Bactericidal capacity of peripheral blood leucocytes in relation to bacterial infections in acute lymphoblastic leukaemia in childhood

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SYNOPSIS Phagocytosis and bacterial capacity of peripheral blood leucocytes were studied in 49 children with acute lymphoblastic leukaemia. No impairment of phagocytic ability was found, but 36% of children in relapse and 25% in remission had varying degrees of diminished intracellular killing capacity of ingested *Staphylococcus aureus*. Serial values in the same individual were constant irrespective of drugs used or stage of the disease. There was a highly significant positive correlation between bactericidal capacity ratio and the number of episodes of infection per patient; there was no significant correlation with death from infection.

The prognosis in acute lymphoblastic leukaemia in childhood has significantly improved in recent years due to multiple agent combination chemotherapy (Pinkel, Hernandez, Borella, Halton, Aur, Samoy, and Pratt, 1971) and early craniospinal irradiation (Aur, Simone, Hustu *et al*, 1971 and 1972). However, infection is still a major problem and a significant cause of death whether the disease is in relapse (Hersh, Bodey, Nies, and Freireich, 1965; Viola, 1967) or remission (Nies, Bodey, Thomas, Brecher, and Freireich, 1965; Simone, Holland, and Johnson, 1972). Although many of these infections are caused by viruses, fungi, and organisms of low pathogenicity (Holland, Simone, and Johnson, 1970; Aur *et al*, 1972) and may result from prolonged immunosuppression (Simone *et al*, 1972), bacterial infections are still a major problem and a significant cause of death (Nies *et al*, 1965; Bodey, Nies, and Freireich, 1965). We were particularly interested in the latter infections, often due to *Staphylococcus aureus* and *Esch. coli* since resistance is principally by non-specific host defence mechanisms such as phagocytosis and intracellular digestion. These mechanisms have been studied in leukaemia and found to be normal by some workers (Braude, Feltes, and Brooks, 1954; Silver, Beal, Schneiderman, and McCullough, 1957). However, we have reported a definite and consistent partial dysfunction in bactericidal capacity of polymorphonuclear leucocytes using *Staphylococcus aureus* in some

children with acute lymphoblastic leukaemia (Gregory, Williams, and Thompson, 1972) and similar findings using *Esch. coli* were reported by Strauss, Paul, Jacobs, Simmons, and Sharra (1970) in leukaemic polymorphonuclear leucocytes both in relapse and in remission. A transient defect has recently been reported (Baehner, Neiburger, Johnson, and Murrman, 1973) following craniospinal irradiation and the suggestion that this therapy produced the dysfunction in polymorphonuclear leucocytes. In view of these variable findings and the fact that few studies have been performed serially in the same individual, we have extended our study and related the dysfunction in polymorphonuclear leucocytes to the incidence of bacterial infection. The bacterial capacity against *Staphylococcus aureus* was therefore tested in 49 children with acute lymphoblastic leukaemia in relapse and remission during a follow-up period of three to 64 months.

Materials and Methods

Forty-nine children (30 boys and 19 girls) with acute lymphoblastic leukaemia aged 3 months to 13 years were studied. Thirty-three were studied in relapse and 45 in remission, 22 were retested two or three times in subsequent months while still in remission. The overall follow-up period ranged from three to 64 months. Two children failed to go into remission and died within three months of presentation. Initial therapy consisted of prednisone (40 mg/m² daily)

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for six weeks and vincristine (1.5 mg/m²/weekly) for three doses. Cranial radiotherapy (2500 rads) at six weeks was used as prophylactic meningeal therapy in 32, intrathecal methotrexate only in five; 12 had no prophylactic treatment. Maintenance therapy consisted of rotational 6-mercaptopurine (70 mg/m²/daily), methotrexate (10-15 mg/m²) twice weekly, or five day blocks every four to six weeks, cyclophosphamide (80 mg/m² daily) for six weeks or isolated injections (300 mg/m²) every three months. Reinduction with vincristine and prednisone was given for three weeks every three months. No tests were done while the patient was on corticosteroids. The controls, young adult volunteers or children in hospital for minor surgical procedures (all tests done before surgery), were run in parallel with the patient samples.

