Defective neutrophil function and microbicidal mechanisms in the myelodysplastic disorders

S MARTIN,*† SC BALDOCK,*† ATM GHONEIM,† JA CHILD*

From the *Department of Haematology, Leeds General Infirmary, and the †Department of Microbiology, University of Leeds, Leeds

SUMMARY Neutrophil function studies have been carried out in a series of 44 patients with primary myelodysplastic syndromes (MDS). In vitro tests of phagocytosis and killing of *Candida guilliermondii* and *Staphylococcus aureus* identified 13 patients with abnormal neutrophil function at presentation and a further 10 who developed abnormalities during the course of their disease. The incidence of defective function in the five disease categories in this series was: refractory cytopenia (RC) 8/17; refractory cytopenia with sideroblastic change (RC + SC) 5/8; acquired idiopathic sideroblastic anaemia (AISA) 2/4; refractory anaemia with excess blasts (RAEB) 7/11; chronic myelomonocytic leukaemia (CMML) 1/4. Eleven of 23 patients with defective neutrophil function experienced severe infective complications; in only three of these patients were neutrophil counts $<1 \times 10^9/l$ and susceptibility to infection was considered to reflect, at least partially, qualitative neutrophil abnormalities. There was no correlation between absolute neutrophil count and defective function. Abnormal overall neutrophil microbicidal activity was equally associated with impaired and normal phagocytosis. Some patients with intracellular killing defects had reduced myeloperoxidase (MPO) activities and one had reduced hexose monophosphate shunt (HMPS) activity. In two patients, whose neutrophils showed markedly impaired candidacidal activity, levamisole corrected function when added in vitro at $10^{-7}M$ and also when administered in therapeutic dosage. It is suggested that deranged function, probably reflecting abnormalities in maturation of the granulocyte series, occurs across the myelodysplastic spectrum and that several microbicidal mechanisms may be defective.

Neutrophil abnormalities are recognised as one feature of "preleukaemia." Neutropenia is common and hypogranularity and Pelger-Huet-like changes are frequently observed in blood films.^{1,2} An increased susceptibility to infection which is not necessarily simply a reflection of neutropenia (if present) has been noted and neutrophil dysfunction suggested as an underlying cause.^{3,4} The data substantiating this are sparse but clear-cut bactericidal and candidacidal defects have been described, notably by Breton-Gorius *et al.*^{4,5} Although there are other reports of clinically significant microbicidal defects in such patients,⁵⁻⁸ the numbers investigated have been small.

It is now apparent that there is a spectrum of related disease states which include "preleukaemia" but which show considerable variation in the risk of transformation to a blastic phase.^{9,10} The range of

disorders have been collectively described as comprising the myelodysplastic syndromes (MDS). In the French-American-British (FAB) Co-operative Group classification¹⁰ there are four basic categories: (i) refractory anaemia (RA); (ii) RA with ring sideroblasts (to include acquired sideroblastic anaemia (AISA)); (iii) RA with excess blasts (RAEB) and (iv) chronic myelomonocytic leukaemia (CMML); a fifth category, RAEB in transformation has also been recognised recently.¹⁰ Evidence of dysgranulopoiesis is seen particularly in RAEB but similar features may be found in the other disorders. In RA, where the marrow is normo- or hypercellular and shows dysmyelopoietic features without excess of blast cells, anaemia is not invariable: neutropenia and/or thrombocytopenia may be the dominant features and, for this reason, we have preferred the term refractory cytopenia (RC) to describe this group. We have also distinguished between RC with sideroblastic change and AISA

without discernible abnormalities in granulopoiesis or thrombopoiesis (as assessed by light microscopy). The present study, of 44 patients, was initiated because of the need for an investigation of the neutrophil function of a larger series of patients across the MDS spectrum of disorders; the principal aims were to assess the incidence of defective phagocytosis and intracellular killing of bacteria and fungi and to compare the findings in the definable categories. The further investigation and treatment of some of these patients also provided data relating to possible underlying abnormal microbicidal mechanisms.

