

Increased α :non- α globin chain synthesis ratios in myelodysplastic syndromes and myeloid leukaemia

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SUMMARY Globin chain synthesis ratios (α : $\beta + \gamma$) in leucocyte free reticulocytes from six of 11 patients with various myelodysplastic syndromes were high, ranging from 1.28 to 2.43. High ratios were also found for reticulocytes from two of four patients with acute myeloblastic leukaemia. Of the eight cases in which high ratios were found, seven were in patients who were either undergoing leukaemic transformation or who had already transformed. The reason for these findings is not known, but an understanding of the mechanism may give us further insight into the process of leukaemic transformation.

During a previous study on reticulocyte globin chain synthesis in sideroblastic anaemia (SA)¹ two of nine patients with primary acquired SA transformed to leukaemia. During this transformation their α : $\beta + \gamma$ ratios increased from normal to 1.36 and 1.45. As it was thought that there might be a link between the high ratios and transformation it was decided to measure such ratios on a group of patients with a high likelihood of developing leukaemia. Patients with various myelodysplastic syndromes, especially those with increased blast cells in their bone marrows, were therefore selected for study.

Material and methods

MYELODYSPLASTIC SYNDROMES (MDS)

Eleven patients, four women and seven men, with ages ranging from 41 to 83 years (mean 69.9) were investigated. Using the classification of Bennett *et al*² one patient had thrombocytopenia only and was classified as refractory anaemia (RA), two patients had RA with excess of blasts (RAEB), two patients had chronic myelomonocytic leukaemia (CMML), and six patients had RAEB in transformation to leukaemia (RAEB'T'). Two patients with RAEB'T' had previously been CMML. Except for the patient with RA, all were transfusion dependent. One patient (CMML) had been treated with 15 mg/day cytosine arabinoside (araC) for two weeks three months before the study. Despite the transfused cells three patients had marginally increased HbF concentrations of 1.1%, 1.2%, and 2.3%, and two had gross increases

of 19.5% (RAEB) and 44% (CMML). The patient with 44% HbF had decreased HbA₂ (1.0%) and a α : $\beta + \gamma$ ratio of 1.2. None had HbH inclusions. Two patients were studied more than once. The first (case 2, table) was studied on two occasions with one month in between. The other patient (case 8) was studied three times with six weeks between the first two investigations and 10 months between the second and third investigations. Eight weeks before the final study this patient was treated for two weeks with low dose araC.

ACUTE MYELOID LEUKAEMIA (AML)

In this group were two men and two women aged 58-85 years. Two had recently undergone leukaemic transformation from RAEB'T', and two had transformed from non-myelodysplastic syndrome conditions. One of the latter two was diagnosed two years previously as having follicular lymphoma (case 14) and had been receiving 2 mg/day chlorambucil until transformation to AML one month before the study. The other was a patient with blast cell transformation of chronic myelogenous leukaemia (CML) (case 15) and, until one month previously, had been treated with 1 g/day hydroxyurea.

CONTROLS

Our normal range had already been established from 38 haematologically normal people. During the course of this study 13 more were investigated, including five within the same age group as the patients (51-80 years, mean 71 years).

All blood samples were obtained in a manner approved by the University of Wales College of

Medicine and South Glamorgan Health Authority joint ethical committee.

GLOBIN CHAIN SYNTHESIS STUDIES

Reticulocyte enriched red cells from which leucocytes had been removed³ were studied as described previously.¹ Essentially, the reticulocytes were incubated for one hour with L-(1-¹⁴C leucine) (59 mCi/mmol) in a leucine free reagent mixture prepared according to the method of Lingrel and Borsook,⁴ with pooled, heat inactivated human AB serum instead of plasma, with 8 μ M (final concentration) 36% saturated human transferrin (Behringwerke) instead of ferrous ammonium sulphate and 0.89 mM (final concentration) L-asparagine. Whole cells were used in making the globin and the $\alpha:\beta + \gamma$ ratio estimated by calculating the total counts incorporated into the α and the β - γ peaks separated by ion exchange chromatography on CM Sepharose CL-6B.

Results

The table shows the results. Eight controls aged 22 to 45 years gave $\alpha:\beta$ ratios of 0.98 to 1.19, and five controls aged 51 to 80 years gave ratios of 1.02 to 1.16. These ratios were within the previously established normal range of 0.97 to 1.19.

Six of 11 patients with myelodysplastic syndrome had high ratios, ranging from 1.28 to 2.43. Five were classified as RAEB" T" and one as CMML.

Two of four patients with AML had high ratios. One of the patients who had transformed from MDS had a ratio of 2.1, and the patient who transformed from CML had a ratio of 1.8.

The rest of the patients gave normal $\alpha:\beta + \gamma$ ratios (one RA, one CMML, two RAEB, one RAEB" T" and two AML). The two patients with high HbF concentrations were in this group.

