Abnormal haem biosynthesis in the chronic anaemia of rheumatoid arthritis

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Abstract

***Objectives*—The chronic microcytic anaemia of rheumatoid arthritis (RA) occurs despite the presence of adequate reticuloendothelial iron stores. The red cell microcytosis is evidence of impaired haemoglobin production. This study has examined possible abnormalities of erythroid haem biosynthesis that may contribute to the anaemia.**

***Methods*—5-Aminolaevulinate (ALA) synthase and ferrochelatase activities were assayed in whole bone marrow and in purified erythroblasts from patients with RA and in control subjects. All patients were iron replete with demonstrable iron in the bone marrow.**

***Results*—ALA synthase activity was significantly reduced in both whole bone marrow and purified erythroblasts from patients with the anaemia of RA. Erythrocyte protoporphyrin levels were raised in nine of 12 patients tested while ferrochelatase activity was normal.**

***Conclusion*—These abnormalities provide absolute evidence of abnormal erythroblast haem biosynthesis and iron metabolism in the anaemia of RA and most likely reflect decreased ALA synthase mRNA translation and some abnormality of erythroblast iron transport. Further studies using highly purified erythroblast populations will attempt to identify the causal factors leading to this abnormal erythroblast metabolism.**

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The anaemia of rheumatoid arthritis (RA) is multifactorial and in many cases is related to gastro intestinal blood loss associated with the use of nonsteroidal anti-inflammatory agents. In 60% of cases, however, the cause is unknown and the anaemia attributed to the unexplained effects of chronic disease.[1] This chronic anaemia is characterised in most cases by iron deficient red cell indices[2] with reduced serum iron levels despite a paradoxical increase in reticulo endothelial iron stores. Most studies of this anaemia have concentrated on the possible roles of reduced marrow iron supply and impaired erythropoietin (Epo) production or response for which both interleukin 1 (IL-1) and tumour necrosis factor (TNF) have been proposed as possible mediators.[3 4]

The strong inverse correlation between Epo levels and haemoglobin concentration that characterises most other anaemias is lost. Epo levels may be raised in the majority of patients but the response is blunted by comparison to levels found in anaemias of other aetiologies.[5] The erythroid marrow in inflammation retains the ability to respond to Epo but hormone production is not increased.[6] These studies have led to costly clinical trials of Epo in the anaemia of RA.[7–9] Such trials have shown at best only modest benefit and fail to address the microcytosis which is not a feature of Epo deficiency.

Hypoferraemia and red cell microcytosis may be evidence of reduced marrow iron supply. It has been suggested that either synovial or marrow macrophages may effect reticulo endothelial iron blockade either through lactoferrin release[10] or inappropriate apoferritin synthesis.[11] It is postulated that this blockade leads to erythroblast iron deprivation. Ferrokinetic studies in RA, however, failed to provide evidence of restricted erythroblast iron supply.[12–14] In vitro the erythroblasts do not show features of iron deficiency and do not show the increase in iron uptake from transferrin as would iron deficient cells.[15] A combination of Epo deficiency and reduced marrow iron supply would not suffice as the microcytosis of iron deficiency requires discrepancy between iron supply and Epo drive.[16]

The hypochromic microcytic red cells in the chronic anaemia of RA provide absolute evidence of haemoglobin deficiency. Elevated erythrocyte protoporphyrin levels have been recognised[13] and so localise the defect in haemoglobin synthesis to either the haem biosynthetic pathway or the iron transport mechanism. We have investigated haem biosynthesis with measurement of 5-Aminolaevulinic acid (ALA) synthase activity and ferrochelatase activity in whole bone marrow and in purified bone marrow erythroblasts. Both enzymes have been proposed as candidate regulators of erythroid haem biosynthesis.[17 18] Protoporphyrin levels were measured in circulating erythrocytes.

