Granulopoietic activity in Felty's syndrome

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Gupta, R., Robinson, W. A., and Albrecht, D. (1975). *Annals of the Rheumatic Diseases*, 34, 156. **Granulopoietic activity in Felty's syndrome.** The levels of urinary and serum granulopoietic factor have been determined in nine patients with Felty's syndrome and compared with similar levels in patients with rheumatoid arthritis without Felty's syndrome and patients with neutropenic disorders of other causes. These studies have shown that urinary and serum levels of colony stimulating activity (CSA) in patients with Felty's syndrome are low (mean urine CSA 10.0 and serum CSA 4.0) when compared to patients with neutropenic disorders without rheumatoid arthritis (mean urinary CSA 26.8 and serum CSA 9.4). These findings suggest that Felty's syndrome may be, in part, the result of decreased production or activity of granulopoietic factors rather than due simply to increased granulocyte destruction.

A. R. Felty (1924) described 5 cases of chronic rheumatoid arthritis with splenomegaly and leucopenia. Since then this syndrome has become increasingly recognized and occurs in about 1% of patients with rheumatoid arthritis (Short, Bauer, and Reynolds, 1957). Leucopenia that occurs is almost exclusively the result of a reduction in granulocyte numbers in the peripheral blood. The mechanism whereby this occurs is unknown. None of the various theories put forth to explain the granulocytopenia been satisfactory. Those that have been suggested are chronic sepsis (Singer and Levy, 1936), sequestration of white blood cells by hypersplenism (Hirschboeck, 1946; Wright, Doan, Bouroncle, and Zollinger, 1951), and inhibition of normal granulocytes by a splenic factor (Hutchison and Alexander, 1954). The most commonly accepted mechanism is hypersplenism with sequestration of peripheral granulocytes and their subsequent destruction in this organ. A common recurrence of granulocytopenia after splenectomy suggests, however, that this is not the only mechanism involved in the development of this disorder, and indicates that extrasplenic factors must be involved (Ruderman, Miller, and Pinals, 1968). In some patients white blood cell antinuclear factors have been shown (Faber, Elling, Norup, Mansa, and Nissen, 1964; Barnes, Turnbull, and Vernon-Roberts, 1971), but their relationship to the development of this syndrome is not clear.

Recently a system has been developed in which single bone marrow cells can be stimulated to divide

and form colonies of granulocytes and macrophages in a semisolid tissue culture medium in the presence of an appropriate stimulus (Pluznik and Sachs, 1965; Bradley and Metcalf, 1966). This system has been used extensively to study factors affecting and regulating granulocyte maturation and production. Using this system, Greenberg and Schrier (1973) have recently shown that the number of granulocyte colony forming cells in the bone marrow of patients with Felty's syndrome was less than that found in the bone marrow of patients with idiopathic neutropenia or splenomegaly or other disorders. They suggested that 'decreased production appears to contribute significantly to the neutropenia present in patients with Felty's syndrome', while not explaining the possible nature of this defect. Using this same system, a factor has been described in normal human and animal serum and urine which will stimulate the growth of granulocytic and macrophage colonies *in vitro* (Foster, Metcalf, Robinson, and Bradley, 1968; Stanley, Robinson, and Ada, 1968; Robinson and Pike, 1970). This factor has been characterized as a glycoprotein with a molecular weight of approximately 45000 which migrates electrophoretically as an α -globulin (Stanley and others, 1968; Stanley and Metcalf, 1969). A large body of evidence exists which strongly suggests that this factor represents, or is, a true granulopoietin stimulating maturation and production of the granulocyte cell line *in vivo* as well as *in vitro* (Metcalf and Stanley, 1971; Metcalf and Moore, 1971; Robinson, 1974). The following studies were undertaken to determine the levels of this granulopoietic factor in the urine of patients with Felty's

syndrome and to compare them with values obtained in the urine of other patients with rheumatoid arthritis without Felty's syndrome and patients with neutropenia of other causes. These studies have shown that urinary levels of granulopoietic factors in patients with Felty's syndrome are low compared with those found in patients with rheumatoid arthritis or patients with neutropenic disorders without rheumatoid arthritis. These data suggest that Felty's syndrome may partly result from decreased production or activity of granulopoietic factors rather than simple increased granulocyte destruction.

