Technical methods

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Letters to the Editor

Erythrocyte ferritin concentration in patients with myelodysplastic syndromes

We have investigated the erythrocyte ferritin concentrations of 45 patients with myelodysplastic syndromes. The mean concentration of heart type ferritin was five times normal and spleen type ferritin was 15 times normal, with no significant difference between the various diagnostic subgroups.

The diagnostic criteria used were those of the FAB group,¹ slightly modified by threshold values for abnormal blood counts and with the addition of a further group of idiopathic macrocytosis.² Of the 45 patients studied, nine had refractory anaemia, 15 had idiopathic sideroblastic anaemia, 11 had refractory anaemia with excess blasts, two had refractory anaemia with excess blasts in transformation, four had chronic myelomonocytic leukaemia, and four had idiopathic macrocytosis. Patients were aged 26-82 years (mean 62.6 years). Twenty four patients had received no blood transfusions at the time of investigation. Erythrocyte ferritin concentration was measured in leucocyte free samples by a two site immunoradiometric assay with antibodies to heart and spleen ferritin,³ and serum ferritin assay was used as a measure of iron stores. Results are shown in the Table.

Patients with myelodysplastic syndromes may have extremely high concentrations of

Erythrocyte ferritin concentration in normal subjects* and patients with myelodysplastic syndromes (ag/cell)

	No of subjects	Mean	Range
Normal subjects*	37		
Heart type		123.1	65-357
Spleen type		12.4	5-34
All patients with myelodysplastic syndromes	45		
Heart type		314.2	43-7556
Spleen type		48.9	4-4243
Refractory anaemia	9		
Heart type		187.9	43-7556
Spleen type		24.8	4-4243
Sideroblastic anaemia	15		
Heart type		392.7	80-1313
Spleen type		66.2	11-467
RAEB plus RAEBT	12		
Heart type		354.2	158-2397
Spleen type		61.3	17-1713

*From Peters *et al*³

RAEB = refractory anaemia with excess blasts.

RAEBT = refractory anaemia with excess blasts in transformation.

erythrocyte ferritin and the mean concentrations of both heart type and spleen type ferritin are greater than normal. Twenty seven of the 45 patients had either heart type or spleen type ferritin concentrations above the normal range, and in 19 patients the concentrations of both were increased. There was no significant difference between patients with refractory anaemia, idiopathic sideroblastic anaemia, or those with marrow blasts in excess of 5%. Those patients who had received one or more transfusions had a mean serum ferritin of 1337 µg/l with a mean erythrocyte ferritin of 410 ag/cell (heart) and 73 ag/cell

(spleen). Those who had not been transfused had a mean serum ferritin of 160 µg/l, with a mean erythrocyte ferritin of 239 ag/cell (heart) and 33 ag/cell (spleen). These values represent a significantly higher serum ferritin concentration in the transfused group ($p < 0.001$), but there is no significant difference for either type of red cell ferritin. When only the 24 patients who had no transfusions are considered, 17 had serum ferritin concentrations in the normal range (below 300 µg/l), but of these seven had abnormally high concentrations of both heart and spleen type erythrocyte ferritin and two had abnormally

high concentrations of spleen type alone. Of the patients with high serum ferritin concentrations (473–762 $\mu\text{g/l}$), two had increases in both heart and spleen type ferritin and two of spleen type ferritin alone.

Patients with idiopathic sideroblastic anaemia have considerably increased intracytoplasmic ferritin concentrations in addition to mitochondrial iron deposition.^{3,4} This study shows that although high concentrations of erythrocyte ferritin are common in patients with myelodysplastic syndromes, these are often associated with transfusional iron overload and in such cases it is difficult to determine whether the increase in ferritin is due solely to an intracorporeal abnormality. Among the non-transfused patients, however, high erythrocyte ferritin concentrations were found in all four patients with idiopathic macrocytosis, seven of nine patients with sideroblastic anaemia, one of seven patients with refractory anaemia, and one of three patients with chronic myelomonocytic leukaemia.

The reason for this increase in red cell ferritin content is not clear. It does, however, indicate that an abnormality of erythroid iron metabolism is common in this condition and is not confined to patients with sideroblastic anaemia, thus supporting the suggestion that sideroblastic anaemia is not a discrete entity but merely signifies a more prominent abnormality in some patients with myelodysplastic syndromes.^{2,5}

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Opsonophagocytosis of group B streptococci: the role of sialic acid

Dr Hindocha *et al*¹ have shown that serotypes Ib and II of group B streptococci are resistant to opsonophagocytosis as measured by neutrophil chemiluminescence. The resistant strains were unaffected by neuraminidase treatment, while trypsin was able to induce susceptibility within the same strains.

We have studied serotypes Ia, Ib, Ic, II, and III group B streptococci by chemiluminescence before and after neuraminidase treatment. Human neutrophils were separated by dextran sedimentation (60 min at room temperature) and Ficoll-Paque centrifugation (30 min at 500 g) to eliminate mononuclear cells. Reference group B streptococci (supplied by the Public Health Laboratory Service, Colindale, London) were cultured overnight in Todd-Hewitt broth with or without neuraminidase (0.43 U/ml, Sigma), washed three times in phosphate buffered saline (PBS) and the suspension adjusted to an optical density of 0.8 at 620 nm ($3 \times 10^8 - 10^9$ CFU/ml). Opsonisation of group B streptococci was performed by rotating bacteria with human pooled serum at 37° for 30 min. Forty group B streptococci wild strains isolated from neonates and asymptomatic mothers were also studied with the same technique. Two hundred microlitres of bacteria at 3×10^7 CFU/ml were mixed with 2×10^5 neutrophils in PBS Ca, Mg, and with 200 μl of 2×10^{-5} M Luminol (Lumac) in the counting chamber of a Picolite luminometer.² Sialic acid measurement was performed with the thiobarbituric acid assay.³

No correlation was found between the absolute sialic acid content of the five group B streptococci serotypes or of the 40 wild strains and chemiluminescence (data not shown). Serotypes Ib and Ic were particularly resistant to opsonophagocytosis (Fig. 1). The removal of sialic acid (more than 60% of the initial amount was lost after enzyme treatment) caused an increase of chemiluminescence in all serotypes except Ia (Fig. 2). The extracellular production of neuraminidase in the five group B streptococci serotypes has been investigated previously⁴ but no correlation was found with chemiluminescence results. Our experiments suggest that sialic acid influences interactions between bacteria and neutrophils, although intrastrain differences in sialic acid content could influence opsonisation.⁵

The role of contaminating proteolytic

Fig. 1 Chemiluminescence (expressed as relative light units (RLU)) of the five group B streptococci serotypes.

Fig. 2 Chemiluminescence (RLU) of the five group B streptococci before and after neuraminidase treatment.

enzymes could not be excluded from our experiments; however, the inability of high neuraminidase doses (we used 0.43 U/ml) used by Dr Hindocha¹ to influence chemiluminescence seems to indicate that proteolytic contamination, if present, is negligible. The experiments of Jennings^{6,7} have indicated that sialic acid associates with the backbone of the native streptococcal antigen (type III); these and our results from five group B streptococci serotypes and 40 wild strains suggest that sialic acid plays a role in the interactions between bacteria and neutrophils by modulating an articulated antigenic complex.

The differences between Dr Hindocha's work and our findings are difficult to explain. Technical reasons, such as separation of the blood cells, heat killing of the group B streptococci, or opsonic capacity of the sera, could be implicated. In our opinion, however, these discrepancies