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Stromal cell activity in bone marrow from the tibia and iliac crest of patients with rheumatoid arthritis

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Abstract Bone marrow aspirates were obtained from the iliac crest and tibial epiphysis in 23 patients with rheumatoid arthritis (RA) who were undergoing total knee arthroplasty. The number of fibroblast colony-forming units (CFU-F), which contain osteogenic precursor cells, and alkaline phosphatase (ALP) activity, as a marker of the osteoblastic phenotype, were compared between the iliac and tibial marrow for each patient. The prevalence of CFU-F in tibial marrow was similar to that in iliac marrow (96% *vs* 100%, respectively). However, the average number of CFU-F per 4×10^5 bone marrow mononuclear cells was significantly lower in tibial marrow than in iliac marrow (8.2 *vs* 28.1, respectively; $P < 0.01$). Although ALP activity was detected in all iliac and tibial marrow specimens, it was significantly lower in tibial marrow compared with iliac marrow (3.7 *vs* 11.9 nmol/min/mg protein, respectively; $P < 0.01$). In addition, there was a significant correlation between the patient's age and the number of CFU-F in iliac marrow ($r = -0.547$; $P < 0.01$), although there was no correlation in tibial marrow. These results demonstrate that the osteogenic activity of bone marrow varies at different sites in patients with RA. The data may also contribute to further investigation into the differential effects of various disease processes on systemic as well as local stromal cell activity in bone marrow.

Key words rheumatoid arthritis · bone marrow · stromal cell activity · iliac crest · tibial epiphysis

Introduction

Numerous studies have demonstrated that osteoblastic precursors are present in bone marrow from animals [1–5], but there have been few reports on osteoblastic progenitors in human marrow [6–8]. Although the results have varied, it appears that human marrow contains osteoprogenitor cells that can differentiate studies into osteoblastic cells [9]. However, most previous studies have used bone marrow from the iliac crest, and little is known about the number or prevalence of osteoprogenitor cells in bone marrow from other sites. In addition, it is not known how the progenitor cell population may be affected by age, gender, and disease state. It is well-known that patients with rheumatoid arthritis (RA) have osteoporotic bone adjacent to the affected joints. Therefore, we hypothesized that osteogenic activity in bone marrow adjacent to joints with RA may be suppressed. The first aim of the present study was to investigate whether or not fibroblast colony-forming units (CFU-F), which contain osteogenic precursor cells, were present in bone marrow adjacent to joints affected by RA. In addition, the number of CFU-F and alkaline phosphatase (ALP) activity, which is a marker of the osteoblastic phenotype, were compared between bone marrow from the iliac crest and the tibial epiphysis in order to investigate the effect of RA on the osteogenic activity of bone marrow in arthritic joints.

Materials and methods

Twenty-three patients with RA who were scheduled to have total knee arthroplasty at our university hospital were enrolled in the present study, including 19 women and four men. Their mean age at the time of surgery was 50.2 years (range 33–61 years). Six patients had stage III (advanced) disease and 17 had stage IV (late) disease according to the classification of Steinbrocher et al. [10]. The functional level was determined as being class II (patients can perform daily activities despite joint pain, but have restricted movement) in seven patients and was class III (almost unable to perform daily activities) in 16 patients [10]. All patients were receiving corticosteroid therapy (less than 5 mg/day prednisolone).

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The study protocol was approved by the ethics committee of our hospital. All patients were provided with an explanation of the purpose of the study, and a detailed informed consent form was signed by each patient.

Preparation of bone marrow

After anesthesia had been induced and before the operation was started, bone marrow was aspirated from the anterior iliac crest on the same side as the knee that was scheduled for total knee arthroplasty. Bone marrow was obtained using a standard needle, as described elsewhere [11,12]. Briefly, the bone marrow aspiration needle was advanced into the medullary cavity at a site 2 cm posterior and 1 cm distal to the anterior superior iliac spine. Then, a 10-ml syringe containing 1 ml heparin solution (1000 units/ml) was connected to the needle. Five milliliters bone marrow was aspirated and, subsequently, an additional 5 ml bone marrow was aspirated from another site adjacent to the first. The marrow aspirates (total volume 10 ml) were subjected to centrifugation on a Ficoll–Hypaque density gradient (1.077 g/ml) and the mononuclear cell fraction thus obtained was washed three times with phosphate-buffered saline (pH 7.4) and used for the subsequent colony-forming assay. Another 10 ml bone marrow was aspirated from two sites at the tibial epiphysis (5 ml each) before surgery and was also prepared for the colony-forming assay.