Subjects and methods

All the patients reported here were seen at the University of Colorado Medical Center between June 1973 and January 1974. The diagnosis of Felty's syndrome was based on a history of classical rheumatoid arthritis, the presence of rheumatoid factors in the serum, clinical splenomegaly, and a peripheral granulocyte count below $2.5 \times 10^9/l$ ($2500/mm^3$). Control patients were those with classical rheumatoid arthritis (A.R.A. criteria) without clinical splenomegaly or granulocytopenia. Patients with granulocytopenia without rheumatoid arthritis represented a wide spectrum of disorders, mostly the result of bone marrow hypoplasia of varying causes. Clinical details of all patients presented in this study will be found in Tables I, II, and III. The method for determination of urinary granulopoietic stimulating activity has been published in detail elsewhere (Foster and others, 1968; Robinson and Pike, 1970). In brief, 0.15 ml of dialysed, sedimented, filter sterilized urine is placed in triplicate 35 mm plastic Falcon petri dishes to which is added a mixture of 0.3% agar, McCoy's 5A medium, and 75000 nucleated bone marrow cells from the femurs of C57/b1 mice. The total volume in each petri dish is 1 ml. After mixing urine with the culture medium, the plates are allowed to

gel at room temperature and then incubated at 37°C in a fully humidified incubator for 7 days. Colony counts are then done as an index of the level of granulopoietic material present in the specimen. Only colonies containing 50 or more cells are counted. Previous data have shown a linear relationship between the number of such colonies counted and the amount of granulopoietic activity present, allowing a quantitative estimate (Stanley and Metcalf, 1969).

Results

Table I shows the clinical details, haematological parameters, and colony stimulating activity of nine patients with Felty's syndrome. Also shown are minimal details of bone marrow biopsy studies when these were available. It can be seen that the majority of these patients had bone marrows which were read as hyperplastic with a maturation arrest in the granulocytic series. Table II shows the same data, without bone marrow biopsies, in nine control patients with rheumatoid arthritis. All patients studied here had absolute granulocyte counts above $2.5 \times 10^9/l$ ($2500/mm^3$) and the majority were above $5.0 \times 10^9/l$ (5000). Table III shows the haematological parameters and bone marrow findings of twelve patients without rheumatoid arthritis who had neutropenic disorders, and shows that the majority of these patients had hypoplastic bone marrows either on the basis of idiopathic neutropenia or as the result of chemotherapy.

Fig. 1 shows the levels of urinary colony stimulating activity in these three groups of patients. The mean value of urinary colony stimulating activity of seven patients with Felty's syndrome was 10.1 and is significantly lower than that found in patients with rheumatoid arthritis and in patients with neutropenic

Table I Clinical details, haematological parameters, and colony stimulating activity in patients with Felty's syndrome

Case no.	Age (years)	Duration of rheumatoid arthritis (years)	Duration of Felty's syndrome (years)	Absolute PMN counts* ($\times 10^9/l$)	% PMN	Serum CSA†	Urine CSA	Bone marrow‡
1	49	26	10	1.872	36	2	0	Maturation arrest
2	36	6	1½	0.185	5	1	9	Hyperplasia, maturation arrest
3	47	12	1	2.336	73	0	3	—
4	61	34	1	2.209	47	4	35	—
5	60	22	3	0.850	34	0	—	Hyperplasia, patchy serous fat atrophy
6	54	10	1	0.216	36	5	5	Hyperplasia, maturation arrest
7	29	10	4	1.728	64	—	5	Normal to hyperplasia
8	55	6	½	0.896	32	16	14	Maturation arrest
9	45	1	½	0.690	23	4	—	—

* PMN = polymorphonuclear cells.

† CSA = colony stimulating activity expressed as number of colonies.

‡ Description related to granulocyte series.

Conversion: SI to Traditional Units—PMNs: $1.0 \times 10^9/l \approx 1000/mm^3$.

