Mechanism of anaemia in rheumatoid arthritis: demonstration of raised interleukin 1 β concentrations in anaemic patients and of interleukin 1 mediated suppression of normal erythropoiesis and proliferation of human erythroleukaemia (HEL) cells in vitro

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SUMMARY The pathogenesis of the anaemia associated with rheumatoid disease is unclear. It has previously been shown that the degree of the anaemia correlates with the severity of the inflammatory disease and that serum from patients with arthritis inhibits erythropoiesis. This study was designed to examine whether interleukin 1 could be a mediator of the anaemia in rheumatoid arthritis. Radioimmunoassay of interleukin 1 β in serum showed that patients with rheumatoid arthritis and associated anaemia had significantly higher interleukin 1 β concentrations than patients with rheumatoid arthritis without anaemia. Pure recombinant human interleukin 1 α and interleukin 1 β , in concentration ranges similar to those found in the arthritic patients, markedly suppressed the colony formation of the erythroid, but not the granulocyte-macrophage progenitor cells in cultures of normal bone marrow. Natural human interleukin 1 and recombinant interleukin 1 β , but not interleukin 1 α , suppressed in a dose dependent manner the proliferation of the human erythroleukaemia cell line (HEL) in cultures, suggesting that the interleukin 1 effect is a direct one. The results show that interleukin 1 is a humoral inhibitor of erythropoiesis and suggests that interleukin 1 is involved in the development of anaemia in association with rheumatoid arthritis.

Key words: pathogenesis, recombinant interleukin 1 α and 1 β , serum erythroid inhibitory activity, anaemia of chronic disease.

Anaemia of chronic disease is a frequently encountered form of anaemia in adults. The anaemia occurs in association with chronic inflammatory diseases, chronic infections, and malignant diseases.¹⁻³ Despite intensive research the pathogenesis of anaemia of chronic inflammatory disease has remained unclear.

Recent studies have demonstrated serum inhibition of in vitro erythropoiesis in adult and juvenile

patients with chronic arthritis.⁴⁻⁶ Inhibition of erythropoiesis corresponded with the severity of the anaemia⁶ and with inflammatory activity.^{4,6} We investigated the possibility that interleukin 1 (IL1), the humoral mediator of various facets of the acute phase reaction characteristic of inflammatory diseases,^{7,8} could also be an inhibitor of erythropoiesis and hence be involved in the pathogenesis of anaemia of chronic inflammatory disease. Our results show that circulating immunoreactive IL1 β concentrations are significantly higher in patients with rheumatoid arthritis (RA) and associated anaemia than in those without anaemia, and that recombinant human IL1 in concentration ranges

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similar to those found in the patients with arthritis suppresses colony formation of the erythroid, but not the granulocyte-macrophage precursor cells, in normal bone marrow. The suppressive effect appears to be a direct one as natural IL1 and recombinant human IL1 β suppress the proliferation of an erythroid-like malignant cell line, HEL, in culture.

Subjects and methods

SUBJECTS

Serum samples from 20 patients with classical or definite RA were studied. Ten of the patients (eight women, two men, mean age 55.5 years, range 26–81) had no anaemia (haemoglobin 139 (SD 10.1) g/l, range 126–162) and 10 patients (all women, mean age 64.7 years, range 38–77) had mild anaemia (haemoglobin 105 (6.3) g/l, range 97–113). No patient had signs of gastrointestinal bleeding or renal impairment, and no cause of the anaemia other than the underlying RA was found.

Twenty one blood donors (10 women, 11 men, mean age 40 years, range 21–72) served as normal control subjects.

