Stimulation of bone marrow erythropoiesis by T lymphocytes of anaemic patients with rheumatoid arthritis

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SUMMARY An inappropriate response of the bone marrow is implicated in the aetiology of the anaemia of chronic disease complicating rheumatoid arthritis.¹ T lymphocyte subsets have been shown to inhibit early erythroid development in vitro in association with some cases of bone marrow failure, and an expanded peripheral blood pool of these cells is reported in rheumatoid arthritis. We have studied the role of peripheral blood T lymphocytes in erythroid bone marrow culture from seven normal volunteers and nine anaemic patients with rheumatoid arthritis and found comparable stimulation of growth in both groups.

Key-word: bone marrow culture.

Rheumatoid arthritis is frequently complicated by an anaemia of varying degree which is usually related to disease severity and adds to the ill health of the patient. The mechanism of this 'anaemia of chronic disease' is poorly understood, though a failure of the erythroid marrow to respond appropriately is implicated.¹

It is now possible to assay very primitive progenitors of all haematopoietic lineages in tissue culture. The cells which give rise to colonies of mature cells in semisolid medium are characterised both by the type of progeny they produce and by their maturation time in culture. Erythroid progenitors in the marrow give rise in small seven-day colonies (colony forming units, CFU-e) and to large 14-day colonies (burst forming units, BFU-e), and these clonal assays have been used to study erythropoiesis both in normal subjects and in the anaemia of chronic disorders. At present evidence suggests that the more primitive BFU-e develop under the influence of local factors produced by adherent cells/macrophages and T lymphocytes.²⁻³ T lymphocytes may normally modulate BFU-e development; recent studies of T lymphocyte subpopulations in culture

have shown that certain subsets fail to stimulate BFU-e growth, whereas others may inhibit it.⁴ Erythroid or myeloid suppression by T cells in vitro has also been demonstrated in chronic lymphatic leukaemia,⁵ aplastic anaemia,⁶ and pure red cell aplasia.⁷

Active rheumatoid arthritis (RA) is characterised by disturbed T lymphocyte/macrophage immunoregulation,⁸ and we have used the bone marrow BFU-e assay to evaluate a possible role for T lymphocytes in the pathogenesis of the anaemia.

In this study both normal control and anaemic RA patients were studied using bone marrow cells deprived of endogenous sources of growth factors by specifically removing T lymphocytes and macrophages. Addition of autologous peripheral blood T lymphocytes to culture resulted in stimulation of BFU-e numbers, which was significant and comparable for both groups.

Patients and methods

PATIENTS (Table 1)

All nine patients studied had classical rheumatoid arthritis as defined by the criteria of the American Rheumatism Association. In all patients disease activity was assessed as active, with polyarthritis, systemic illness, and anaemia. All patients had normal or increased bone marrow iron stores,

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Table 1 Patient details

Patient	Sex	Age	IgM RF	Disease duration (years)	Morning stiffness (min)	Articular index (Lansbury)	Drugs*	Hb (g/dl)	ESR (mm/h)	Fe (µmol)	IBC (µmol)	Urea (mmol/l)	Marrow Fe
1	M	60	+	16	180	177	1,3,4	7.9	135	4	42	11.0	+
2	F	53	+	4	120	140	1,2	9.2	55	4	48	6.6	+
3	F	72	+	8	120	134	1	8.2	101	4	51	8.5	+
4	F	37	+	7	30	88	1	8.7	106	6	50	4.2	+
5	F	67	+	1.2	90	104	1	7.3	103	4	48	6.6	++
6	F	29	+	6	45	140	1,2	8.3	73	4	60	3.1	+
7	F	58	-	2	120	84	1,2	9.9	101	4	48	5.5	++
8	F	69	+	2	15	122	1	8.6	111	4	45	8.4	+
9	M	69	+	32	30	94	1	9.9	75	7	45	11.1	++

*Drugs: 1=non-steroidal anti-inflammatory drugs; 2=chloroquine; 3=sodium aurothiomalate; 4=prednisolone. RF=rheumatoid factor. Hb=haemoglobin. ESR=erythrocyte sedimentation rate. IBC=iron binding capacity.

alternative causes for the anaemia were excluded, and no patient had received previous blood transfusions. All patients were taking non-steroidal anti-inflammatory drugs. No patient was receiving a cytotoxic drug. Four patients were on a disease modifying drug (chloroquine or sodium aurothiomalate), and one patient was receiving prednisolone 5 mg on alternate days.

BONE MARROW

Posterior iliac crest aspirates were taken with the informed consent of healthy volunteers and from patients as part of the routine investigation of their anaemia. Non-adherent bone marrow mononuclear cells (NA-BMC) were obtained as previously described⁹ and were used to enumerate BFU-e numbers. In six of nine patients studied and in all controls T lymphocyte depletion was performed by a one-stage method using neuraminidase treated sheep red blood cells.¹⁰ Further macrophage removal was carried out on the T lymphocyte depleted fraction by centrifugation over a sucrose gradient (5-15%) at 100 g for 7 minutes and harvesting the upper light density fraction.¹¹ This fraction was designated as the low density fraction minus T lymphocytes (LDF, T⁻). Non-adherent bone marrow mononuclear cells from the first three patients studied were depleted of macrophages by the sucrose gradient technique only. 40 ml of peripheral blood was taken at the time of bone marrow aspiration, and mononuclear cells separated on a Ficoll gradient were subjected to the one-stage E rosette procedure outlined above. T lymphocytes were recovered after lysis of the pellet with NH₄Cl. Previous enumeration had shown T lymphocytes to constitute more than 95% of the 'T lymphocyte fraction'.