Table II *Clinical details, haematological parameters, and colony stimulating activity in patients with rheumatoid arthritis*

Case no.	Age (years)	Duration of rheumatoid arthritis (years)	Absolute PMN counts* ($\times 10^9/l$)	% PMN	Serum CSA†	Urine CSA
10	43	10	5.159	67	4	29
11	54	30	3.234	66	2	48
12	50	6	4.672	64	2	14
13	65	19	7.720	80	9	5
14	34	6	8.132	76	2	27
15	53	9	8.453	79	2	4
16	34	3	2.870	70	0	23
17	55	3	5.040	70	3	—
18	52	1½	3.658	59	1	87

* PMN = polymorphonuclear cells. See Table I for conversion.

† CSA = colony stimulating activity expressed as number of colonies.

Table III *Clinical details, haematological parameters, and colony stimulating activity in patients with other neutropenic disorders*

Case no.	Age (years)	Diagnosis	Duration of neutropenia (years)	Absolute PMN counts* ($\times 10^9/l$)	% PMN	Serum CSA†	Urine CSA	Bone marrow‡
19	71	Cyclic neutropenia	1	0.368	23	4	42	Maturation arrest
20	30	Pancytopenia	1/12	1.323	49	4	—	Hypoplasia and maturation arrest
21	62	Neutropenia	1	1.449	69	58	7	Maturation arrest
22	43	Megaloblastic anaemia	2/12	2.201	71	4	70	Normal maturation, erythroid hyperplasia
23	35	Aplastic anaemia	6/12	0.008	1	2	62	Marked aplasia
24	51	Aplastic anaemia	5	0.713	30	0	13	Hypoplasia
25	29	Aplastic anaemia	3	0.092	4	3	23	Aplasia
26	21	Neutropenia	6/12	1.850	50	9	3	Maturation arrest
27	37	Chemotherapy suppression	3/12	1.904	76	6	2	Granulocytic hypoplasia
28	21	Aplastic anaemia	2/12	0.040	8	0	17	Hypoplasia, serous fat atrophy
29	48	Pancytopenia	2	0.608	38	15	23	Hypoplasia, maturation arrest
30	24	Neutropenia, secondary to chemotherapy	0.5/12	1.724	49	9	33	Normal

* PMN = polymorphonuclear cells.

† CSA = colony stimulating activity expressed as number of colonies.

‡ Description related to granulocyte series.

disorders with means of 29.6 and 26.8, respectively. There is a wide range of scatter, particularly in the group of patients with neutropenic disorder, probably representing the wide spectrum of disease in this group. Normal values for urinary CSA in our laboratory are 0–40 with a mean of 25.

Fig. 2 shows the serum colony stimulating activity of the same three groups of patients. Eight patients with Felty's syndrome had a mean serum colony stimulating activity level of 4.0, which is significantly lower than that of other neutropenic patients but is not significantly different from that of other patients with rheumatoid arthritis or normal humans (range

0–10 with a mean of 5 in our laboratory). The inability to show clearly low levels of serum CSA probably reflects the insensitivity of the assay system when low concentrations of granulopoietic factors are present.

Discussion

It has long been assumed that granulocytopenia occurring in Felty's syndrome was the result of a hypersplenic state; and on this basis splenectomy has frequently been performed. However, several reports have indicated the lack of uniform success with this

FIG. 1 Urinary granulocyte colony stimulating factor levels in patients with rheumatoid arthritis, Felty's syndrome, and neutropenic disorders of other causes. Each ● on the curve represents a single patient and is the mean colony count of 3 plates. The mean of all the CSA values obtained is indicated by the bar. Normal values for urinary CSA in our laboratory are 0–40 with a mean of 25

FIG. 2 Serum granulopoietic colony stimulating activity in patients with rheumatoid arthritis, Felty's syndrome, and neutropenic disorders of other causes. Each ● represents a single patient and is the mean colony count of 3 plates. Bars indicate mean values

method in alleviating the granulocytopenia and the recurrent infections that occur in association with this syndrome (Barnes and others, 1971; Moore, Brunner, Sandusky, and Leavell, 1971). In most reported series the neutrophil count returns to normal after splenectomy in 60–70% of patients, although most patients have a transient increase in neutrophils in the peripheral blood. These data suggest that Felty's syndrome, at least in some cases, is not simply the result of hypersplenism alone, and previous data have suggested that this might be the result of an antinuclear factor, specific for leucocytes, found in high titres in the serum of patients with rheumatoid arthritis, specifically in patients with Felty's syndrome (Faber and others, 1964; Barnes and others, 1971). The direct pathogenic role played by this serum factor in the development of Felty's syndrome, however, is still open to question.