BONE MARROW CULTURES

Bone marrow was obtained from five normal bone marrow donors. Erythroid progenitor cells BFU-E (burst forming units, erythroid) and CFU-E (colony forming units, erythroid) were cultured in methylcellulose as previously described by Iscove *et al*⁹ with the modification of Guilbert and Iscove.¹⁰ The colony formation was stimulated with 2 units of erythropoietin (step III sheep erythropoietin, Connaught Laboratories, Willowdale, Canada) per 2×10^5 nucleated cells. Granulocyte-macrophage precursors CFU-GM (colony forming units, granulocyte-macrophage) were cultured in methylcellulose using leucocyte feeder layer as the source of colony stimulating activity according to the method of Pike and Robinson.¹¹ Colonies were scored for CFU-E on the seventh day of culture and for BFU-E and CFU-GM on the 14th day of culture. Duplicate or triplicate plates at nucleated cell concentration of 2×10^6 /l were cultured in all experiments. Recombinant human interleukin 1 α and 1 β were tested in the marrow culture experiments.

HEL CELL CULTURES

The human erythroleukaemia cell line¹² was grown (2×10^4 cells/well) in RPMI 1640 medium supplemented with 50 mg/l transferrin. DNA synthesis was measured by [³H]thymidine incorporation techniques (30 kBq/well).¹³ Human monocyte derived purified IL1 and recombinant human IL1 α and IL1 β

were tested in these experiments. All determinations were done in triplicate.

MEASUREMENT OF SERUM IL1 β

Immunoreactive IL1 β concentrations in serum were measured by a competitive radioimmunoassay (IL1 β ¹²⁵I kit, Cistron Biotechnology, Pine Brook, New Jersey, USA). The antiserum used in the assay is specific for IL1 β ; no cross reactivity was apparent with IL1 α , interleukin 2, tumour necrosis factor, or interferon gamma.

SERUM AMYLOID A AND C REACTIVE PROTEIN ASSAYS

These measurements were carried out by radial immunodiffusion as described elsewhere.¹⁴

IL1 PREPARATIONS

The specific activity of purified recombinant human IL1 α and IL1 β was approximately 10^8 thymocyte mitogenesis units/mg.¹⁵ The natural human IL1 used in the experiments had been purified from stimulated human monocytes by immunoabsorption chromatography (Genzyme, Koch-Light Ltd, Suffolk, England).¹⁶ The specific activity of the preparation was 8 U/pg,¹⁷ and it was endotoxin free.

Fig. 1 Serum IL1 β concentrations in blood donors (Co) and in patients with rheumatoid arthritis (RA) with or without anaemia. The horizontal line indicates the mean concentration. The difference between the anaemic and non-anaemic patients with RA is significant ($p < 0.01$).

STATISTICS

Two tailed Student's *t* test and Wilcoxon's rank sum test (non-parametric data) were used in the analyses.

Results

CIRCULATING IL1 β AND ACUTE PHASE PROTEIN CONCENTRATIONS

Healthy subjects had very low concentrations of IL1 β immunoreactive material in serum (median <0.05 μ g/l). Patients with RA had raised IL1 β concentrations (Fig. 1); those with anaemia had significantly higher ($p<0.01$) concentrations than those without (Table 1). The serum concentrations

of the acute phase reactants serum amyloid A and C reactive protein were significantly higher ($p<0.01$) in the anaemic patients than in the non-anaemic subjects (Table 1).

EFFECT OF IL1 ON ERYTHROPOIESIS

In cultures of normal bone marrow progenitor cells recombinant human IL1 α and IL1 β suppressed the colony formation of the erythroid precursors BFU-E and CFU-E but had no inhibitory effect on the granulocyte-macrophage progenitors CFU-GM (Fig. 2). The IL1 effect on the erythroid colony formation was dose dependent; an IL1 concentration of 0.1 μ g/l caused on average about 50% inhibition (Fig. 2).

Table 1 Serum immunoreactive interleukin 1 β , serum amyloid A, and C reactive protein concentrations in patients with rheumatoid arthritis with or without anaemia

Patient group	Haemoglobin (g/l)		Serum interleukin 1 β (μ g/l)		Serum amyloid A (mg/l)		Serum C reactive protein (mg/l)	
	Mean (SD)	Range	Mean (SD)	Range	Median	Range	Median	Range
RA with anaemia (n=10)	105 (6.3)	97-113	0.31 (0.17)*	0.15-0.62	152	68-546†	41	26-190†
RA without anaemia (n=10)	139 (10.1)	126-162	0.13 (0.06)	<0.05-0.20	37	<5-189	10	<5-52

* $p<0.01$ (two tailed *t* test).