COLONY ASSAYS FOR BFU-e

All cultures were performed in 1% methyl cellulose Iscove's complete medium, 1% deionised bovine serum albumin (Sigma), 30% fetal calf serum (Sera Lab), 10⁻⁴ M 2-β-mercaptoethanol, and sheep step III erythropoietin (Connaught) at a concentration of 2 U/ml. In all experiments bone marrow progenitor cells were cultured at 0.5 or 1.0×10⁵ cells/ml and T lymphocytes added at 5 and 10×10⁵/ml. All cultures were in duplicate and incubated at 37°C and 5% CO₂ in a fully humidified incubator until 14 days when erythroid bursts were scored.

STATISTICAL ANALYSIS

Mean values for colony numbers were derived from log transformed data, and unpaired Student's *t* tests were used to assess differences between control and

RA assays. Mean values were given together with their 95% confidence intervals. Assay results with T lymphocyte addition were log transformed, and paired *t* tests performed to assess the significance of increased colony numbers.

Results

ERYTHROID COLONY NUMBER—NORMAL AND PATIENT BONE MARROW
Cultures of marrow progenitor cells (NA-BMC) from eight of the nine anaemic patients gave a mean BFU-e number of 42 (19–100) per 1×10^5 cells, which was not significantly different from the mean value of 30 (8–98) for our seven control marrows, confirming our previous observation.⁹

THE EFFECT OF T LYMPHOCYTES ON BFU-e IN NORMAL AND PATIENT MARROW
In seven normal individuals the addition of T lymphocytes to the LDF, T° (at 5 and 10×10^5 cells/ml) resulted in a significant stimulation of BFU-e growth ($p < 0.025$, $p < 0.01$) (Table 2). In three initial studies of anaemic patients T cells were added to autologous marrow depleted for macrophages but not for T lymphocytes, and in all

Table 2 Effects of autologous peripheral blood T lymphocytes on macrophage and T lymphocyte depleted bone marrow BFU-e

Control	Erythroid bursts per 0.5×10^5 bone marrow cells		
	LDF, T° $+5 \times 10^5$ T lymphocytes/ml	LDF, T° $+10 \times 10^5$ T lymphocytes/ml	LDF, T° $+10 \times 10^5$ T lymphocytes/ml
1	6	24	
2	4	48	
3	3	7	20
4	10		22
5	32		87
6	64	140	120
7	23	66	98
Patient			
1	16	45	85
2	27	39	30
3	23	183	181
4	12	25	34
5	12	33	44
6	6	8	16
Paired <i>t</i> statistic			
Control	$+5 \times 10^5$ T lymphocytes/ml		$p < 0.025$
	$+10 \times 10^5$ T lymphocytes/ml		$p < 0.01$
Patient	$+5 \times 10^5$ T lymphocytes/ml		$p < 0.025$
	$+10 \times 10^5$ T lymphocytes/ml		$p < 0.01$

Fig. 1 Growth of BFU-e from patient non-adherent bone marrow cells (NA-BMC) and from bone marrow depleted for macrophages (LDF), demonstrating stimulation of LDF derived BFU-e numbers by addition of autologous peripheral blood T lymphocytes.

three BFU-e growth was stimulated (Fig. 1). Subsequent assays on six patients whose marrows were depleted for both T lymphocytes and macrophages (LDF, T°) showed significant stimulation by T lymphocyte addition at 5 and 10×10^5 cells/ml ($p < 0.025$, $p < 0.01$) (Table 2), which was not different from that seen with the seven normal individuals.

Discussion

The T lymphocyte dependence of erythropoiesis has been shown in vivo in animals¹² and in vitro in man by clonal assays for BFU-e. Clonal assays with peripheral blood BFU-e not only showed stimulation by T lymphocytes but in addition a synergistic action of the two cell types.² More recently either failure to stimulate¹³ or inhibition of haematopoietic progenitors by peripheral blood or bone marrow T lymphocytes have been described, and T lymphocyte subsets bearing Fc γ receptors or 'Ia like' antigen have been implicated.^{14 15} In RA there is evidence of an enlarged pool of activated lymphocytes bearing Ia antigen,¹⁶ and thus it was possible that T lymphocytes might contribute to suppression of erythropoiesis by either of these mechanisms.

Populations of marrow progenitors are not pure, and as macrophages or T lymphocytes may stimulate progenitors in culture, failure to remove one cell type adequately may give spurious results with addition of the other. In our experience it is extremely difficult to remove marrow macrophages and macrophage precursors by the usual methods, and this may be the reason for the previous failure to

find T lymphocyte stimulation of marrow erythroid progenitors.¹⁷ However, with our present technique of adherence followed by density centrifugation over sucrose there is almost complete removal of macrophages, and no macrophages were visible after 14 days culture. Our data demonstrate stimulation of marrow BFU-e by T lymphocytes, as previously noted for peripheral blood BFU-e.³ T lymphocytes from RA patients also stimulate BFU-e growth, and their effects were comparable to those from our control studies.

It was not technically feasible to obtain sufficient T lymphocytes from bone marrow for coculture experiments so it is still possible that an imbalance of T lymphocyte subsets in marrow rather than blood could suppress erythropoiesis.

We have recently reported stimulation of BFU-e in culture by marrow macrophages⁹ from RA patients, and the present data appear to refute a cellular basis for inhibition of erythropoiesis. Serum from anaemic RA patients profoundly suppresses erythroid colony formation,⁹ and it seems possible that a soluble inflammatory or immune product circulates to the bone marrow where it is responsible for suppression of its erythropoietic proliferative capacity.

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