Neutrophil function in children with acute lymphoblastic leukaemia in the presence and absence of viral infections

M. M. REID, A. W. CRAFT, AND W. T. LOW

Royal Victoria Infirmary, Newcastle upon Tyne

SUMMARY Neutrophil function was assessed regularly in 26 children with acute lymphoblastic leukaemia (ALL) in remission, both when they were well and during viral infections. Tests of candidacidal ability when these children were apparently free of infection showed a trend towards lower levels compared with controls. The most pronounced depression of candidacidal ability and chemotaxis was during viral infections, and these two functions of neutrophils were more likely to be abnormal than when the children were free of infection. In children with ALL in remission, whose neutrophils may function abnormally even when they are well, the risk of acquiring bacterial or fungal infections may be made greater by virus infections.

Infection is a major cause of death for children with ALL, particularly during remission (Hughes 1971; Craft *et al.*, 1977). Although viruses are increasingly being incriminated in the deaths of such children, overwhelming bacterial or fungal infections still account for many deaths. Profound neutropenia is not always associated with such deaths. Several of the deaths recently reported from Newcastle were associated with coexistent viral and bacterial infections (Craft *et al.*, 1977). The interaction of such infections is poorly understood. As part of a larger survey of infection and immunity in children with ALL in remission, neutrophil function was assessed to discover whether any defects were detectable and, if so, whether they could be related to the presence of infection.

Patients

Between June 1975 and October 1976, 27 children with ALL presenting consecutively to one of two major hospitals in Newcastle upon Tyne were entered into the study. Their ages at diagnosis ranged from one to 10 years. One patient died before remission

was induced. The remaining 26 patients were followed up, while in remission, for periods ranging from 4 months to 2 years. All were receiving a modified version of an ALGB 6801 regimen (Reid *et al.*, 1977). The children were assessed if possible at regular monthly intervals and when infection was suspected. At times, insufficient blood was obtained and some tests could not be performed. Routine testing was not carried out at diagnosis because it was usually impossible to obtain adequate numbers of neutrophils.

Controls

Neutrophil function tests were controlled with cells from laboratory staff. Reference ranges were derived from results obtained from 20 staff members and from 6 children, aged 3 to 11 years, admitted to hospital with noninfective conditions.

Methods

Neutrophils were separated by centrifuging white blood cell-rich plasma from dextran-sedimented blood over a Ficoll-Triosil (Lymphoprep, Nygaard) gradient.

Stimulated nitrobluetetrazolium (NBT) reduction was assessed on whole blood samples by the method of Segal and Peters (1975). Results were expressed as arbitrary spectrophotometer units/ 10^6 neutrophils.

Chemotaxis was assessed by a method based on that of Wilkinson (1974). Results were expressed as

Paediatric Department, Newcastle General Hospital, Newcastle upon Tyne

M. M. REID, registrar

Department of Child Health, Royal Victoria Infirmary, Newcastle upon Tyne

A. W. CRAFT, consultant paediatrician

Victoria Hospital, Kirkcaldy, Fife

W. T. LOW, senior chief technician

the distance in μm travelled by the leading front of neutrophils into the micropore filter.

Killing ability was assessed using *Candida albicans* as the test organism by a method based on that of Goldman and Th'ng (1973). Results were expressed as the percentage of *C. albicans* killed.

Virus infections were diagnosed by immunofluorescent techniques and culture (Gardner and McQuillin, 1974).

During the period of the survey there was no

occasion on which adequate numbers of neutrophils were obtained from children with bacterial or fungal infections.

Results

The results of tests of NBT reduction, killing ability, and chemotaxis in the children with ALL, both in the presence and absence of virus infections, are shown in Figs 1, 2, and 3. The reference adult and child

Fig. 1 Stimulated NBT reduction in children with ALL when well and during virus infections with reference ranges (mean $\pm$ SD) for children and adults.

RSV = respiratory syncytial virus
CMV = cytomegalovirus
HVH = herpesvirus hominis
H. zoster = Herpes zoster

Fig. 2 Neutrophil candidacidal ability in children with ALL when well and during virus infections, with reference ranges (mean $\pm$ SD) for children and adults.

Fig. 3 Neutrophil chemotaxis in children with ALL when well and during virus infections, with reference ranges (mean $\pm$ SD) for children and adults.

Table 1 *Killing ability. Summary of 'normal' and 'abnormal' results in noninfected and infected children with ALL*

	Noninfected	Infected
Normal tests	96	14
Abnormal tests	47	17
Totals	143	31

Table 2 *Chemotaxis. Summary of 'normal' and 'abnormal' results in noninfected and infected children with ALL*

	Noninfected	Infected
Normal tests	134	21
Abnormal tests	22	12
Totals	156	33

ranges are given for comparison. Each point in the noninfected group represents a mean of all tests performed in an individual child when no infection could be detected. One of the 26 children was at no time tested when free of infection. All other points represent the results of a single test. The mean value of killing ability ($\pm$ SD) in the children with ALL when they were free of infection is 19% ($\pm$ 4). The reference adult range is 25% ($\pm$ 6). This difference is significant (Student's *t*, $P < 0.01$).

Tables 1 and 2 give a summary of the results of all the individual tests of killing ability and chemotaxis. Results which are described as 'abnormal' are all below the lowest point on the reference ranges for the appropriate test. Killing ability was more likely to be 'abnormally' low ($<18\%$) in infected than non-infected children ($\chi^2 = 5.3$, $P < 0.05$). Chemotaxis was also more likely to be 'abnormal' ($<84 \mu\text{m}$) in infected children ($\chi^2 = 4.6$, $P < 0.05$).

There was no obvious change in the pattern of results with time, nor did the stage of treatment appear to affect them.

Discussion

There was no difference in NBT reduction and chemotaxis between the control populations and the children with ALL when not infected. However the results of tests of killing ability in such children (Fig. 2) suggest there was some impairment, as there was a trend towards lower levels than was found in the normal population. Quantitative assessment of killing ability is simplified if *C. albicans* is used as the test organism. It is probably killed by the hydrogen peroxide mechanism, as are many bacteria.

However, tests of candidacidal ability may not be comparable with those of bactericidal ability. Few defects in neutrophil function during remission of

ALL have been reported. Strauss *et al.* (1970) reported a bactericidal defect in 4 of 6 patients in remission. Gregory *et al.* (1972) described a bactericidal defect in a group of children most of whom were in remission. Baehner *et al.* (1973) found a temporary bactericidal defect associated with craniospinal irradiation, but not at any other time in remission. Sarab-el-Nakeeb and Thompson (1977) reported abnormal chemotaxis in children with ALL in remission. Bactericidal defects have also been described during relapse of ALL (Strauss *et al.*, 1970; Humbert *et al.*, 1976).

Depression of neutrophil function by viruses in normal subjects has been demonstrated *in vitro* (Larson and Blades, 1976; Larson *et al.*, 1977; Ruutu *et al.*, 1977). Normal children with virus infections also have abnormally functioning neutrophils (Anderson *et al.*, 1976; Craft *et al.*, 1976). The present study did not show significant depression of NBT reduction in children with ALL by virus infections. However a striking depression of killing ability and chemotaxis in some of these children took place during virus infections.

Virus infections may therefore be associated with depression of neutrophil function in a group of children whose neutrophils may already be adversely affected by their leukaemia or by treatment. This depression of function, in addition to the risks of neutropenia, may make children with ALL even more susceptible to bacterial and fungal infections, and shows the seriousness of comparatively common viruses to such children.

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Correspondence to Dr M. M. Reid, Department of Haematology, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP.

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