Material and methods

Forty-four patients with myelodysplastic syndromes (MDS) were investigated. Their ages ranged from 40 to 87 (mean 65) yr. The following categories were distinguished on the basis of peripheral blood and bone marrow films examined by light microscopy: refractory cytopenia (RC) (17 patients); refractory cytopenia with sideroblastic change (RC + SC) (8 patients); acquired idiopathic sideroblastic anaemia (AISA) (4 patients); refractory anaemia with excess blasts (RAEB) (11 patients); chronic myelomonocytic leukaemia (CMML) (4 patients) (Table 1). Neutrophil function studies were in many cases carried out longitudinally during the course of the disease as well as at the time of diagnosis; the period of follow-up, which in some cases was as long as two years, averaged three months. Neutrophil function tests were not carried out during episodes of clinically or microbiologically diagnosed infection because of the possibility of misleading results. Neutrophils from patients were always tested in parallel with neutrophils from normal control subjects (chiefly laboratory staff volunteers). Control data obtained over a period of three years was also used in the statistical method for determining the indices of function.

Table 1 Patient categories

Diagnosis	Patients
RC	AG, AM, ES, MS, FW, TD, EW, FL, BR, PW, RW, GC, DK, KK, JW, KM, MG.
MG.	
RC + SC	ED, EL, MC, HTh, JH, DS, CS, HJ.
AISA	RL, WS, HP, DB.
RAEB	MF, HD, PF, AH, JM, SS, JB, MCh, BB, IM, HT.
CMML	JJ, JS, JSt, MM.
RC	= refractory cytopenia.
RC + SC	= refractory cytopenia with sideroblastic change.
AISA	= acquired idiopathic sideroblastic anaemia.
RAEB	= refractory anaemia with excess blasts.
CMML	= chronic myelomonocytic leukaemia.

NEUTROPHIL PREPARATION

Heparinised blood samples were sedimented in Dextran and the neutrophils separated from the leucocyte-plasma by centrifugation over Ficoll-Isopaque, SG 1-077.¹¹ The neutrophils were washed twice in Hank's balanced salt solution (HBSS) and resuspended to 5 × 10⁶ neutrophils/ml. The leucocyte suspension was >90% neutrophils with viability by trypan blue dye exclusion >95%.

SERUM

Pooled AB serum from healthy donors, stored in liquid nitrogen (−196°C), was used in all assays.

NEUTROPHIL FUNCTION STUDIES

All incubations were carried out at 37°C on an orbital shaker (170 rpm).

A *microscopic candidacidal assay* involved the examination of smears prepared from a mixture of *Candida guilliermondii* and neutrophils incubated for 60 min at a ratio of 3:1 in HBSS + 10% AB serum (HBSS-AB).^{12,13} The smears were stained with May-Grünwald-Giemsa and the number of viable and non-viable candida within 100 neutrophils was counted.

A *⁵¹chromium release candidacidal assay* measured neutrophil candidacidal activity by the release of ⁵¹chromium from labelled *C guilliermondii*.^{14,15} Triplicate assays were set up comprising 5 × 10⁵ neutrophils and 1.5 × 10⁶ ⁵¹Cr *C guilliermondii* in 0.5 ml HBSS-AB. After 60 min incubation 0.1 ml sodium deoxycholate (24 mg/ml) and 0.2 ml DNase (0.1 mg/ml) were added for 15 min and the tubes then centrifuged for 10 min at 2000 g: 0.2 ml of each supernatant was removed for gamma counting. The results were expressed by the following formula:

% release = $\frac{X - S}{T - S} \times 100$

where X is the radioactivity released in the presence of neutrophils, S is the spontaneous release after incubation in medium, T is the total radioactivity in 0.2 ml of assay mixture.

Phagocytosis of candida

The technique of Yamamura *et al*¹⁶ was adapted for use with *C guilliermondii* whereby the uptake of ³H-uridine by non-ingested organisms was used as a measure of phagocytosis.¹⁷ Ingested candida were unable to incorporate the uridine. Triplicate assays comprised 5 × 10⁵ neutrophils and 5 × 10⁶ *C guilliermondii* in 0.5 ml HBSS-AB. Control assays contained candida but no neutrophils. After 30 min

incubation 1 mg phenylbutazone in 0.05 ml HBSS was added to the assays to inhibit further phagocytosis.¹⁸ Duplicate 0.1 ml aliquots were then removed and incubated in microtitre wells containing 5 μ l ³H-uridine (0.5 μ Ci:5Ci/mmol). After 30 min at 37°C, 0.85% TRIS-NH₄Cl was added to each well to lyse contaminating red cells. The candida-associated radioactivity was collected on to GF/C glass fibre filters using an automated cell harvester. The filters were washed in water and methanol and placed into NE 233 scintillant for liquid scintillation counting. Results were expressed in terms of the neutrophil-induced inhibition of uridine uptake into candida:

$$\% \text{ inhibition} = \frac{\text{cpm candida} - \text{cpm (candida + neutrophils)}}{\text{cpm candida}} \times 100$$

Bactericidal assay

Duplicate assays comprised 10⁶ neutrophils and 10⁷ *Staphylococcus aureus* NCTC 6571 in 0.5 ml HBSS-AB. After 20 min incubation the tubes were put on ice and 4.5 ml sterile distilled water added. Duplicate 0.1 ml aliquots were taken for serial dilution and viable counting to determine the percentage of bacteria killed¹⁸ at 20 min.