Colony-forming assay of stromal precursors

The CFU-F were assayed in the iliac and tibial aspirates of 23 patients according to the adherent cell colony method of Nara et al. [13] and Castro-Malaspina et al. [14] with minor modifications. Briefly, 4×10^5 bone marrow mononuclear cells were cultured in 2 ml α-minimal essential medium (Gibco, Grand Island, NY, USA) supplemented with 20% fetal calf serum (Gibco) in 35-mm diameter dishes (Falcon; Nippon Becton Dickinson, Tokyo, Japan) at 37°C under a humidified atmosphere of 5% CO_2 in air. Cultures were performed in triplicate and the medium was totally renewed every 3 days. On day 14, the medium was discarded, and the culture dish was dried and stained with Wright's stain. Colonies of more than 50 fibroblasts were counted.

Assay of ALP activity

Cultures were maintained by changing the medium with 2×10^{-8}M 1,25-dihydroxy vitamin D_3 (1a,25$(OH)_2D_3$) every 3 days. After the cells became confluent, the culture was continued for 4 days in 0.5% bovine serum albumin (BSA) with 2×10^{-8} M1a,25$(OH)_2D_3$. The ALP activity was determined using *p*-nitrophenyl

Table 1. Details of the 23 subjects with rheumatoid arthritis

Case no.	Age (years)	Sex	CFU-F iliac marrow[a]	CFU-F in tibial marrow[a]	ALP in iliac marrow[b]	ALP in tibial marrow[b]
1	33	F	40	16	21.2	5.4
2	45	F	30	18	20.0	8.3
3	61	M	11	6	4.0	1.1
4	58	F	13	7	3.2	2.1
5	40	F	32	0	14.5	0.8
6	46	M	41	16	15.2	5.5
7	57	F	31	5	10.3	2.0
8	50	F	43	6	11.2	1.8
9	43	F	25	13	18.7	6.3
10	46	F	29	10	10.5	1.4
11	48	F	32	7	9.2	1.9
12	51	M	30	19	15.3	10.8
13	60	M	14	3	5.8	1.5
14	53	F	12	3	12.6	1.0
15	55	F	19	15	9.2	7.7
16	43	F	34	3	18.0	5.1
17	58	F	39	2	6.5	2.3
18	52	F	35	7	13.8	4.1
19	51	F	38	11	10.6	7.4
20	52	F	22	7	13.6	3.1
21	57	F	16	5	11.2	1.5
22	43	F	28	1	13.1	2.1
23	54	F	29	9	6.4	1.8

[a] CFU-F, fibroblast colony-forming units/4×10^5 mononuclear cells
[b] ALP, alkaline phosphatase (nmol/min/protein)

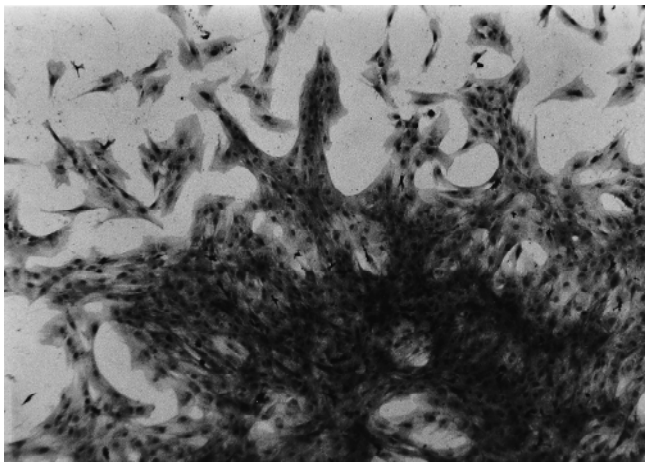

Fig. 1. Photomicrograph of fibroblast colony-forming units (CFU-F) obtained from the cultured tibial bone marrow mononuclear cells of a patient with rheumatoid arthritis (case 2). (Wright's stain; ×100)