† $p<0.01$ (Wilcoxon's rank sum test).

Fig. 2 Effect of recombinant human IL1 α and IL1 β on the colony formation of erythroid (BFU-E and CFU-E) and myeloid (CFU-GM) progenitor cells. The data are expressed as percentage of control (culture medium without IL1 additions) and represent the mean of five separate experiments (marrow samples from five different donors) done in duplicate or triplicate (IL1 β) or the mean of two separate experiments (marrow samples from two different donors) done in duplicate (IL1 α).

EFFECT OF IL1 ON HEL CELLS

To examine whether IL1 can affect cell proliferation directly without the mediation of other cells we studied the effect of IL1 on the proliferation of a human malignant cell line with erythroid characteristics, HEL, in culture. A dose dependent antiproliferative effect was found with both natural human IL1 and recombinant human IL1 β (Fig. 3). In contrast, at the concentrations tested IL1 α had no measurable suppressive effect in the incorporation of [3 H]thymidine in DNA of the HEL cells (Fig. 3).

Fig. 3 Effect of purified human IL1 and recombinant human IL1 α and IL1 β on [3 H]thymidine incorporation in DNA of the human erythroleukaemia cell line HEL. Data are expressed as percentage of control and were pooled from two or three separate experiments done in triplicate. For conversion of units to nanograms see 'Subjects and methods'.

Discussion

IL1 is a regulatory cytokine elaborated mainly by activated monocytes/macrophages.^{7,8} IL1 has been implicated in a variety of biological phenomena, including T and B cell activation,¹⁸⁻²⁰ acute phase protein synthesis,²¹ regulation of fever,^{7,22} fibroblast proliferation,²³ muscle proteolysis,²⁴ bone resorption,²⁵ and induction of procoagulant activity.²⁶ IL1 has also been shown to be cytostatic or cytotoxic for some tumour cells.^{27,28} It has become increasingly evident that IL1 is a key mediator of several metabolic alterations associated with the acute phase reaction, and there is evidence to suggest that IL1 is also involved in the pathogenesis of various manifestations of chronic inflammatory diseases.^{7,8} This study was designed to examine whether IL1 could be involved in the pathogenesis of the anaemia of chronic inflammatory joint disease. The results show that pure recombinant IL1 α and IL1 β selectively suppress the colony formation of erythroid progenitor cells in marrow culture experiments. Moreover, both natural and recombinant IL1 β suppressed the proliferation of the erythroleukaemia cell line HEL, suggesting that the suppressive effect of IL1 on the erythroid cells is a direct one.

We do not know why IL1 α at the concentrations tested had no significant effect on the malignant cells, but this may be related to a structural/functional alteration of IL1 receptors of the HEL cells. IL1 α and IL1 β are structurally distinct proteins.²⁹ The cDNA nucleotide sequences suggest that they are initially translated as precursor polypeptides of 271 and 269 amino acids respectively, which are subsequently processed to yield the Mr ~ 17 500 products usually associated with IL1 activity. The exact functional relations between IL1 α and IL1 β are not known, but they appear to have some biological effects in common,^{30,31} and appear to share the same plasma membrane receptor in certain cells.^{32,33}

With respect to the pathogenesis of the anaemia associated with RA it is of interest to note that recombinant IL1 α and IL1 β had no inhibitory effect on the colony formation of the granulocyte-macrophage progenitors in the marrow culture experiments. This observation is in accordance with the haematological findings in most anemic patients with RA. Recent in vitro studies show that IL1 can in fact stimulate fibroblasts and marrow stromal cells to produce granulocyte-macrophage and granulocyte colony stimulating factors.³⁴⁻³⁶ As these factors have, under certain in vitro conditions, also been shown to stimulate the growth of BFU-E³⁷ the effects of IL1 on haematopoiesis are complex and involve both stimulatory and inhibitory signals.