The patient with RAEB" T" who was studied twice had a ratio of 1.34 on the first occasion and 1.80 on the second. The patient with RAEB who was studied three times gave a ratio not significantly different from 1.0 on all three occasions.

Specific activity ratios were estimated on seven occasions using at least three tubes from each globin chain peak.⁵ These were always fairly close to the total counts ratio (table).

Discussion

This study confirms that increased $\alpha:\beta + \gamma$ ratios are common in reticulocytes of patients with a high likelihood of developing leukaemia, in particular those with excess blasts in their marrows. The number of

Table Globin chain synthesis ratios on patients with myelodysplastic syndromes and acute myeloid leukaemia

Diagnosis at time of first study	Case No	Bone marrow		Peripheral blood		Globin synthesis			
		Per cent blasts (+) = + Auer + atypical rods	Per cent blasts atypical mononuclear cells	No white cells ($\times 10^9/l$)	No of monocytes ($\times 10^6/l$)	Per cent blasts	Per cent blasts + atypical mononuclear cells	$\alpha:$ non α total counts	$\alpha:$ non α specific activities
RA	1	2		8.3	83	0		1.12	
RAEB	2a	7		2.0	40	1		1.00	
	b	24		2.2	22	1		1.01	
	c	4		7.8	234	0		0.99	0.98
CMML	3*	8		3.6	72	1		1.20	
	4**	7		3.8	304**			1.08	0.96
	5	4		3.1	1550	0		1.67	1.57
RAEB" T"	6	18		18.6	8184	11	22	1.37	
(from CMML)	7	5(+)		8.1	1296	1		1.06	
RAEB" T"	8a	6(+)		22.8	684	12		1.34	
	b	15(+)		42.5	5525	17		1.80	1.65
AML	9	11(+)	35	2.1	273	<1		2.43	
	10	15	25	3.5	0	<1		1.36	1.28
	11***							1.28	1.25
AML (from RAEB" T")	12	21	25	90.0	7200	21	46	1.10	
	13	47		6.2	1302	1		2.10	1.94
AML	14			11.4		19		1.17	
Controls	15			120.0		90		1.80	
ages 22-45 (n = 8)								0.98-1.19	
ages 51-80 (n = 5)								1.02-1.16	
Previous normal range								0.97-1.19	0.92-1.15

*19.5% HbF; **44% HbF; ***Unusually low result—monocytosis was a consistent finding before ($1.22-2.66 \times 10^9/l$) and after ($1.03-1.89 \times 10^9/l$) the study; ****Information not available—developed leukaemia within two weeks of study, six months previously had 11% blasts in the bone marrow.

patients is too small to test any meaningful correlations, but it should be said that there was no obvious relation between the α : β + γ ratios and the percentage of blasts in the marrow or the percentage of blasts in the peripheral blood. Furthermore, red cell hypochromia, basophilic stippling, microcytosis, and macrocytosis were not restricted to the patients with abnormal α : β + γ ratios. A more quantitative assessment of red cell abnormalities in these patients was impossible because of the overwhelming presence of transfused red cells.

Several reports of abnormal globin synthesis ratios in preleukaemic conditions have described low α : β + γ ratios,⁶⁻⁸ but this was not borne out by our own experience of patients with primary acquired SA, except in the case of acquired HbH disease.¹ High α : β + γ ratios have been reported in these conditions but only in association with either high HbF concentrations,⁹⁻¹¹ or a reticulocytosis.¹² Our results differ in that the two patients with high HbF had normal α : β + γ ratios, and none of the patients had a reticulocytosis.

Measurement of globin chain synthesis ratios is prone to error when non-globin protein synthesis becomes a large fraction of the whole. It is therefore possible that the variability of published reports is caused by variable contamination of the globin peaks by non-globin proteins. Removal of white cells, as carried out in this study, eliminates most of such contamination, mainly from the β - γ regions,¹³ but if the reticulocytes from these patients are synthesising an unusual protein, or relatively more of a normal protein with a charge similar to that of one of the globin chains, then this could conceivably affect the results. A changed pattern of globin degradation might also generate high ratios. Investigation of these possibilities was not pursued owing to the small amount of material available for study, as these patients have very low reticulocyte counts.

Apart from technical reasons, the high ratios may be a result of the genetic instability of differentiating bone marrow cells in these conditions¹⁴ with a selective advantage of the cells with the high ratios. They might, however, equally well be a more direct result of the genetic changes associated with leukaemic transformation in these patients. Such changes might entail the globin genes themselves or other areas of the genome important for the expression of globin genes. Haemoglobin synthesis is an area of metabolism that has been extensively investigated, and tools for detailed investigation of abnormality are already avail-

able. It would, therefore, be well worth tracing the mechanism of this abnormality back to its origin to gain more biochemical information about the process of leukaemic transformation, and methods to investigate these changes in bone marrow cells from similar patients are currently being established.

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