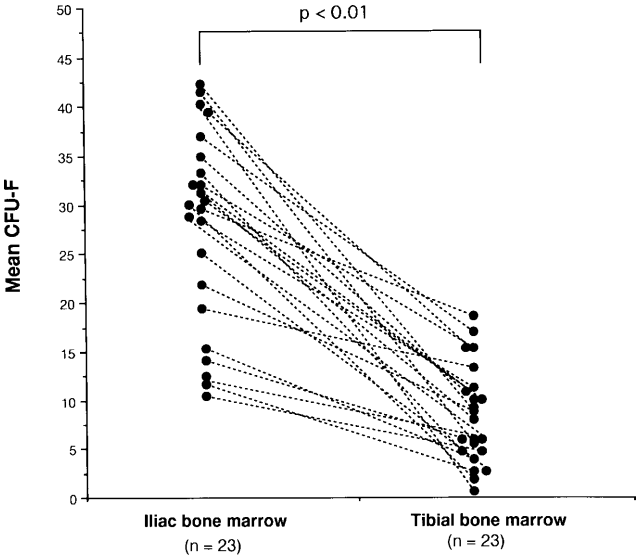

Fig. 2. Comparison of the mean number of fibroblast CFU-F per 4×10^5 bone marrow mononuclear cells in iliac and tibial marrow from each patient. Data are the mean number of CFU-F determined by triplicate assays. Data from individual patients are connected by dashed lines

phosphatate as the substrate [15]. Results were normalized for the protein content, which was determined by the bicinchoninic acid assay (Pierce, Rockford, IL, USA).

Statistical analysis

The mean number of CFU-F was compared between bone marrow from the iliac crest and the tibial epiphysis in each patient using the Wilcoxon rank sum test, and correlations between the number of CFU-F and gender or age were analyzed by calculating Pearson's correlation coefficients. ALP activity was also compared between iliac marrow and tibial marrow using the Wilcoxon rank sum test.

Results

CFU-F in tibial and iliac marrow

Table 1 presents data on the number of CFU-F and ALP activity in iliac and tibial marrow obtained from 23 patients with RA. The CFU-F (Fig. 1) were detected in all iliac marrow samples, and were also detected in all but one sample of tibial marrow. However, the number of CFU-F in tibial marrow was significantly lower than that in iliac marrow for each patient, with the mean (±SD) number being 8.2 ± 5.6 and 28.0 ± 9.8, respectively ($P < 0.01$; Fig. 2). Although the number of CFU-F in tibial marrow was not correlated with age ($r = -0.268$) or gender ($r = 0.231$), the number of CFU-F in iliac marrow was negatively correlated with age ($r = -0.547$; $P < 0.01$; Fig. 3).

ALP activity in tibial and iliac marrow

ALP activity was detected in all cultures from tibia and iliac marrow. However, ALP activity was significantly lower in tibial marrow than in iliac marrow from each patient, with the mean value being 3.7 ± 2.8 and 11.9 ± 4.9, respectively ($P < 0.01$; Table 1). The ALP activity in bone marrow cultures (iliac or tibial) was positively correlated with the number of CFU-F ($r = 0.497$; $P < 0.05$).

Discussion

To our knowledge, this is the first study that has compared the stromal cell activity of human bone marrow at two different sites in the same subjects. The results of the present study show that the prevalence of CFU-F in tibial bone marrow is similar to that in iliac bone marrow from RA patients. However, the number of CFU-F was significantly lower in tibial marrow compared with iliac marrow. In addition, the ALP assay indicated that osteogenic activity was significantly lower in tibial marrow than in iliac marrow from these patients.

Bone marrow has been shown to contain osteogenic precursor cells in many animal studies [1–5,16]. However, only a few studies have investigated CFU-F (which contain osteogenic precursor cells) in human bone marrow, and the results have varied widely [6–9]. A recent study by Muschler et al. [11] revealed a mean of 36 fibroblast colonies per 10^6 nucleated cells in human iliac bone marrow from 36 normal subjects,