Materials and methods

PATIENTS AND CONTROLS

Ethical approval was obtained from Lanarkshire and Glasgow Royal Infirmary Ethical Committees. Bone marrow samples were

Table 1 Haemotological parameters in patients and controls, mean (1SD)

	Patients (Anaemia of rheumatoid arthritis)	*Controls*
Hb(g/dl) Male	9·6 (1·1)	14·1 (1·0)
Female	9·0 (0·7)	12·3 (0·5)
MCV (fl)	82·0 (7·0)	89·0 (4·0)
Ferritin (ng/l)	138 (95)	80 (27)
EPP (nmol/l)	1360 (632)	<900
ESR (mm h)	66 (23)	12 (7)

EPP = erythrocyte protoporphyrin.

aspirated from the posterior iliac crest in 14 patients with sero positive RA and the anaemia of chronic disease and from 10 normal subjects (table 1). The patients studied were taking only non steroidal anti-inflammatory agents for disease control. Patients on gold, penicillamine or other such third line agents were excluded. The normal bone marrow samples were obtained from normal volunteers having day surgery for minor urological disorders. Patients with iron deficiency (serum ferritin value less than 20 ng/ml) were excluded and in all cases reticulo endothelial iron was demonstrated by standard morphological techniques in bone marrow samples. The ESR was recorded in both groups.

BONE MARROW FRACTIONATION

Erythroblasts reside within the marrow as a minority member of a heterogenous population. The cells in the marrow both proliferate and differentiate. We have developed methods to enrich bone marrow erythroblasts with positive selection of early and intermediate erythroblasts which are the cells most active with regard to haem biosynthesis.

Bone marrow neutrophils and granulocyte precursors were lysed using monoclonal antibody TGI, IgM in type and specific for myeloid cells from promyelocytes to polymorphs.[19] Residual erythroblasts were then fractionated using Percoll equilibrium density centrifugation to concentrate early and intermediate erythroblasts.[20] These cells were removed from the Percoll gradients between density bands 1·062 and 1·075 g/ml. The more mature late erythroblasts and the erythrocytes are denser than these cells and pass further through the density gradients. Myeloid cell lysis and Percoll fractionation achieved a mean total threefold increase in percentage erythroblast content of each bone marrow sample and further effected enrichment of early and intermediate erythroblasts over the late erythroblasts which have less haem biosynthetic activity.

HAEM BIOSYNTHESIS

ALA synthase and ferrochelatase activities were assayed in whole bone marrow and in purified marrow erythroblasts using sensitive radiochemical methods which utilise high performance liquid chromatography for product isolation.[20 21]

Erythrocyte protoporphyrin levels were measured in 12 of the 14 patients and in the 10 normal subjects using a haematofluorimeter (model 206 Aviv Biomedical Inc) and expressed nmol/l RBCs.

Results

ALA SYNTHASE AND FERROCHELATASE ACTIVITY

ALA synthase activity was significantly reduced in both whole bone marrow and fractionated erythroblasts from patients with the anaemia of rheumatoid arthritis (fig 1). Enzyme activity in whole bone marrow will always appear higher than that found in purified erythroblast preparations. In crude whole bone marrow there is a significant myeloid cell contamination which has been shown to contribute some 50% of the total ALA synthase activity.[20] Erythroblast ALA synthase activity is further reduced by 30% during the incubation stage required to achieve TG-1 mediated myeloid cell lysis.[20] Erythrocyte protoporphyrin levels [mean (SD)] were raised in nine of the 12 patients [1360 (632) nmol/l table 1]. The remaining three patients demonstrated EPP values at the higher end of the normal range. Ferrochelatase activity in both whole bone marrow and fractionated erythroblasts fell within the normal range in all patients (fig 2, table 2). The ESR was used as a crude measure of disease activity and was elevated to more than 60 mm hour in eight of the 14 patients. Although the mean (SD) Hb values of these eight patients was not significantly lower than that found in the other six members of the patient group 9·3 (1·0) *v* 10·2 (1·1) g/dl ($p = 0·2$), their whole bone

