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

P J Prouse,1 B Bonner,2 J M Gumpel,1 and C D L Reid2

From the 1Department of Rheumatology, and the 2Section of Haematology, Northwick Park Hospital, and Clinical Research Centre, Watford Road, Harrow, Middlesex

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 immuno-regulation,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.
### Table 1 Patient details

<table>
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<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>IgM RF</th>
<th>Disease duration (years)</th>
<th>Morning stiffness (min)</th>
<th>Articular index (Lansbury)</th>
<th>Drugs*</th>
<th>Hb (g/dl)</th>
<th>ESR (mm/h)</th>
<th>Fe (µmol/l)</th>
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<th>Urea (mmol/l)</th>
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*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.
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 $1\times10^5$ 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 $10^5$ 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 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 $10^5$ 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 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
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find T lymphocyte stimulation of marrow erythroid progenitors. 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. 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 macrophages 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, 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.

References