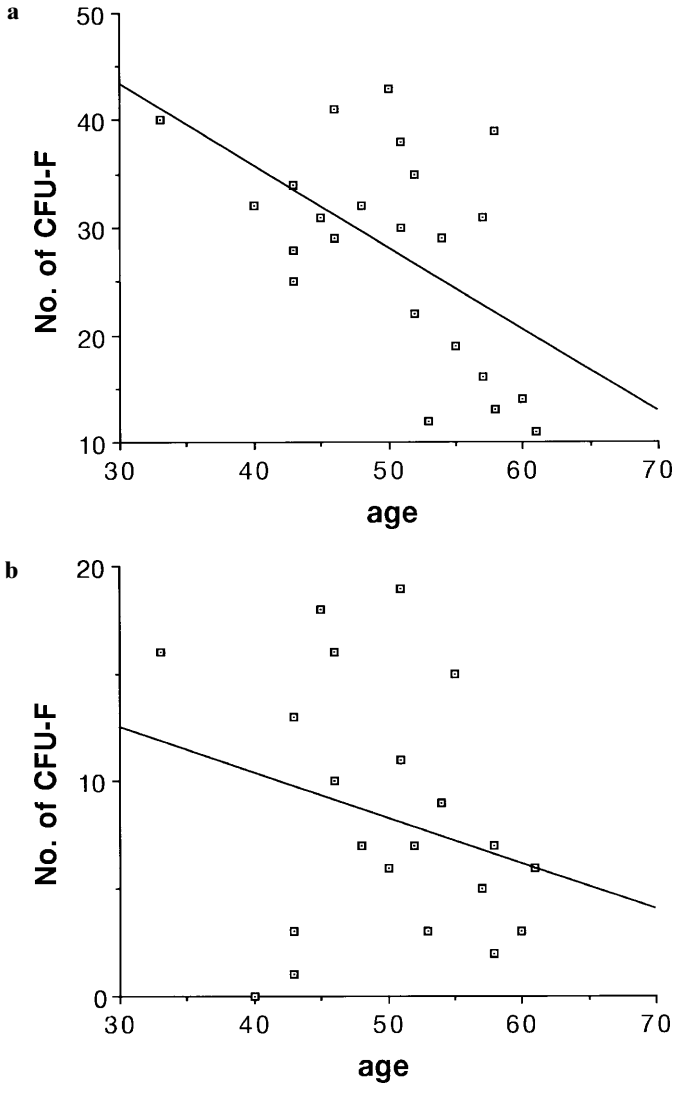

Fig. 3. Relationship between age and the number of CFU-F in bone marrow from rheumatoid arthritis patients. **a** The number of CFU-F in iliac bone marrow was significantly correlated with patient's age ($r = -0.547$; $P < 0.01$). $y = 66.342 - 0.76286x$, $R^2 = 0.300$. **b** The number of CFU-F in tibial bone marrow was not significantly correlated with patient's age ($r = -0.268$). $y = 18.808 - 0.21072x$, $R^2 = 0.071$

while Hernigou and Beaujean [12] found a mean of 19 fibroblast colonies per 10^6 bone marrow cells in marrow from 33 normal subjects. We found a mean of 28 fibroblast colonies per 4×10^5 mononuclear cells. It is impossible to directly compare results between these studies because of differences in the subject profile, culture medium, incubation time, and cell fraction. However, it appears that the number of osteogenic precursor cells in the iliac bone marrow of patients with RA is within the range reported for normal subjects. We found a significant negative correlation between the number of CFU-F and the patient's age. This finding is consistent with previous reports [11,17,18], suggesting that the tendency for a decrease in the number of osteoprogenitor cells with advancing age may occur irrespective of disease status.

It has been reported that active bone marrow is not present in any limb bone distal to the femur in normal adults [19]. In contrast, the present study detected osteogenic activity in tibial bone marrow of RA patients, although the number of CFU-F and the ALP activity were significantly lower compared with those in iliac bone marrow. We have reported previously that hematopoietic activity in the bone marrow adjacent to joints affected by RA may be influenced by several cytokines produced in nearby synovial tissues [20]. Therefore, it seems likely that stromal cell activity in the tibial epiphysis, which should support hematopoietic activity, is also promoted by these cytokines. It is not known whether the presence of osteogenic activity in tibial bone marrow is specific to RA patients, because we have not found any data in the literature on the osteogenic activity of tibial marrow from normal subjects. Therefore, we need information on the osteogenic activity at the iliac crest and tibia in normal subjects to determine whether the differential bone marrow activity shown in the present study is specific to RA. Currently, we are investigating the osteogenic activity at these two sites in patients with osteoarthritis and the results of our ongoing study may be helpful in elucidating this point. In addition, the possible effect of corticosteroids on bone marrow activity should not be ignored. However, there may be only a little effect of low-dose corticosteroids (less than 5 mg/day) on bone marrow activity. This is because the number of CFU-F in iliac bone from RA patients found in the present study is almost identical to that from normal subjects in previous reports [11,12]. Even if a systemic effect of corticosteroids on bone marrow activity is present, the present data clearly show the differential activity of stromal cells between iliac and tibial marrow in RA patients. To determine whether there is a systemic effect of corticosteroids on stromal cell activity in the bone marrow, we would need to compare the present data with findings in RA patients who are not on corticosteroid therapy.

The results of the present study suggest that inflammation of the joints affected by RA may influence the microenvironment of bone marrow near the joints, which results in less osteogenic activity compared with iliac bone marrow where inflammation does not take place. This may explain, in part, why marginal osteoporosis or atrophy adjacent to joints affeted by RA occurs frequently. In conclusion, the present study has demonstrated differential osteogenic activity between bone marrow from the iliac crest and tibial epiphysis in RA patients. Our findings may contribute to further investigations of the effects of several disease processes

on systemic, as well as local, stromal precursor cell activity associated with osteoblastic differentiation in bone marrow.

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