkable difference in terms of association with particular parameters at clinical presentation. The vast majority of patients with cALL and a high WBC, as well as all patients with pre-B immunophenotype and all patients under the age of 1 year, showed clonal diversity with respect to one or several immune system genes.

Multiple immune system gene rearrangements within immunoglobulin loci have been the subject of different studies in patients with B-precursor ALL [5, 6, 8, 19]. Kitchingman and associates [8] as well as Beishuizen and colleagues [5] suggested that B-precursor ALL with multiple IgH gene rearrangements may have a higher tendency to relapse. However, Beishuizen's study failed to reveal a significant difference in disease-free survival between patients showing J_H multiclonality and patients with a single J_H clone [5]. Both Katz and associates [9] and Forestier and associates [19] found no correlation between J_H multiclonality and clinical outcome. In the present study, we improved the detection of clonal diversity in two ways. First, we analysed both Ig and TCR immune system loci rather than Ig genes only, for the presence of oligoclonality, and second we set out criteria to detect oligoclonality including not only the number but also the relative density of bands. We were able to demonstrate that half of the patients with clonal diversity (6/12) showed diversity in loci other than J_H. The observation that oligoclonality can be restricted to TCR loci, as shown here by simultaneous subclone formation in both Jγ and Jδ loci, is novel as is the observation that simultaneous subclone formation in B-precursor ALL can include both Cκ and Jγ loci.

A comparison of the two groups of patients with and without clonal diversity stratified according to the criteria described showed they had a significant difference in disease-free survival. This is the first study to show that patients with B-precursor clonal diversity have a significantly higher tendency to relapse. One may argue that the association between clonal diversity and other poor prognostic features of B-precursor ALL, such as a high WBC, age under 1 year and a pre-B immunophenotype may be the reason why patients with clonal diversity have a poor outcome. Such a suggestion has already been made by Katz and colleagues [8] and was anticipated by Beishuizen and colleagues [5]. We suggest that clonal diversity may represent a mechanism of disease progression which is common to different types of aggressive B-precursor ALL (infant ALL, pre-B ALL and cALL with high WBC). It is possible that clonal diversity provides a source of clonal variation which leads gradually to the selection of a resistant clone.

The fact that clonal diversity is associated with other high risk features of B-precursor ALL may be important from the perspective of following minimal residual disease. Patients with clonal diversity and other poor prognostic features bear a higher risk of relapse and, therefore, an early assessment of minimal residual disease, as well as assessment at regular intervals in this group may be of special importance. In addition, as clonal diversity can involve any of the immune system loci it may be useful to expand the assessment of minimal residual disease to as many immune system gene loci as possible.

Several studies have analysed the change of pattern of clonality in relapse of B-precursor ALL [12, 20–23] and in some studies it has been shown that the relapsed clone, when compared with the clone at presentation, may show the same TCR but different Ig rearrangement [19]. For example, a leukaemic progenitor cell with a TCR gene rearranged and Ig genes in germline configuration can give rise to two different clones, each of which bears the same parental TCR rearrangement but with different Ig rearrangements. It is also possible that additional rearrangement within the joining segment of a particular gene can take place during the progression of disease. In our study, we undertook a reassessment of gene rearrangements at three different loci (J_H, Jδ and Jγ) in 2 patients with B-precursor ALL, at both the end of the induction treatment and after completion of treatment. Both were patients with no evidence of clonal diversity and, therefore, J_H, Jδ and Jγ rearrangements observed at presentation were related to the same leukaemic clone. We found a simultaneous disappearance of J_H, Jδ and Jγ rearranged bands in both patients, with the reappearance of the germline bands. The disappearance of all three rearrangements was the consequence of the loss of the single leukaemic clone. This finding was consistent with morphological analysis of the bone marrow smears, which suggested remission. Conversely, in 1 of 2 patients with a mixed lineage leukaemia, the follow-up of immune system gene rearrangements appeared to be a good means of detecting a change in the pattern of clonal diversity during the progression of disease.

The rearrangements in T-ALL were lineage specific. Heterogeneity of immune system gene rearrangements observed in our group of patients with T-ALL and ANLL did not seem to reflect clonal diversity. Although it is difficult to draw any conclusion from the small number of analysed patients, it is possible that the heterogeneity of immune system gene rearrangements in ANLL and T-ALL reflects a mechanism that is different from clonal diversity.

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Feature Articles

Renal Cell Carcinoma and Interleukin-2: A Review

J. Wagstaff, J.W. Baars, G.-J. Wolbink, K. Hoekman, A.J.M. Eerenberg-Belmer and C.E. Hack

CANCERS ARISING in the kidney are relatively uncommon, accounting for approximately 1.4% of all cancers in Northern Europe and 1.5% of all cancer deaths. The disease is twice as common in men as in women, and has a peak age of around 60 years [1]. Outside of paediatric practice, the dominant histology is adenocarcinoma with less than 10% being squamous or transitional carcinomas of the renal pelvis. The former histological type has also been termed hypernephroma [2] or Grawitz tumour [3].

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Renal cell carcinoma (RCC) may remain clinically occult for most of its course. The presenting clinical features of RCC have been well described and are usually indicative of advanced disease [4–6]. Although the classical triad of gross haematuria, abdominal mass and pain are only seen in 9% of patients, individual symptoms including haematuria (59%), abdominal mass (45%), pain (41%), weight loss (29%) and anaemia (21%) are common. Approximately 30% of patients have metastatic disease at presentation, another 25% have locally advanced disease, leaving 45% with localised tumour [7]. The TNM staging system provides an accurate method of classifying the extent of tumour involvement, although the staging has been simplified by dividing the patients into four groups [8]. The shortcomings of this system become obvious, however, when it is noted that the survival rate of patients with stage II is less than that of stage III (Table 1), indicating an inappropriate assignment to prognostic groups. The grouping of renal vein,