Phagocytosis of bacteria

The assay mixture comprised 10⁶ neutrophils and 10⁷ ³H-thymidine labelled *S aureus* in 0.5 ml HBSS-AB.^{17,19} Tubes were set up in duplicate to determine:

- the total radioactivity in the assay;
- percentage phagocytosis of bacteria by neutrophils.

After 20 min incubation 2 ml phosphate-buffered saline (PBS) were added to tubes (a) which were then centrifuged for 10 min at 1500 g to collect all neutrophil and bacteria-associated radioactivity in the cell pellet. Lysostaphin (5 μ g) and 1 mg phenylbutazone in 0.1 ml HBSS were added to tubes (b) and incubated for 20 min at 37°C to lyse non-ingested bacteria. After washing twice in PBS for 5 min at 200 g the neutrophils and ingested bacteria were resuspended in 0.85% TRIS-NH₄Cl and harvested on to GF/C fibre filters for liquid scintillation counting.

$$\% \text{ phagocytosis} = \frac{\text{neutrophil-associated cpm}}{\text{total cpm}} \times 100$$

BIOCHEMICAL STUDIES

Neutrophil hexose monophosphate shunt activity was measured by the oxidation of glucose-1-¹⁴C to ¹⁴CO₂.^{17,20} The assay was set up in duplicate in 25 ml Warburg flasks with 0.2 ml 15% TCA in the sidearm. The assay mixture comprised 2 $\times$ 10⁶ neutrophils and 2 $\times$ 10⁷ heat-killed *C guilliermondii* in 2.0 ml Ca⁺⁺-free Kreb's Ringer phosphate + 10% AB serum and 0.05 ml glucose-1-¹⁴C (1 μ Ci:61 mCi/mmol). Control flasks contained neutrophils or candida alone. The flasks were tightly closed with stoppers from which a piece of filter paper, soaked in 0.1 M hyamine hydroxide, was suspended inside each flask. After 30 min incubation the TCA was tipped into the flasks to stop the reaction and drive the ¹⁴CO₂ out of the solution on to the filters. These were then dropped into NE 233 scintillant for liquid scintillation counting. The post-phagocytic increase in counts-per-minute (cpm) generated by the neutrophils was expressed: log increase in cpm =

$$\log_{10} (\text{cpm from phagocytosing neutrophils}) - \log_{10} (\text{cpm from resting neutrophils})$$

Assay of neutrophil myeloperoxidase content: neutrophil preparations were freed from erythrocyte contamination by lysis with 0.85% TRIS-NH₄Cl. After washing twice, the neutrophils were resuspended in PBS to 10⁶/ml. The neutrophils in 5 ml of this suspension were lysed by sonication, cellular debris removed by centrifugation at 1200 g for 7 min and the supernatant assayed spectrophotometrically for peroxidase activity according to the method of Klebanoff.²¹

The rate of reaction was expressed as absorbance change at 460 nm/min. Serial dilutions of horseradish peroxidase were also assayed to give a standard curve for absorbance change/min v enzyme units/ml. The myeloperoxidase concentration in neutrophil sonicates could thus be determined.

LEVAMISOLE STUDIES

Preservative-free crystalline levamisole hydrochloride (Janssen-Pharmaceutica Ltd) was dissolved in HBSS and added to the test system to give a final concentration of 10⁻⁷M. This approximates to the blood levels achieved by administration of usual doses of the drug.²² In two cases levamisole was given by mouth at a dose of 50 mg twice daily, two days/week.