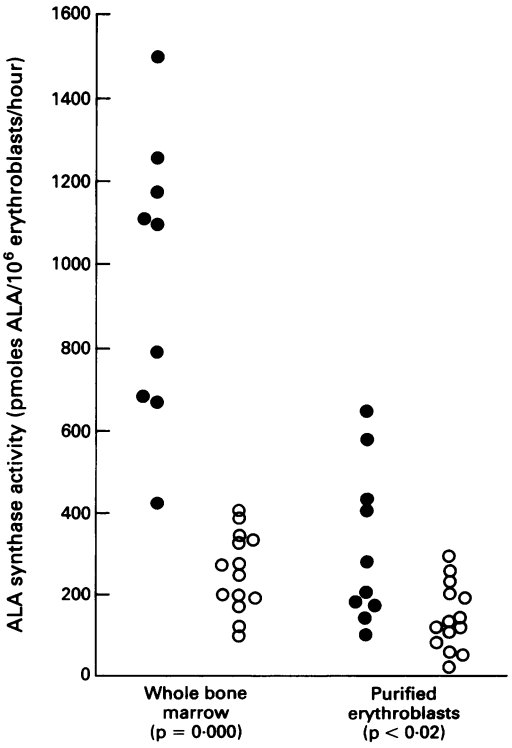

Figure 1 ALA synthase activity in whole bone marrow and purified erythroblasts. Closed circles represent controls. Open circles represent patients with the anaemia of rheumatoid arthritis.

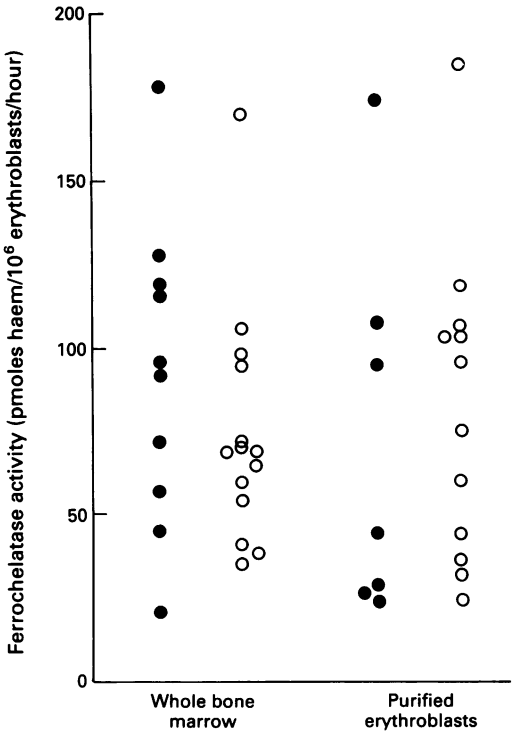

Figure 2 Ferrochelatase activity in whole bone marrow and purified erythroblasts. Closed circles represent controls. Open circles represent patients with the anaemia of rheumatoid arthritis.

Table 2 Enzyme activity in whole bone marrow and purified human erythroblasts, mean (1SD)

	ALA synthase activity (pmoles ALA/10⁶ erythroblasts/hr)		*Ferrochelatase activity (pmoles haem/10⁶ erythroblasts/hr)*	
	Whole bone marrow	*Purified erythroblasts*	*Whole bone marrow*	*Purified erythroblasts*
Controls (n = 10)	996 (337)	316 (191)	92 (46)	71 (57)
RA patients (n = 14)	257 (97)	148 (72)	75 (35)	82 (46)
p value	0·000	<0·02	NS	NS

marrow ALA synthase activity did show a significant reduction 220 (69) *v* 345 (64) pmol ALA. 10^6 erythroblasts hour ($p = 0{\cdot}02$) with enzyme activity in purified erythroblasts showed a similar but not significant trend, 109 (73) *v* 200 (72) ($p = 0{\cdot}057$).

