Late Relapsing Childhood Lymphoblastic Leukemia

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Childhood lymphoblastic leukemia (ALL) is usually assumed to have been permanently eradicated in patients in long-term remission, but occasionally can recur after many years. To learn more about the problem, we studied a group of children whose leukemia had been in remission for 10 or more years before relapse and tried to determine whether they had true recurrences or second malignancies. We studied children treated on Medical Research Council ALL protocols between 1970 and 1984 and followed up by the Clinical Trial Service Unit in Oxford. Detailed clinical and laboratory data was collected from the centers concerned on all who were reported to have had a recurrence of their leukemia after 10 or more years from the time of achieving first complete remission (CR1). To prove that the relapse was a true recurrence rather than a second or secondary leukemia, DNA extracted from archived marrow smears was subjected to polymerase chain reaction (PCR) analysis for the presence of an identical Ig heavy chain (IgH) or T-cell receptor (TCR) gene rearrangement at initial diagnosis and subsequent relapse. A total of 1,134 of 2,746 children had survived 10 years or more (range, 10 to 24 years) in CR1 and of those, 12 (approximately 1%) had subsequently relapsed. Relapse blast cells were shown to express the common ALL antigen (CD 10) in all cases and an identical clonal IgH or TCR gene rearrangement was found on PCR analysis of DNA from diagnosis and relapse in all eight cases where DNA extraction was successful. A further program of therapy was successful in inducing a second CR in all patients, four of whom have succumbed to a second relapse after 12 to 27 months. The remaining eight are in continuing CR2 at a follow-up of 12 to 108 months (median, 52) from relapse. Although the risk of relapse of childhood ALL after 10 years in remission appears to be small (around 1%), it persists. This raises questions about how blasts can survive quiescent for so long and when we can truly be confident of cure, if ever.
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EVEN AFTER TREATMENT with the best current therapy, approximately one in four children with lymphoblastic leukemia (ALL) relapse.[1] The risk of relapse is mainly during the period immediately after stopping treatment and diminishes with time so that patients in complete remission (CR) for 10 years are considered to be effectively cured of their disease.[2] Relapse after that time period has been reported,[3] but until a few years ago, it was difficult to be certain whether these patients had a true recurrence of the original clone or a second or secondary leukemia.

Confirmation of a true relapse can now be obtained by documenting the presence of an identical clone-specific molecular signature in lymphoblasts at diagnosis and recurrence. Immunoglobulin (IgH) and T-cell receptor (TCR) gene loci consist of dispersed gene segments, which undergo somatic recombination early in B- and T-cell ontogeny to produce functional Ig or TCR molecules. Clonal IgH or TCR rearrangements are detectable in almost every case of ALL. Each rearranged gene is unique and therefore provides a good clonal marker to monitor minimal residual disease in patients in apparent remission.

Such molecular techniques have been used by several investigators, including ourselves, to confirm relapse in cases of late recurrence.[4-6] These cases not only raise fundamental questions about the biology of childhood ALL, they also generate anxiety about the resilience of cure in patients whose leukemia has been in long-term remission. To answer the latter question in particular, we studied long-term follow-up data on a large cohort of children treated on the MRC national ALL trials. We tried to estimate the frequency of very late relapse, what type of leukemia gives rise to it, and what type of patient is at risk.

MATERIALS AND METHODS

Patient Population and Data Collection

Since the late 1960s, an increasing proportion, now over 90%, of children diagnosed with ALL in the United Kingdom have been treated on MRC childhood leukemia studies. Long-term follow-up of these patients is undertaken by individual treatment centers and reported every 2 years to the Clinical Trial Service Unit (CTSU) in Oxford. Data are available for nearly all patients treated between 1970 and 1984 with less than 1% lost to follow-up at 10 years. Median follow-up of surviving patients is 18 years. Information on the number of patients surviving for 10 years in first remission and those who subsequently relapsed was extracted from the CTSU database. Where detailed clinical treatment or outcome information on patients with late relapse was unavailable in the database, individual centers were contacted to obtain this information. The actuarial risk of relapse after 10 years in first complete remission (CR1) was calculated using Kaplan-Meier life table analysis.