STATISTICAL ANALYSIS

The results from each assay, for all control neutrophils tested over a period of three years, were accumulated and found to fit a normal distribution pattern.¹⁷ The numbers of controls for the individual

tests ranged from a minimum of 51 to 101. The mean ($\bar{x}$) standard deviation (SD), tolerance factor (TF) at the 5% level of significance and the tolerance limits (TL) were determined for each control population.²³

$$TL = \bar{x} \pm (SD \times TF)$$

When the results of function tests fell outside the appropriate tolerance limits, the patient was considered to have a defect in that particular in vitro function. Following the approach of Hoffman and Bullock²⁴ the results were expressed as indices:

LCI = leucocyte candidacidal index

LBI = leucocyte bactericidal index

LRI = leucocyte (chromium) release index

PCI = phagocytic (candida) index

PBI = phagocytic (bacteria) index

where index =

$$\frac{\% \text{ killing or phagocytosis (patients)} - \text{mean } \% \text{ killing or phagocytosis (controls)}}{SD \times TF}$$

An index < -1.0 indicates significant impairment of neutrophil function.

Results

Overall, 23 of the 44 patients investigated showed defects in neutrophil microbicidal activity. The incidence of abnormalities in the diagnostic categories was: RC 8/17, RC + SC 5/8, AISA 2/4, RAEB 7/11 and CMML 1/4. Of these patients, 13 had abnormal tests at the time of presentation and 10 developed abnormalities subsequently. One of the patients with AISA (with normal tests of neutrophil function) has since developed RC and therefore moved to the RC + SC category. The distribution of values for the various indices of function at presentation is shown in Fig. 1: the incidence of abnormalities in each test was PCI 6/30, LCI 3/13, LRI 7/40, PBI 2/24, LBI 8/40.

Reduced microbicidal activity was associated with impaired phagocytosis in seven patients but normal phagocytosis in eight patients. In a further six patients showing impaired microbicidal activity, phagocytosis was not tested. In two patients, although neutrophils showed reduced phagocytic ability, the overall microbicidal activity was within the normal range.

The incidence of abnormal tests in the 23 patients showing impaired neutrophil function at some time during the study is shown in Table 2, together with an indication as to the occurrence of infective problems: 11 of these patients had one or more severe infections. All three patients who died of infection had RAEB. This may be contrasted with the

Fig. 1 Neutrophil phagocytic and microbicidal function in myelodysplastic syndromes.

LCI = leucocyte candidacidal index

LRI = leucocyte (chromium) release index

LBI = leucocyte bactericidal index

PCI = phagocytic (candida) index

PBI = phagocytic (bacteria) index

The results are from 44 patients on the first occasion they were investigated. An index of < -1.0 indicates abnormal neutrophil function. ○: results of subsequent assays were abnormal.

absence of severe infective problems in the 21 patients who showed no abnormalities of neutrophil function.

RELATIONS BETWEEN NEUTROPHIL FUNCTION, ABSOLUTE COUNT AND INCIDENCE OF INFECTIONS

The possibility that neutrophil function as tested in vitro might show a correlation with the absolute neutrophil count (PMN) was investigated for LCI, LRI and LBI. The correlation coefficients were as follows:

PMN ν LCI (46 values): $r^2 = 0.048$

PMN ν LRI (81 values): $r^2 = 0.078$

PMN ν LBI (89 values): $r^2 = 0.022$

The observed differences in neutrophil function in the groups studied could not, therefore, be attributed to differences in neutrophil count. An absolute count $< 1 \times 10^9/l$ was recorded in 7/33 patients who had no severe infective complications and in 3/11 patients who developed severe infections. The occurrence of serious infection did not, therefore, simply reflect neutropenia.

Table 2 Defective neutrophil function in myelodysplastic syndromes

Patient	No. of abnormal tests				Severe infective problems
	<i>Candida</i> phagocytosis	<i>Candida</i> killing	<i>Bacteria</i> phagocytosis	<i>Bacteria</i> killing	
EW	1/3	3/6	1/1	1/2	+
IM	2/3	1/3	0/3	0/3	+
DS	1/1	1/1	0/1	0/1	-
HD	1/1	1/1	NT	NT	-
MS	0/1	0/1	1/2	1/2	+
RL	1/1	NT	NT	1/2	-
KM	2/3	5/8	NT	1/3	+
CS	1/3	1/3	0/6	3/6	+
JS	1/6	7/12	0/8	6/13	+
MC	0/1	0/3	0/2	1/3	-
GC	0/1	1/1	0/1	0/1	+
MG	0/2	2/2	0/2	1/2	+
ED	0/2	0/4	0/4	1/8	-
AM	0/2	0/5	0/2	1/6	-
ES	0/4	1/3	0/3	0/3	-
MF	NT	1/1	NT	0/1	++
MCh	NT	3/6	NT	2/2	++
BB	NT	1/2	NT	2/2	++
JW	NT	1/4	NT	2/4	-
AH	NT	0/1	NT	1/1	-
JH	NT	1/1	NT	NT	-
HT	0/1	0/2	1/2	0/1	-
DB	1/1	0/1	0/1	0/1	-

*Patient had infective death.

