

Granular acute lymphoblastic leukaemia of childhood: a morphological phenomenon

P J DARBYSHIRE, J S LILLEYMAN*

From the Department of Haematology, The Children's Hospital, Birmingham, and the *Department of Haematology, Sheffield Children's Hospital, Sheffield

SUMMARY Three hundred and twenty consecutive children with lymphoblastic leukaemia (ALL), treated on the Medical Research Council UKALL VIII schedule, had their Romanowsky stained diagnostic marrows reviewed for the presence of azurophil granules in blast cell cytoplasm. Twenty patients (7%) had > 5% blasts showing this feature; 19 had the cell phenotype of "common ALL." Male children and those with French-American-British (FAB) L₂ morphology predominantly showed this feature. There was also a strong correlation between granularity and non-diffuse acid phosphate positivity, but no obvious difference between the 20 patients in their response to treatment emerged during a minimum follow up of 15 months.

The "granular" variant occurs in around 7% of children with ALL, but has no clear prognostic importance. Morphologists should be aware of its existence and incidence to avoid confusion with acute myeloid leukaemia.

Acute lymphoblastic leukaemia (ALL) is not typically associated with the presence of cytoplasmic azurophil granules, which are more commonly a feature of acute myeloid leukaemia (AML). In some case reports and small series of patients, however,¹⁻⁴ ALL has been described, in which such granulation occurs and where the lymphoid nature of the cells has been confirmed by surface marker studies and terminal deoxynucleotidyl transferase (Tdt) content. The nature of the granules in these patients was examined by cytochemistry and electron microscopy in some of these reports,¹⁻⁴ and it is generally believed that the granules are abnormal intracellular organelles containing lysozyme. Their clinical importance, if any, is not clear.

The purpose of our study was to confirm the existence of "granular" ALL in a large unselected series. We also attempted to find out if patients so affected have any common clinical features and whether they respond differently to treatment.

Material and methods

Bone marrow smears, from 320 consecutive newly diagnosed patients submitted to the Medical Research Council UK ALL trials FAB typing panel

up to October 1984, were examined for the presence of granulation. On a count of 500 cells, positive cases were defined as those with more than 5% blast cells showing azurophilic granules of more than 0.5 μm in diameter. Patients were divided into three groups on the basis of the number of granular blasts as follows: group I (<5%); group II (5-10%); group III (>10%).

All patients were treated according to the principles of the UK ALL VIII study and trial.⁵ Cytochemical stains performed at the referral hospital were examined directly for group III patients and by reported data for group II patients. Most tissue samples were stained with periodic acid Schiff, Sudan black, and acid phosphatase stains; in addition, samples from three patients in group III with non-specific esterase stains were available for study. As the cytochemistry was performed at different hospitals slight variations in methods were inevitable.

The clinical features at diagnosis of all the patients with > 5% granular blasts (group II and III) were analysed from the trial returns to the Clinical Trials Service Unit in Oxford. Cell surface marker studies were carried out in most cases and a minimum of antibodies to Ia, common ALL antigen, T and B cell antigens, and Tdt were applied in all but one case. The features of groups II and III were compared with those of the remainder of the study group using χ^2 contingency tables with Yates correction.

Table 1 No of patients in groups I, II, and III

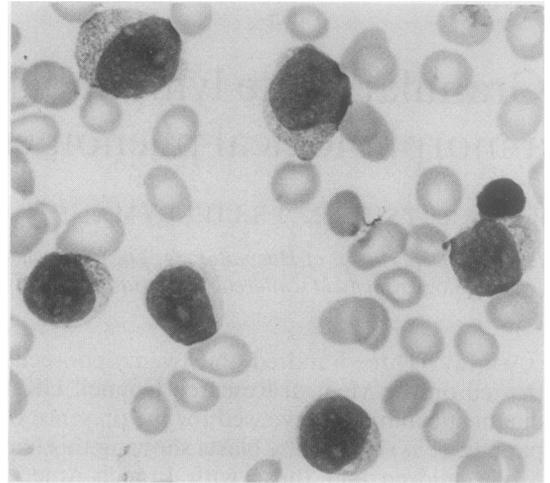
I	< 5% Granular blasts	300
II	5-10% Granular blasts	11
III	> 10% Granular blasts	9
Total		320

Results

A total of 320 marrow smears were examined. Cases with > 5% granular blasts numbered 20 of 320 (7%), with nine cases (3%) having > 10% (table 1). Table 2 details these nine cases from group III. All cases were common ALL, being Ia, c-ALL antigen, and Tdt positive. Apart from one case in which no immune phenotype was known, this also applied to group II patients. There was also a slight excess of boys and cases with L₂ morphology (p = 0.01), although this may have reflected the increased ease of detection of granulation in blasts with more cytoplasm. The figure gives an example of the morphology seen. Case 4 had giant intracytoplasmic inclusions.

The most striking feature associated with granulation was found in the cytochemical reactions. All the cases in group III and eight of 11 in group II had strong punctate positivity of the granular blasts with acid phosphate, considerably greater than that seen in an unselected group with common ALL,⁶ often but not always in conjunction with periodic acid Schiff positivity. Sudan black positivity was not seen in any case. In two of three cases studied, however, the granules showed staining with non-specific esterase. Fluoride inhibition was not performed.