Molecular Analysis

Central morphology review and archiving of marrow smears is undertaken on all cases entered into MRC childhood leukemia studies. Diagnosis and relapse marrow smears were retrieved from the central archive or were kindly provided by individual centers from their local archives where these were unavailable in the central archives.

DNA was extracted from marrow smears archived at diagnosis and relapse using a previously described technique.[7] Briefly, material was scraped from the slide using a sterile blade and DNA was extracted by proteinase K digestion, phenol/chlorofom extraction, and ethanol

precipitation. Polymerase chain reaction (PCR) amplification of the IgH gene (Frameworks 1, 2, and 3) or TCR gene (Vδ2-Dδ3) were performed on 1 μg of patient DNA. The three IgH PCR reactions used a 3′ consensus primer to the joining region of the IgH gene and a consensus 5′ primer to the appropriate framework region of the variable gene.[8,9] The TCR δ region PCR used a 5′ Vδ2 primer and a 3′ Dδ3 primer.[10] PCR products were visualized using polyacrylamide gel electrophoresis and ethidium bromide staining. Clonal products were sequenced with forward and reverse primers using an ABI 373 automated sequencer (Applied Biosystems, Foster City, CA).

RESULTS

Risk of Late Relapse

A total of 2,746 children were treated on Medical Research Council (MRC) ALL trials between 1970 and 1984. Details of study treatment protocols and patient outcome have been published elsewhere.[11-15] A total of 1,134 children survived for 10 years in CR1, of whom 12 subsequently relapsed giving an actuarial risk of 1% over the next 10 years.

Clinical Characteristics of Relapse Cases

Diagnosis. There were equal numbers of males and females within the 12 relapse cases (Table 1). Based on their age and white blood cell count at presentation, all had standard risk disease according to current risk stratification criteria for childhood ALL.[16] Although the diagnosis of ALL was based primarily on cytomorphology and cytochemistry, it was further confirmed in six patients by documenting CD10 expression (common ALL antigen) by the lymphoblasts. Treatment varied according to the then current study protocol, but most patients did not receive any postinduction intensive consolidation therapy,[17] and all could be regarded as having received inadequate treatment by current standards.[1]

Relapse. Although relapse was diagnosed after 10 to 15 years in CR1 in 10 children, it did not occur until after 20 years in two patients (Table 2). All relapses were in the marrow except one, a patient who had a pelvic lymphoma with no morphological evidence of marrow disease. Blast cells were of precursor B lymphoid origin in all cases. Four of seven patients in whom a blast cell karyotype analysis was successful had a clonal

abnormality. One of these was a Philadelphia translocation, which had, in retrospect, probably been present at diagnosis.

All patients were successfully reinduced into a second complete remission and the majority received intensive and continuing chemotherapy to consolidate and maintain the remission without a marrow transplant. Of the 12 patients, eight remain in a second complete remission at a median follow-up of 52 months and the actuarial disease-free survival 5 years after relapse is 56%. Of the four patients who relapsed a second time, two had received an autologous transplant and two chemotherapy alone.

Molecular analysis. Amplifiable DNA could be extracted from both diagnosis and relapse marrow smears in 8 of 12 cases studied. Polyacrylamide gel electrophoresis of the amplified DNA showed an identical size clonal TCR (1 case) or IgH (7 cases) gene rearrangement at diagnosis and relapse in these 8 cases (Tables 1 and 2, Fig 1, patient 1). Automated sequence analysis confirmed complete sequence homology of rearrangement at diagnosis and relapse in seven patients. The remaining patient has been reported previously[5] and was shown to have an identical FR3 IgH rearrangement by dot hybridization using a clone-specific oligonucleotide probe at diagnosis, end of treatment, and relapse. In two cases, a clonal IgH rearrangement could be detected at relapse (Table 2, patients 11 and 12), but we failed to extract DNA from the diagnostic smear.