NT = not tested.

RELATION BETWEEN NEUTROPHIL FUNCTION AND PERIPHERAL BLOOD MORPHOLOGY

All 44 patients showed some morphological abnormalities of the granulocyte series. In many cases there were degrees of cytoplasmic hypogranularity and sometimes apparent agranularity. Evidence of dysplasia with abnormal nuclear configuration was also common with pseudo-Pelger-Huet appearances, "cottage-loaf" nuclei and usually a marked left shift overall. There was no correlation between the degree of morphological abnormality as assessed by light microscopy and the incidence of defective function: of the 23 patients who showed functional defects, nine had marked morphological abnormalities and of 21 patients with normal function, seven had marked abnormalities in the blood film.

MODULATION OF FUNCTION BY LEVAMISOLE

In the two patients investigated in detail for the effects of levamisole, the addition of 10^{-7} M levamisole to the assay system corrected defective candidacidal activity but had no effect on control neutrophil function or on candida viability *per se* (Fig. 2). This was a reproducible phenomenon.

In vitro neutrophil function was also monitored before and during the oral administration of levamisole in "therapeutic" dosage. The changes in candidacidal activity for patient MCh, who had RAEB, are shown in Fig. 3. The LCI improved markedly after four weeks' treatment with

levamisole, 100 mg, two days per week; this improvement was maintained when the dose was halved to 100 mg, one day per week. When the drug was withdrawn, function deteriorated. The enhancement of function occurred despite concomitant decrease in absolute neutrophil counts. The other patient, BB, who also had RAEB, received levamisole, 100 mg, two days per week, for a period of only four weeks after which candidacidal function, as tested by LCI, was restored to normal (from -1.97 to -0.39). Again function deteriorated when the drug was withdrawn.

BIOCHEMICAL ASSAYS

Six of the eight patients with normal phagocytosis but defective intracellular killing of microorganisms were investigated for their neutrophil MPO content and/or HMPS activity.

In four of six cases (ED, AM, ES, CS) tested for MPO content there were fluctuations in both neutrophil microbicidal ability and MPO levels. The assays were repeated in parallel on several occasions and, in each instance, when function was abnormal MPO levels were reduced (Table 3). Two had RC and two RC + SC.

In one of five cases (GC, who had RC) tested for HMPS activity, reduced candida killing ability was associated with abnormally low post-phagocytic HMPS activity (LRI = -1.18 ; log increase in cpm:

Fig. 2 Correction of the defective candidacidal function of neutrophils from two patients by levamisole. Normal control neutrophil function was not affected by levamisole. 2.5×10^6 neutrophils were incubated with 7.5×10^6 *C. guilliermondii* in HBSS-AB for 60 min $\pm 10^{-7}$ M levamisole. Candida killing was assessed by microscopic evaluation of stained smears.

Fig. 3 Effect of oral levamisole on peripheral neutrophil count and in vitro neutrophil function. MCh was on oral levamisole 50 mg bd 2 days/week from August until December and then 50 mg bd 1 day/week until January. 2.5×10^6 neutrophils were incubated with 7.5×10^6 *C. guilliermondii* in HBSS-AB for 60 min and candida killing assessed by microscopic evaluation of stained smears.

GC = 0.57; control range = 0.64–1.65, mean = 1.12).

In one case impaired microbicidal ability could not be explained by abnormalities in either MPO or HMPs activity, which were both normal.

Thirteen of the 21 patients with normal neutrophil function were tested for their MPO levels. In 12 of these 13 cases, neutrophil MPO levels were normal; one patient (WS), investigated on six occasions, was unusual in having normal tests of function but consistently low MPO levels (mean = 0.4 enzyme units/ 10^6 PMN). He had AISA but has since developed RC + SC.

Discussion

The results of the present investigations suggest that defects in neutrophil function, as tested in vitro, are common in patients with the myelodysplastic syndromes: 23 of 44 patients had one or more neutrophil functional abnormalities when tested in vitro. In seven patients there was reduced microbicidal activity associated with impaired phagocytosis; in eight patients phagocytosis was normal but intracellular killing impaired; in six patients the overall microbicidal activity was impaired but phagocytosis was not measured; and in two patients only phagocytosis was impaired.