Bactericidal Capacity

This was assayed by the method of Quie, White, Holmes, and Good (1967). Phagocytic cells were removed from a gravity sedimentated heparinized 10 ml whole blood sample containing 4 ml of 6% dextran in saline. These were washed three times in Hanks solution and then resuspended in this solution containing 20% fresh pooled normal human serum. The organism (Oxford strain of *Staphylococcus aureus*) was obtained from an 18-hour broth culture. The incubation mixture (final ratio of 10 bacteria per phagocyte) was shaken in a reciprocating water bath at 37°C for 20 minutes to allow phagocytosis to occur, after which the tubes were removed and centrifuged at 500 rpm for 10 minutes. The resulting pellets were resuspended in Hanks solution containing penicillin (1000 units/ml) and streptomycin (100 µg/ml). Aliquots were removed at this stage and at 120 minutes, diluted with distilled water to lyse the cells, and then transferred to pour plates. The number of viable bacteria was determined by colony

counts after an overnight incubation. The bactericidal capacity was calculated as the ratio of the number of viable organisms at 140 minutes to the number at 20 minutes, the normal range in our laboratory being 0.03-0.25 (corrected geometric mean 0.06). Two Leishman-stained smears were made at 20 minutes from each sample, 200 cells were counted at random on each slide, and the percentage number of cells containing bacteria was determined.

Clinical Findings

The clinical course of these children was analysed to determine the frequency of bacterial infection. The infections were graded into two groups, severe and minor, dependent on severity and whether treatment in hospital was necessary or not. Major infections included septicaemia, pneumonia, meningitis, cellulitis, and on four occasions a prolonged fever which responded to antibiotics although bacteriological data were negative. The most frequent infecting organisms were *Staphylococcus aureus*, *Strept. pneumoniae*, *Esch. coli*, *H. influenzae*. Injected antibiotics (often intravenous) were always necessary for these infections. Minor infection involved the upper respiratory tract, otitis media, and skin, and was usually treated on an outpatient basis. All these infections occurred during haematological remission (table I). Terminal septicaemia frequently associated with relapse and severe pancytopenia was not included in the analysis of infections.

Bactericidal Assay

The bactericidal ratios obtained are shown in figure 1. Some patients were studied more than once and in these cases the individual mean was taken. All ratios were transformed to the log (base 10) for statistical analysis and the corrected geometric mean and standard error calculated for each group. The range

	Bactericidal Capacity Ratio				
	<0.09	0.1-0.19	0.2-0.29	0.3-0.39	0.4-0.5
No. of patients	25	12	5	6	1
All Infections					
No. of patients	7	7	4	6	0
No. of episodes	13	18	15	35	0
Severe Infections					
No. of patients	2	5	3	6	0
No. of episodes	4	7	7	26	0
Deaths					
Relapse	8 (2) ¹	4 (2)	1	3 (1)	
Remission	1	0	1 (1)	1 (1)	

Table I Incidence of bacterial infections in relation to bactericidal capacity ratio using *Staphylococcus aureus* as test organism

¹Figures in parentheses denote number of patients with terminal septicaemia.

Fig 1 *Bactericidal capacity ratio (using Staphylococcus aureus as test organism) in control patients with acute lymphoblastic leukaemia while in remission and relapse. No significant difference was found between the mean remission and relapse values but both values differed significantly from the control mean ($P < 0.001$).*

of values in leukaemic patients, whether in relapse or remission, was considerably greater than in the controls, but both means for leukaemic patients were significantly different from the control (relapse $t = 4.0$ $P < 0.001$, remission $t = 3.8$ $P < 0.001$). Only one control value exceeded 0.2 compared with 12 of 33 (36%) relapse and 11 of 45 (25%) remission values which exceeded this figure. In 23 patients, serial estimations (one to 18 months apart) were done while on and off therapy (table II). Eleven children had consistently high ratios (> 0.15) and 11 were consistently less than this, irrespective of the stage of the disease or the drugs used; the other child had a normal value initially but low values were obtained later. Ten had received prophylactic cranial irradiation one to 12 weeks before the assays were performed. None of them showed any deterioration in bactericidal capacity.