DISCUSSION

With the large number of children treated on MRC ALL studies between 1970 and 1984, almost all of whom were rigorously followed for more than 18 years, we can be confident that the 1% risk of late relapse uncovered in this study is representative. Therefore, physicians treating children with ALL can be reassured that the vast majority of long-term remitters are truly cured of their disease. Whether even this small risk is faced by patients treated on later protocols is uncertain, as all of the patients reported received less intensive treatment than is used in current protocols.[1,17]

The evidence provided in this study that relapse can occur after 10 years in remission raises some fundamental questions

Table 1. Patient Characteristics and Treatment at Initial Presentation

Patient	Age (yr; diagnosis/ relapse)	Sex	WCC (10⁹/L)	Immuno- phenotype	Karyotype	Treatment	CR1 Duration (yr)	Clonal Marker
1	10/34	M	14	NK	NK	UKALL I	24	IgH FR2 (260 bp)
2	11/21	F	2	NK	NK	UKALL III	10	Failed*
3	3/13	F	18	NK	NK	UKALL V	10	IgH FR3 (125 bp)
4	11/31	M	12	CD10+	NK	UKALL V	20	IgH FR3 (120 bp)
5	4/15	F	10	NK	NK	UKALL V	11	Failed*
6	3/13	M	14	CD10+	Normal	UKALL V	14	IgH FR3 (130 bp)
7	8/22	F	2	NK	NK	UKALL V	14	IgH FR2 (250 bp)
8	7/19	F	8	CD10+	NK	UKALL V	11	IgH FR3
9	7/18	M	60	NK	NK	UKALL VI	11	TCRδ (vδ2-Dδ-Dδ3 240 bp)
10	5/18	F	21	CD10+	NK	UKALL VIII	12	IgH FR3 (120 bp)
11	7/19	M	8	CD10+	NK	UKALL VIII	12	Failed*
12	4/14	M	2	CD10+	46 XY Gq ?Ph	UKALL VIII	10	Failed*
Median (range)	7 (3-11)		11 (2-60)				12 (10-24)	

*Failed DNA extraction.

Table 2. Patient Characteristics, Treatment, and Outcome After Relapse

Patient	Site	Immunotype	Karyotype	Treatment	Response	CR2 Duration (mo)	Current Status	Clonal Marker
1	Marrow + testes	Common	Hypodiploid 45XY	Chemo + XRT	CR2	27	CCR2	IgH FR2 (260 bp)
2	Marrow	Common	NK	Chemo	CR2	26	Relapse death	Failed*
3	Marrow	Pre-B	NK	Chemo	CR2	134	CCR2	IgH FR3 (125 bp)
4	Marrow	Common	46XY,del 14q,t(12;17)	Chemo	CR2	18	CCR2	IgH FR3 (120 bp)
5	Pelvic	Common (APAAP)	NK	Chemo + autograft	CR2	106	CCR2	Failed*
6	Marrow	Common	Normal	Chemo	CR2	113	CCR2	IgH FR3 (130 bp)
7	Marrow	Pre-B	Tetraploid 92-94, del 4q, t(9; 12)del10q	Chemo + autograft	CR2	26	Relapse death	IgH FR2 (250 bp)
8	Marrow	Common	Normal	Chemo	CR2	94	CCR2	IgH FR3
9	Marrow	Common	Normal	Chemo	CR2	96	CCR2	TCRδ (vδ2-Dδ-Dδ3 240 bp)
10	Marrow	Common	NK	Chemo + allograft	CR2	36	CCR2	IgH FR3 (120 bp)
11	Marrow	Common	NK	Chemo + allograft	CR2	12	Relapse death	IgH FR2 (260 bp)
12	Marrow	Common	46XY t(9:22)(q34; q11)	Chemo	CR2	27	Relapse death	IgH FR3 (120 bp)
Median (range)						30 (12-134)		

Patients 4 and 8 have been reported previously.[5,6]

Abbreviations: NK, not known (not performed or failed); CR, complete remission; IgH, immunoglobulin heavy chain gene rearrangement; FR2 and 3, framework 2 and 3; TCR, T-cell receptor rearrangement; CCR, continuing complete remission.