The broadly similar incidence of abnormalities in the main disease categories—RC, RC + SC and RAEB would suggest that such qualitative defects

Table 3 Changes in neutrophil function and myeloperoxidase content

Patient	Interval	LBI	LRI	MPO Enzyme units/10 ⁶ PMN
ED	↓ 6 wk	-1.01 -0.49	NT NT	0.4 2.7
AM	↓ 18 wk	-1.13 +0.37	NT NT	0.8 2.6
ES	↓ 13 wk	NT NT	+0.43 -1.3	1.2 0.7
CS	↓ 59 wk	-1.25	-1.42	0.4 0.3
	↓ 10 wk	-1.21		0.5
	↓ 8 wk	-0.11	-0.49	2.1

LBI = leucocyte bactericidal index.

LRI = leucocyte (chromium) release index.

MPO = myeloperoxidase (normal range 0.7-3.7; mean 1.7 enzyme units/10⁶ PMN).

PMN = neutrophils.

NT = not tested.

reflect the dysgranulopoiesis which may occur in all these disorders, whether relatively "benign" or more obviously preleukaemic. The variability of the abnormalities is consistent with data on the expression of Fc-IgG and C3b receptors by maturing granulocytes, presumably reflecting the heterogeneity of the MDS and variation in the degree of dysgranulopoiesis.²⁵ The demonstration of the platelet dysfunction in these patients²⁶ is consistent with the concept of an underlying stem cell disorder which may be variably expressed in one or more cell lines. The occurrence of abnormalities in AISA supports its inclusion in the MDS "spectrum", though further studies, especially longitudinal, of patients in this category are required.

Increased susceptibility to infection is recognised as a feature of MDS and, although this may chiefly reflect neutropenia, it has been pointed out that frequent and severe infections occur in patients with normal neutrophil counts.^{3,4} This fact, and the often strikingly abnormal morphology in blood and marrow films, have encouraged the view that clinically significant qualitative abnormalities occur in these disorders although relatively few patients have been investigated. In the previous largest series of 17 patients, a tendency towards reduced neutrophil phagocytic and bactericidal activity was reported but the difference between patients and simultaneously tested controls was not striking in any of these cases, consistent with a low incidence of infective problems in this particular group.⁷ In the present study, patients who at some stage had severe infections also tended to show functional defects in the *in vitro* tests and this could not be attributed to infections as the coincidence of active infection and critical testing was avoided. Approximately half of those

patients who had abnormal tests experienced such infective problems. This susceptibility to infection was probably not simply a reflection of neutropenia: low neutrophil counts were observed in patients with and without a history of recurrent infections.

The possibility that qualitative and quantitative abnormalities were directly related required consideration, though previous studies argued against this, no correlation between indices of function and absolute neutrophil count was found. In view of the complexity of the mechanisms involved it is hardly surprising that there was no correlation between morphological appearances as assessed in blood films and tests of function. Hypogranularity and in particular, myeloperoxidase (MPO) deficiency, has been put forward as the explanation for impaired microbicidal activity.^{3,4,5,27} Of the eight patients showing defective neutrophil intracellular killing ability, six were investigated for neutrophil MPO content: four showed reduced levels at some time and coincidence with microbicidal dysfunction was observed. The findings in one other patient who had consistently normal neutrophil function and yet abnormally low MPO levels may appear to add confusion but support the concept that "back-up" killing mechanisms exist, whereby non-enzymatic microbicidal activity is increased to "compensate" for MPO deficiency. In this study the MPO estimations represented the net level of enzyme and did not distinguish subpopulations of neutrophils with differing MPO content as described by Breton-Gorius *et al*,^{4,27} who suggested that in addition to an absolute deficiency of MPO in some neutrophils, the azurophil granules could show considerable variation in MPO content and that degranulation could also be defective. Whatever the mechanisms, a lack

of MPO in the phagocytic vacuole may result in impaired microbicidal activity and variably impaired maturation in the granulocyte series²⁸ would explain abnormalities seen in the MDS. In tests of function which assess the overall competence of neutrophil populations it is the net effect of any deficiency which will determine the result.

Although MPO deficiency may be one likely cause of dysfunction it is clear that other mechanisms concerned with phagocytosis and intracellular killing of microorganisms may also be deranged. Of five patients showing normal phagocytosis but defective intracellular killing ability who were investigated for HMPS activity, one had reduced activity. The metabolic burst, as measured by HMPS activity is triggered by perturbation of the plasma membrane during phagocytosis. It is typically defective in chronic granulomatous disease (CGD)²⁹ associated with NADPH oxidase deficiency³⁰ or absence of membrane-associated cytochrome b.³¹ Our results imply a similar defect in our patient, as yet undefined.