Indirect evidence for raised IL1 concentrations in the patients with RA and associated anaemia was obtained by finding significantly increased concentrations of serum amyloid A and C reactive protein in these patients, as IL1 induces the hepatic acute phase protein synthesis.²¹ Direct evidence was obtained by radioimmunoassay of serum IL1 β . Raised concentrations were found in the patients with RA, those with anaemia having significantly higher concentrations than those without anaemia.

Although not directly comparable, it should be noted that the raised serum concentrations of IL1 β found in the patients with arthritis were in the concentration range that caused a significant suppression of the colony formation of the erythroid progenitor cells in the marrow culture experiments. Although the anaemic patients with RA had significantly higher IL1 β concentrations than the non-anaemic patients, raised concentrations were also found among the patients with RA without anaemia (who also had raised acute phase protein concentrations) and even in a few blood donors. This finding is not surprising in view of the previous demonstrations of IL1-like activity in the plasma of healthy subjects after exercise³⁸ or ovulation,³⁹ and of the role of IL1 as a mediator of the acute phase reaction in inflammatory conditions.⁴⁰⁻⁴² It seems probable that the inhibitory effect of IL1 on erythropoiesis becomes clinically relevant only in situations of long term IL1 overproduction.

The detection of IL1 activity has previously been based on bioassays using various cell lines in culture or on testing the pyrogenicity or leucocytic endogenous mediator activity of biological samples in animals.⁷ The previous difficulties in detecting IL1 in serum in rheumatic diseases⁴³ might have been due to the presence of serum inhibitors of the biological activity^{7,44} or to the lesser sensitivity of the bioassay as compared with radioimmunoassay.

Previously, Dainiak *et al* found that serum from anaemic patients with systemic lupus erythematosus or RA inhibited erythroid colony growth of cultured bone marrow cells.⁴ The presence of this serum inhibitor was related to disease activity and could be removed by plasma exchange. Roodman *et al* have shown that autologous bone marrow adherent cells from patients with anaemia of chronic disease inhibited erythroid colony formation.⁴⁵ Similar results have also been obtained with bone marrow adherent cells from patients with fungal infections.⁴⁶ Zucker *et al* have shown that cancer cells inhibit erythropoiesis by a mechanism which is independent of cell contact.⁴⁷ All these findings are also compatible with the view that IL1 is one of the humoral factors responsible for the suppression of erythropoiesis in rheumatic disease, infections, and malignancy.

Moreover, the hypoferraemia of anaemia of chronic disease may also be explained by a raised IL1 concentration as IL1 has been shown to depress serum iron concentrations under experimental conditions.¹⁸

It has been shown that after activation by a variety of different stimuli,^{7,18} including interferons⁴⁸ and tumour necrosis factor,⁴⁹ macrophages/monocytes produce/release IL1. Some tumour cells are also capable of producing IL1,⁵⁰ which would explain the cancer cell mediated inhibition of erythropoiesis.

Our contention that IL1 has a central role in the pathogenesis of the anaemia of RA does not exclude the possibility that other factors are involved, nor does it rule out interactions between various cytokines. Growing evidence suggests that in addition to having a potential role in the mediation of host defence against neoplastic cell growth and parasite infection tumour necrosis factor α has important regulatory functions in normal cell metabolism.^{51,52} The actions of tumour necrosis factor and IL1 are very similar in many processes associated with inflammation. In addition, recombinant tumour necrosis factors appear to mediate the haematopoietic colony inhibitory activity of natural killer cells, showing synergism with interferon gamma in this respect.^{53,54} The colony inhibiting activity of natural killer cells, however, is primarily directed against the granulocyte-macrophage cell line and not the erythroid cell line. Moreover, in a recent report Peetre *et al* showed that the erythroid progenitors were less susceptible to the action of tumour necrosis factor than were the granulocyte-macrophage progenitors.⁵⁵

In conclusion, based on the results of the present study, it is suggested that in situations of protracted overproduction IL1, a hormone-like intercellular mediator, which is primarily involved in host defence mechanisms, may not only be involved in the pathogenesis of muscle breakdown, coagulopathy, and reactive amyloidosis of chronic inflammatory diseases⁸ but also in the development of the anaemia of these conditions.

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