*Failed DNA extraction.

about the biology of childhood ALL. Like the requirement for a prolonged ''maintenance'' treatment program, late relapse seems to be unique to this cancer. Together with recent evidence that PCR techniques, similar to those used in this study, can detect residual leukemic cells in a substantial proportion of patients in long-term remission,[18] our findings suggest that cells from the original clone can remain dormant for long periods of time. Although the mechanisms responsible for this dormancy are not known, they are likely to involve either an ability of the clonal cells to remain out of cell cycle (in G_0) for long periods, or the ability of host immune surveillance to maintain minimal residual leukemia in limbo, or a combination of the two. Clinical recurrence would only arise when there was either a breach in immune surveillance or a return to cell cycle triggered by the acquisition of new mutations within the dormant cell genome allowing clonal escape. The absence of subclones with mutated IgH or TCR genes at late relapse (there was complete sequency homology between the clonal rearrangements found at diagnosis and relapse) in the patients reported suggests the

former to be a more likely explanation. However, we do not have sufficient information to exclude immunophenotypic or karyotypic clonal evolution at relapse, so this hypothesis remains unsubstantiated.

The relative ease with which the leukemia could be reinduced into an easily maintained second remission suggests that the lymphoblasts retain chemosensitivity. If so, there is every reason to suppose that such patients can be salvaged simply with more intensive conventional chemotherapy than that which they received on the first occasion and without recourse to progenitor cell transplantation.
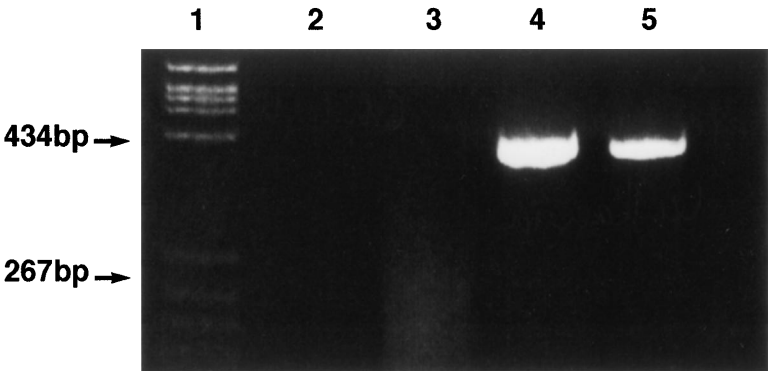
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Fig 1. Ethidium bromide-stained 4% polyacrylamide gel of IgH framework 1 PCR products obtained at diagnosis and relapse in patient 1. Lane 1, PBR 322/HaeIII size marker. Lane 2, water blank. Lane 3, normal bone marrow sample showing a typical polyclonal smear. Lane 4, patient 1 at diagnosis. Lane 5, patient 1 at relapse.

REFERENCES

1. Chessells JM, Bailey CC, Richards SM: Intensification of treatment and survival in all children with lymphoblastic leukaemia; results of UK Medical Research Council Trial UKALL X. Lancet 345:143, 1995

2. Chessells JM, Hardisty RM, Richards SM: Long survival in childhood lymphoblastic leukaemia. Br J Cancer 55:315, 1987

3. Nygaard R, Moe PJ, Brincker H, Clausen N, Nyman R, Perkkio M, Eilertsen EM, Johansen OJ, Vare M, Brinch L, Siimes MA: Late relapses after treatment for acute lymphoblastic leukaemia in childhood: A population-based study from the Nordic countries. Med Pediatr Oncol 16:45, 1989