We have demonstrated that levamisole, whether simply added to the test system in vitro or administered to patients in therapeutic dosage, can enhance previously depressed neutrophil function. Although the mechanism of action of levamisole is still obscure,¹³ one possibility is that it may have an effect on cyclic nucleotide levels and therefore on degranulation.³² The possible link between cyclic nucleotide levels and degranulation in patients with these disorders requires further study.

The microbicidal defects found in our investigation were, in some instances, severe and approached the degree of change seen in chronic granulomatous disease. In other cases the indices of function were either mildly abnormal or at the lower end of the normal range. While it is difficult to assess the clinical significance of in vitro tests, particularly in the "grey area" of mild abnormality, there was a striking correlation between the findings of clear-cut neutrophil defects and the liability to severe infections. It has been suggested that even mild functional defects may assume importance at low neutrophil counts.³³ Furthermore, micro-organisms which are not killed following ingestion may be readily disseminated.³⁴ Impaired neutrophil function is probably an important factor underlying life-threatening infections in this category of patient. It would seem worthwhile to monitor neutrophil function in the MDS in order to identify patients who may be particularly at risk. Although improved methodology makes the simultaneous investigation of several aspects of function feasible it can be difficult, and is sometimes impossible, to carry out a complete range of tests in patients who are also

severely neutropenic; close monitoring of such patients will require further technical advances.

The authors are grateful for the encouragement and support of Professor EM Cooke, Professor DH Watson, Dr BE Roberts and Dr C Toothill.

The study was supported by grants from the Special Trustees, Leeds General Infirmary and the Yorkshire Cancer Research Campaign.

References

- Dacie JV, Smith MD, White JC, Mollin DL. Refractory normoblastic anaemia: a clinical and haematological study of seven cases. *Br J Haematol* 1959;**5**:56-82.
- Linman JW, Bagby GC. The preleukemic syndrome (hemopoietic dysplasia). *Cancer* 1978;**42**:854-64.
- Breton-Gorius J, Houssay D, Vilde JL, Dreyfus B. Partial myeloperoxidase deficiency in a case of preleukaemia. II. defects of degranulation and abnormal bactericidal activity of blood neutrophils. *Br J Haematol* 1975;**30**:279-88.
- Breton-Gorius J, Coquin Y, Vilde JL, Dreyfus B. Cytochemical and ultrastructural studies of aberrant granules in the neutrophils of two patients with myeloperoxidase deficiency during a preleukaemic state. *Blood Cells* 1976;**2**:180-209.
- Lehrer RI, Goldberg LS, Apple MA, Rosenthal NP. Refractory megaloblastic anemia with myeloperoxidase deficient neutrophils. *Ann Intern Med* 1972;**76**:447-53.
- Solberg CO, Schreiner A, Hellum KB, Hamre E. Neutrophil granulocyte function in the early diagnosis of acute myelomonocytic and myeloblastic leukaemia. *Acta Med Scand* 1975;**197**:147-51.
- Ruutu P, Ruutu T, Vuopio P, Kosunen TU, De La Chappelle A. Function of neutrophils in preleukaemia. *Scand J Haematol* 1977;**18**:317-25.
- Houghton JB. Neutrophil function and diagnosis of preleukaemic states. *Postgrad Med J* 1979;**55**:396-9.
- Sultan C, Pierre RV, Hast R, Imbert M. Refractory anemias and dysmyelopoietic syndromes (DMPS). In: McArthur JR, ed: Hematology/Hematologie Transfusion 1980. The education program of the American Society of Hematology, 1980:22.
- Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan, C. The French-American-British (FAB) Co-operative Group. Proposals for the classification of the myelodysplastic syndromes. *Br J Haematol* 1982;**51**:189-99.
- Boyum A. Separation of leucocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968;**21**:suppl 97.
- El-Maalllem H, Fletcher J. Defective neutrophil function in chronic granulocytic leukaemia. *Br J Haematol* 1976;**34**:95-103.
- Child JA, Martin S, Cawley JC, Ghoneim ATM. Defective microbicidal function of neutrophils in haematological malignancies and lymphomas: correction by levamisole in vitro. *Biomedicine* 1978;**29**:159-61.
- Yamamura M, Boler J, Valdimarsson H. A ⁵¹chromium release assay for phagocytic killing of *Candida albicans*. *J Immunol Methods* 1976;**13**:227-33.
- Martin S, Ghoneim ATM, Child JA. A new neutrophil candida killing test: ⁵¹chromium release from *Candida guilliermondii*. *J Clin Pathol* 1980;**33**:757-61.
- Yamamura M, Boler J, Valdimarsson H. Phagocytosis measured as inhibition of uridine uptake by *Candida albicans*. *J Immunol Methods* 1977;**14**:19-24.
- Martin S. Study of neutrophil function in haematological malignancies. University of Leeds: PhD thesis, 1981.
- Solberg CO. Influence of phenylbutazone on the phagocytic and