Phagocytosis

The percentage number of polymorphonuclear leucocyte cells containing organisms after 20 minutes' incubation was identical in the groups tested. The range of values in the controls was 77-94% (mean 89%) and in the remission cells 70-96% (mean 92%). Only three patients in relapse were studied and values of 80, 85, and 86% respectively were obtained. The number of organisms per phagocyte ranged from 3 to 25 in all groups.

Relationship of Clinical Findings to Leucocyte Function

The incidence of infective episodes in relation to bactericidal capacity ratio is shown in table I. The ratios have been subdivided into five groups to emphasize the increased incidence of infection, severe infection in particular, with an increasing bactericidal capacity ratio. Twenty-four children had at least one infection. Fourteen of the 37 children with a ratio of < 0.2 had 31 episodes of infection while 10 of the 12 children with a ratio of > 0.2 had 50 infections. For severe infections, seven children with a ratio of < 0.2 had 11 infections while nine children with a ratio of > 0.2 had 33 infections. Twenty-five children had no infective episodes at all, 23 of whom had a ratio of < 0.2 .

Statistical analysis comparing observed and expected numbers by the chi-squared test, showed positive relationships between bactericidal capacity ratio and the proportion of children with an infection ($P < 0.01$), the number of infective episodes ($P < 0.001$), and the number of infective episodes per child ($P < 0.001$). For severe infections alone there were similar significant positive relationships ($P < 0.001$, $P < 0.001$, and $P < 0.005$ respectively). In none of the children was severe neutropenia ($0.5 \times 10^9/l$) associated with the onset of an infection, but three had polymorphonuclear leucocyte counts of $0.5-1.0 \times 10^9/l$ within a week of an episode and the remainder had counts between 1.0 and $3.0 \times 10^9/l$.

On reviewing serial neutrophil counts in the 49 children, we found 22 children with periods of profound neutropenia ($0.5 \times 10^9/l$) for two to seven days on 18 occasions, and 24 children with counts between 0.5 and $1.0 \times 10^9/l$; but apart from the three already referred to, no association with an infection was found on these occasions. The duration of follow up of patients in each subgroup was similar and bore no relation to the incidence of infection, or bactericidal capacity ratio. Nineteen children died, 16 of them in relapse (five terminal septicaemia) and three in remission (two terminal septicaemia). The two who died of a terminal septicaemia while in

Patient	Bactericidal Capacity Ratio						Time of Death from Diagnosis (months)	
	Relapse	Remission						
		No Treatment	Vincristine	6-Mercapto- purine	Metho- trexate	Cyclophos- phamide	After Radiation ¹	Relapse
G.T.	0.30		0.30	0.32		0.18 (3)	28	
L.H.	0.31	0.30	0.25	0.17			54 ^a	
M.H.	0.27	0.12	0.28			—	19 ^a	
A.G.		0.20	0.18	0.20		—	44	
S.J.	0.20	0.06	0.03	0.05	0.06			
P.W.				0.11	0.08	0.07 (4)		
H.J.	0.07			0.02	0.07			
P.K.	0.08			0.05	0.06	0.07 (3)	35	
G.V.	0.11		0.08		0.04	0.08 (12)	20 ^a	
K.W.	0.11		0.10	0.11		0.11 (12)		
L.H.	0.06		0.05	0.07			31 ^a	
H.H.	0.02		0.03	0.04				
P.L.	0.16		0.17		0.13	0.13 (6)		
L.W.	0.19			0.16		0.08 (8)		
J.G.			0.08	0.12				
N.D.	0.30			0.23	0.32	0.24	54	
S.G.	0.19		0.25	0.14			24	
T.P.	0.15			0.18	0.11			
K.D.	0.40			0.24		0.32 (4)		11 ^a
N.J.	0.09		0.09	0.06		0.06 (1)		10
M.L.	0.12			0.11			21 ^a	
A.H.	0.04			0.05		0.04 (4)		
C.B.	0.32			0.25				10 ^a