4. Levasseur M, Maung ZT, Jackson GM, Kernahan J, Proctor SJ, Middleton PG: Relapse of acute lymphoblastic leukaemia 14 years after presentation: Use of molecular techniques to confirm true re-emergence. Br J Haematol 87:437, 1993

5. Potter MN, Steward CG, Oakhill A: The significance of detection of minimal residual disease in childhood acute lymphoblastic leukaemia. Br J Haematol 83:412, 1993

6. Frost L, Goodeve A, Wilson G, Peake I, Barker, Vora AJ: Clonal stability in late relapsing childhood lymphoblastic leukaemia. Br J Haematol 98:992, 1997

7. Fey M, Pilkington SP, Summers C, Wainscoat JS: Molecular diagnosis of haematological disorders using DNA from stored bone marrow slides. Br J Haematol 67:489, 1987

8. Owen RG, Johnson RJ, Evans PA, Smith GA, Child JA, Morgan CJ: Detection of clonal immunoglobulin gene rearrangements in the PBPC harvest of patients with acute lymphoblastic leukaemia. Bone Marrow Transplant 16:831, 1995

9. Aubin J, Davi F, Nguyen-Salomon F, Leboeuf D, Debert C, Taher M, Valensi F, Canioni D, Brousse N, Varet B, Flandrin G, Macintyre EA: Description of a novel FR1 IgH PCR strategy and its comparison with three other strategies for the detection of clonality in B cell malignancies. Leukemia 9:471, 1995

10. Cave H, Guildal C, Rohrlich P, Delfau MH, Broyart A, Lescoeur B, Rahimy C, Fenneteau O, Monplasir N, d'Auriol L, Elion J, Vilmer E, Grandchamp B: Prospective monitoring and quantitation of residual blasts in childhood acute lymphoblastic leukaemia by polymerase reaction study of δ and γ T-cell receptor genes. Blood 83:1892, 1994

11. Medical Research Council: Treatment of acute lymphoblastic leukaemia: The effect of 'prophylactic' treatment again CNS leukaemia. Br Med J 2:381, 1978

12. Medical Research Council's Working Party on Leukaemia in Childhood: The treatment of acute lymphoblastic leukaemia in childhood, UKALL III: The effects of added cytosine arabinoside and/or asparginase and a comparison of continuous or discontinuous mercaptopurine in regimens for standard risk ALL. Med Pediatr Oncol 10:501, 1982

13. Report to the Council by the Working Party on Leukaemia in Childhood: Medical Research Council Leukaemia Trial — UKALL V: An attempt to reduce the immunosuppressive effects of therapy in childhood acute lymphoblastic leukaemia. J Clin Oncol 4:1758, 1986

14. Eden OB, Lilleyman JS, Richards SM, on behalf of the Medical Research Council on Leukaemia in Childhood: Testicular irradiation in childhood lymphoblastic leukaemia. Br J Haematol 75:496, 1990

15. Eden OB, Lilleyman JS, Richards SM, Shaw MP, Peto J: Results of Medical Research Council Leukaemia Trial UKALL VIII (Report to the Medical Research Council on behalf of the Working Party on Leukaemia in Childhood). Br J Haematol 78:187, 1991

16. Chessells JM, Richards SM, Bailey CC, Lilleyman JS, Eden OB: Gender and treatment outcome in childhood lymphoblastic leukaemia: Report from the MRC UKALL trials. Br J Haematol 89:372, 1995

17. Report to the Council by the Working Party on Leukaemia in Childhood: Improvement in treatment for children with acute lymphoblastic leukaemia. Lancet 1:408, 1986

18. Roberts WM, Estrov Z, Ouspenskaea MV: Measurement of residual leukemia during remission in childhood acute lymphoblastic leukaemia. N Engl J Med 336:317, 1997