- bactericidal activities of neutrophil granulocytes. *Acta Pathol Microbiol Scand [B]* 1974;**82**:258–62.
- ¹⁹ Verhoef J, Peterson PK, Quie PG. Kinetics of staphylococcal opsonization, attachment, ingestion and killing by human polymorphonuclear labelled bacteria. *J Immunol Methods* 1977;**14**:303–11.
 - ²⁰ Odeberg H, Olofsson T, Olsson I. Granulocyte function in chronic granulocytic leukaemia: bactericidal and metabolic capabilities during phagocytosis in isolated granulocytes. *Br J Haematol* 1975;**29**:427–41.
 - ²¹ Klebanoff SJ. Inactivation of rat uterine preparation. *Endocrinology* 1965;**76**:301–11.
 - ²² Symoens J. Levamisole, an antiangergic chemotherapeutic agent, an over view. In: Chirigos MA, ed. *Control of neoplasia by modulation of the immune system*. New York: Raven Press, 1977:1–24.
 - ²³ Eisenhart H, Hastay W, Wallis D. Techniques in statistical analysis. London: McGraw-Hill Book Company, 1947.
 - ²⁴ Hoffman TA, Bullock WE. A statistical approach to the polymorphonuclear leukocyte bactericidal assay. *J Lab Clin Med* 1973;**81**:148–56.
 - ²⁵ Bynoe AG, Scott CS, Hough D, Roberts BE. Granulocyte Fc-IgG and C3b receptor expression in the primary myelodysplastic syndromes (MDS)—relationship with dysgranulopoiesis and evidence for heterogeneity within morphological subgroups. Submitted for publication.
 - ²⁶ Pamphilon DH, Roberts BE, Davies JA. Platelet dysfunction in myelodysplastic syndromes (MDS) (Abstract). *Br J Haematol* 1983. (in press.)
 - ²⁷ Breton-Gorius J, Houssay D, Dreyfus B. Partial myeloperoxidase deficiency in a case of preleukaemia. I. Fine structure and myeloperoxidase synthesis of promyelocytes. *Br J Haematol* 1975;**30**:273–8.
 - ²⁸ Breton-Gorius J. Abnormalities of granulocytes and megakaryocytes in preleukaemia syndromes. In: Schalzl F, Hellriegel K-P, eds. *Preleukaemia*. Berlin: Springer, 1979:24–34.
 - ²⁹ Holmes B, Page AR, Windhorst DB, Quie PG, White JG, Good RA. The metabolic pattern and phagocytic function of leukocytes from children with chronic granulomatous disease. *Ann NY Acad Sci* 1968;**155**:888–901.
 - ³⁰ Hohn DC, Lehrer RI. NADPH oxidase deficiency in X-linked chronic granulomatous disease. *J Clin Invest* 1975;**55**:707–13.
 - ³¹ Segal AW, Jones OTG. Absence of cytochrome b reduction in stimulated neutrophils from both female and male patients with chronic granulomatous disease. *FEBS Letters* 1980;**110**:111–4.
 - ³² Anderson R, Glover A, Koornhof JH, Rabson AR. *In vitro* stimulation of neutrophil motility by levamisole: maintenance of cGMP levels in chemotactically stimulated levamisole-treated neutrophils. *J Immunol* 1976;**117**:428–32.
 - ³³ Child JA, Martin S, Robinson AE, Ghoneim ATM. Microbicidal function of neutrophils from patients with leukaemia and lymphomas and from donors: therapeutic implications. *Exp Hematol* 1978;**6** suppl 3:103.
 - ³⁴ Cline MJ. Defective mononuclear phagocytic function in patients with myelomonocytic leukemia and in some patients with lymphoma. *J Clin Invest* 1973;**52**:2185–90.

Requests for reprints to: Dr JA Child, Department of Haematology, Leeds General Infirmary, Leeds LS1 3EX, England.