Table II Serial bactericidal capacity ratios (using *Staphylococcus aureus*) measured in leukaemic patients in remission and relapse, on and off therapy, and following cranial irradiation

¹Cranial irradiation 2500 rads. Figures in parentheses denote time in weeks from date of radiotherapy to bactericidal test.

*Denotes terminal septicaemia.

remission (K.D., C.B., table II), had mean ratios of 0.32 and 0.29 respectively and had suffered frequent infections during 10 and 11 months of follow up. The other (N.J., table II) had a ratio of 0.08 but had no infection in the preceding 10 months. However, statistical analysis failed to show a significant relationship between leucocyte function and death from infection.

We were unable to show any difference in leucocyte function following radiotherapy, but this is possibly due to the fact that none of the children received spinal irradiation.

Discussion

The data presented confirmed our previous findings (Gregory *et al*, 1972) that leucocyte bactericidal capacity is significantly reduced ($P < 0.001$) in acute leukaemia in childhood when compared with non-leukaemic controls. Although the majority of children in relapse (21 of 33) and remission (34 of 45) had normal function, 36% in relapse and 25% in remission showed varying degrees of leucocyte dysfunction. These findings are similar to those reported by Strauss and his colleagues (1970) using a different organism (*Esch. coli*) and method. Serial studies in our patients showed that the degree of

dysfunction was constant irrespective of the therapy or stage of the leukaemia. Treatment schedules in these children were not identical but all received the same drug at some time or another in approximately the same dose. The lack of uniformity in leucocyte function while on drugs, and the similar findings before therapy was started, is suggestive that the drugs did not significantly affect leucocyte function nor produce it. Although the degree of dysfunction was not gross, the constancy of findings in an individual and the positive correlation with the clinical incidence of infection, particularly severe and recurrent infections, is suggestive that this mechanism is important in some leukaemic individuals and may even contribute to death.

Many factors, often as a direct consequence of therapy are known to affect host resistance. These include neutropenia (Bodey, Buckley, Sathe, and Freireich, 1966), the effect of steroids on the immunological response (Kass, Kendrick, and Finland, 1955; Spain, Molomut, and Haber, 1952), phagocytosis (Moeschlin, Zurukzoglu, and Crabbé, 1953), and chemotactic ability (Holland, Senn, and Banerjee, 1971). In addition, the depression of humoral and cell-mediated immunity due to prolonged immunosuppression (Keye and Magee, 1956) and as a sequel to craniospinal irradiation (Campbell,

Hersey, MacLennan *et al*, 1973) also contribute. Thus a combination of factors probably antecedes each infective episode.

However, considerable variation in the clinical incidence of infection is known in leukaemic patients with apparently comparable degrees of neutropenia and immunosuppression. We feel that impaired polymorphonuclear cell function could make certain children more vulnerable to infection. Although the overall survival for children with leukaemia has unquestionably improved in recent years, reports of unexplained death in remission are disturbing (Simone *et al*, 1972; Aur *et al*, 1972) and may possibly be related to a specific defect as we have described. Whereas we felt that leucocyte dysfunction was causally related to the severity of infections in two of the three children who died in remission, we do not know how important this was in relation to their premature death or whether the association was fortuitous.

In the light of our observations and the well recognized variations in susceptibility to infection we feel it is necessary to try and identify individuals who are at particular risk of infection. In these patients particular care should be taken to avoid undue myelotoxicity and antibiotics administered as soon as infection is suspected.

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