The finding of Greenberg and Schrier (1973) of normal or low numbers of granulocyte colony forming cells in the bone marrow of patients with Felty's syndrome also suggested that hypersplenism alone does not produce granulocytopenia in this disorder. It would be expected, in contradistinction to their

findings, that if the disorder were due to increased destruction of granulocytes, that increased numbers of granulocyte-producing cells might be found in the bone marrow of these patients. In the patients reported here, no study was done of the colony forming capacity of bone marrow cells. However, the majority of these patients had bone marrows which were read as hyperplastic, which must be viewed with caution in light of the findings reported by Greenberg and Schrier and those reported here. It is suggested that this is more likely the result of a maturation arrest in the granulocyte series rather than increased cellular proliferation.

The evidence that factors other than hypersplenism may lead to the development of granulocytopenia in this disorder led us to study the levels of granulopoietic factors. Previous studies have indicated a physiological role for the colony stimulating factor described here in the stimulation of maturation and production of the granulocyte cell line (Metcalf and Moore, 1971; Metcalf and Stanley, 1971; Robinson,

1974). Furthermore, it has been shown that the levels of this factor in the serum and urine of animals and humans with various granulocytopenic disorders are highest during times when the granulocyte levels are decreased (Dale, Brown, Carbone, and Wolff, 1971; Shadduck and Nagabhushanam, 1971; Quesenberry, Morley, Stohlman, Rickard, Howard, and Smith, 1972). Contrary to the expectation of high levels of granulopoietic factor in the urine of these patients, not only on the basis of peripheral granulocytopenia, but also on the basis of increased destruction of these cells within the spleen, we found markedly reduced levels in this disorder.

Recent data suggest that the major source of colony stimulating factor in humans is the monocyte-macrophage system (Golde and Cline, 1972; Chervenick and LoBuglio, 1972; Moore, Williams, and Metcalf, 1973). On the basis of cell separation studies, it appears that these cells actively manufacture this material by a process of secretion in response to increased demands. Several possible explanations may be offered. The first possible mechanism is decreased or altered production of such factors by the reticuloendothelial system. Such a mechanism, however, does not satisfactorily explain the response of 60–70% of patients with this disorder to splenectomy. If such a mechanism were involved, removal of this organ with its large component of reticuloendothelial cells might be expected to aggravate rather than alleviate granulocytopenia. A second possible explanation is the production of a factor inhibiting normal granulopoiesis which is partially, but not wholly, produced by the spleen itself. Inhibitory factors against the colony stimulating material have been previously described but have not been well characterized as to specificity, biochemical nature, source, or mode of action (Metcalf and Moore, 1971), and though this was not investigated in the present study, it is a possible mechanism and is being investigated at the present time.

Such inhibitory material might be directed either against the granulocyte precursor cells in the bone marrow or the colony stimulating factor itself. The present data do not give any indication as to which of these might be operative. Mixture of serum or urine from patients with Felty's syndrome with other materials of known colony stimulating activity to determine the presence of inhibitory materials would not answer this question. More sophisticated techniques are required and are now being employed.

Consideration should be given to the possibility that the splenomegaly occurring in this disorder is the result of the granulocytopenia rather than the cause. Splenomegaly, with resultant extramedullary haematopoiesis, is known to occur in a variety of disorders in which production of haematopoietic cellular elements is interfered with. Removal of this spleen would be expected to increase peripheral granulocyte numbers due to increased life span of these cellular elements without effectively altering granulocyte production. Although this may be a useful therapeutic manoeuvre in this disorder, as it is in idiopathic thrombocytopenic purpura, it may not attack the primary problem (Crosby, 1973). We would not suggest at the present time changes in the current recommended indications for splenectomy in Felty's syndrome as this may, as previously shown, be a life-saving manoeuvre in these patients. Serious consideration should, however, be given to other mechanisms for the development of granulocytopenia in patients with Felty's syndrome. Studies are now underway to further delineate the role of granulopoietic factors and cellular responsiveness in this disorder.

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