Discussion

This study has identified previously unrecognised abnormalities of erythroid haem biosynthesis and iron metabolism in the anaemia of RA. The erythroblast is the major haem forming cell in the body. Beyond its requirement for haemoglobin synthesis free intracellular haem plays an essential role in the regulation of erythroblast metabolism and differentiation.[22–27] A close, but as yet unresolved coordinate relationship between the haem biosynthetic pathway and erythroblast iron uptake/transfer is also a clear requirement in normal erythropoiesis. Haem biosynthesis occurs by way of an unbranched pathway consisting of a series of irreversible reactions and a total of eight enzymes. The final reaction catalysed by ferrochelatase brings together the haem biosynthetic pathway and intracellular iron transport mechanisms with the insertion of ferrous iron into the protoporphyrin ring which results in haem formation. The association between erythroblast haem biosynthesis and iron metabolism also exists at the level of erythroid ALA synthase as enzyme activity may be controlled by iron responsive elements (IRE) on erythroid ALA synthase mRNA[28] similar to the IREs already identified and essential for the translation of transferrin receptor and ferritin mRNAs.[28 29] Erythroid ALA synthase gene transcription is triggered by developmental signals and enzyme activity thereafter is attenuated by post transcriptional factors.[30] Regulation is thought to be achieved by interaction of the IRE with a high affinity binding protein (IRE-BP) now thought to be an aconitase.[31] Iron deficiency leads to binding of the IRE and IRE-BP with consequent inhibition of translation of ferritin and erythroid ALA-synthase mRNA and stabilisation of transferrin receptor mRNA.[29 32]

The regulation of hepatic haem biosynthesis is well defined. ALA synthase has been identified as the rate limiting enzyme subject to negative feedback control by haem.[33] The regulation of erythroid haem biosynthesis is, however, poorly understood but is clearly different from that in the liver. Haem stimulates rather than inhibits erythroblast ALA synthase activity,[27] hepatic ALA synthase mRNA does not contain an IRE and different genes on chromosomes 3 and X respectively have been identified for the hepatic and erythroid iso enzymes.[34]

This study has identified reduced ALA synthase activity in whole bone marrow and fractionated erythroblasts of patients with the chronic anaemia of RA. This reduction in enzyme activity appears to be related to disease activity as assessed by simple measurement of ESR. The cause of the reduced ALA synthase activity is unclear. This may reflect increased activity of ALA synthase protease already shown in RA.[35] An isolated reduction of ALA synthase, however, would be expected to reduce erythrocyte protoporphyrin levels as in congenital sideroblastic anaemia.[36] Reduced ALA synthase activity and increased EPP is also found in erythroblast iron deficiency. In iron deficiency reduced ALA synthase activity is thought to relate either to intracellular haem deficiency or iron deficiency.[37] These results would be consistent with the belief that the anaemia of chronic disease results from reticulo endothelial iron blockade resulting in reduced erythroblast iron uptake. However, the in vivo ferrokinetic studies[12–14] and the in vitro erythroblast iron uptake studies[15] do not support simple erythroblast iron deficiency. We suggest that the abnormalities found in this study most likely reflect decreased ALA synthase mRNA translation and, as yet, an unspecified abnormality of intracellular erythroblast iron transport.

The potential clinical significance of reduced ALA synthase activity in the pathogenesis of anaemia was first recognised in the sidero-

blastic anaemias where anaemia is almost invariably associated with reduced enzyme activity.[38] The significance in the development of microcytic anaemia has recently been established with the recognition of erythroid ALA synthase gene mutations as the primary abnormality in congenital microcytic sideroblastic anaemia.[39 40] The combined findings of increased erythrocyte protoporphyrin levels despite normal ferrochelatase activity can only be explained by the non availability of ferrous iron to protoporphyrin and so provides absolute evidence of abnormal erythroblast iron metabolism in the anaemia of RA.

The nature of this abnormality in erythroblast iron metabolism and its relationship to reduced erythroblast ALA synthase activity is currently under investigation.

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