The survival of the whole group with > 5% granular blasts was good: one patient in group III died after



Sudan black negative "granular" childhood lymphoblastic leukaemia. (Romanowsky stain.)

a haematological relapse; two patients relapsed in group II, one within the central nervous system and one within the bone marrow. A further patient failed to attain full remission. Two of these patients had conventionally poor prognostic features: one a chromosome 4/11 translocation, another a white cell count above $100 \times 10^9/l$. It must be emphasised, however, that only 14 (70%) of the 20 patients were off treatment at the time of writing. No other features were common to the groups with granular blasts in terms of age, clinical features, white cell count or cytogenetic abnormalities.

Table 2 Group III patients with > 10% granular blasts

Case No	Age	Sex	Presenting white cell count $\times 10^9/l$	Chromosomes	Cytochemistry	Surface markers	Survival months off treatment	% Granular blasts	FAB type
1	11	F	3.2	46XX	SB- NSE NP	AP++ PAS++	Common ALL	36+	1
2	7	M	2.1	Hyperdiploid	SB- NSE NP	AP++ PAS+	Common ALL	25+	2
3	12	M	4.1	Not performed	SB- NSE NP	AP++ PAS-	Common ALL	On treatment	1
4	13	M	2.9	Hyperdiploid	SB- NSE NP	AP++ PAS-	Common ALL	8+	1
5	5	M	4.5	Not performed	SB- NSE NP	AP+ PAS-	Common ALL	Died after haematological relapse	1
6	3	F	15.5	Not performed	SB- NSE ++	AP++ PAS-	Common ALL	On treatment	1
7	4	M	9.6	46XY	SB- NSE NP	AP++ PAS++	Common ALL	2+	1
8	8	M	5.9	Pseudodiploid	SB- NSE-	AP++ PAS-	Common ALL	On treatment	2
9	6	F	17.5	46XX	SB- NSE++	AP++ PAS-	Common ALL	On treatment	2

SB = Sudan black; + = positive; AP = acid phosphatase; NSE = non-specific esterase; ++ = strongly positive; PAS = periodic acid Schiff; NP = not performed.

Discussion

There now exist several well described morphological variants of ALL, and the FAB classification system⁷ is apparently capable of predicting response to treatment.⁸ Other features, such as the degree of vacuolation of L₁ lymphoblasts,⁹ the presence of hand mirror forms,¹⁰ and the degree of periodic acid Schiff positivity, have been described, but their importance is as yet unclear.

The patients reviewed support the suggestion that "granular" ALL is a distinct morphological variant, defined by the presence of azurophilic cytoplasmic granules between 0.5 µm to 2.0 µm in diameter. It occurs in children with the common ALL phenotype, more often in males and those of FAB L₂ subgroup. The cytochemical staining of strong acid phosphatase positivity in the granules is the most consistent finding, with the granules often additionally showing periodic acid Schiff and non-specific esterase activity. The overall incidence of "granular" ALL depends on the precise criteria chosen; we found 7% of cases with > 5% granular blasts, which is of the same order as that recently found by the St Jude's group (7.6%) in a smaller series of patients.¹

In one of our cases we saw giant intracytoplasmic inclusions. These have been reported previously⁴ and studied by electron microscopy: the granules were abnormal mitochondria and were found to be acid phosphate negative. Our case, however, was positive with acid phosphatase.

Overall, the cytochemical reactions we observed are in keeping with those previously described,^{2,3} though the St Jude's study reported that the granules were more consistently periodic acid Schiff positive than has been our experience.¹ The St Jude's study further suggested that, based on electron microscopic findings, the granules are lysozyme in dysplastic intracellular organelles. Lysozyme is known to be present in the normal large granular lymphocyte fraction,¹¹ particularly natural killer cells; but there is no other evidence to suggest that the leukaemia described above had such a cell of origin. Natural killer leukaemia is thought to have a phenotype similar to that of the normal natural killer cell, expressing a mixture of T cell and myeloid antigens,¹² but not expressing the common ALL antigen, quite unlike the cases we have described.

Further study of granular ALL should include the use of wider panels of monoclonal antibodies against cells of B lineage, such as B1 or BA1, which are more specific than Tdt staining. Ultrastructural cytochemistry may also detect features not readily detected by light microscopy.

"Granular" ALL is not presently of any obvious prognostic importance. Eighty per cent of our

patients are still in their first remission at the time of writing, but only 70% have stopped treatment; another three to four years will need to elapse before any long term effect on prognosis can be discerned.

In summary, "granular" ALL is a true morphological phenomenon of as yet unknown importance. Most importantly, it should be recognised as such and not confused with acute myeloid leukaemia. This mistake can easily be made on Romanowsky stains, particularly if heavy granulation is seen in conjunction with FAB L₂ morphology. Immunology and cytochemistry should point to the correct diagnosis and the most appropriate treatment.

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Requests for reprints to: Dr PJ Darbyshire, Department of Haematology, The Children's Hospital, Ladywood Mid-dleway, Ladywood, Birmingham B168ET, England.