# Phenotypic Characteristics of Bone Marrow Cells in Patients with Rheumatoid Arthritis

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*ABSTRACT. Objective.* Our previous study showed the presence of abnormal myeloid lineage cells in the epiphyseal bone marrow adjacent to joints affected with severe rheumatoid arthritis (RA). Now, we investigated whether there were any changes of other marrow cell populations related to RA, and whether there were any pathologically characteristic changes in the iliac bone marrow, which is one of the major systemic hematopoietic organs.

***Methods.*** 2-Color flow cytometry was carried out to analyze the phenotypes of mononuclear cells (MNC) fractions in bone marrow aspirates and venous blood from 56 patients with RA and 7 non-RA controls.

***Results.*** The absolute number of MNC in the iliac bone marrow was increased by 3-fold in the RA patients compared with the non-RA controls. In contrast, no significant increase of MNC was observed in the tibial epiphyseal bone marrow or peripheral blood. The ratio of each MNC fraction in the iliac bone marrow did not differ significantly between the RA patients and the non-RA controls. In lymphocyte subsets, the percentage of HLA-DR+CD8+ cells to all CD8 cells in the iliac bone marrow increased significantly in the RA patients compared with the non-RA controls. Abnormal myeloid cells (MX-GA+MY4+ cells), specific to severe RA, were found to be more concentrated in the iliac bone marrow than in the tibial epiphyseal bone marrow.

***Conclusion.*** Characteristic pathologic changes of the iliac bone marrow suggest an important role of systemic bone marrow in the progression of RA. (*J Rheumatol 1994;***21:1608–14**)

*Key Indexing Terms:*
RHEUMATOID ARTHRITIS
BONE MARROW
MYELOID LINEAGE CELLS

Preceding the induction of polyarthritis, maturation and proliferation of bone marrow cells were observed, and interleukin 1 (IL-1) and IL-6 levels in bone marrow serum were elevated in collagen induced arthritis and adjuvant arthritis in rats[1-3]. These pathological changes were maintained while arthritis continued. The results suggested that bone marrow plays an important role in inducing polyarthritis.

Our studies have demonstrated the existence of abnormal myeloid cells, which strongly express the difucosyl or trifucosyl type 2 chain (dimetric or trimetric $Le^x$, a specific marker of human undifferentiated cells[4,5]), in the epiphyseal bone marrow adjacent to joints affected with active severe subset of RA[6]. These myeloid lineage cells, which accumulate in the epiphyseal bone marrow in various stages of maturity, were found to exist in severe and active subset of RA. However, these cells were not found in the epiphyseal bone marrow of normal controls, nor in patients with mild RA, osteoarthritis or infectious arthritis. Thus, these myeloid lineage cells appear to be specific for severe RA. Maintaining these cells *in vitro* could not be achieved using commercially available culture medium, but was possible when epiphyseal bone marrow serum from patients with severe RA was added to the medium[7]. These results show the characteristic changes in epiphyseal bone marrow in RA. Recently other investigators have also reported abnormalities in bone marrow in patients with RA[8-10].

These results left us with 2 major questions to be answered: (1) are there any changes in other bone marrow cell populations related to RA and (2) are there any characteristic pathological changes of the iliac bone marrow, which is one of the major sites of systemic hematopoietic organs.

To answer these questions, we studied bone marrow blood cells from a large number of patients with RA. Our test subjects were obtained from the iliac crest and the tibial proxymal epiphysis at the time of surgical procedures.

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## MATERIALS AND METHODS

*Subjects.* Bone marrow blood samples and peripheral blood samples were obtained from 56 patients with RA (51 women, 5 men) who met American College of Rheumatology criteria[11], and 7 healthy volunteers (non-RA controls) (4 women, 3 men) after informed consent was obtained. All the patients were operated on at Osaka University Hospital or the related facilities from 1990 to 1993. The average age of the RA patients and the non-RA controls was 55 and 40 years, respectively (range: 30–75, and 26–55, respectively). The average duration of the disease was 9.3 years (range: 3–30). According to our reported criteria[12,13], we classified patients with RA into 3 disease subsets; the subset with least erosive disease (LES), the subset with more erosive disease (MES) and the subset with mutilating disease (MUD). In the LES, erosive articular changes were primarily limited to the smaller peripheral joints. In the MES, the larger axial joints were also involved. In the most severely affected subset (MUD), which involves mutilating disease, almost all joints were extensively damaged. There were 17 patients in LES, 26 in MES, and 13 in MUD, respectively.

*Cell preparations.* At the studies of bone marrow cells, peripheral blood contamination of aspirates is thought to be inevitable[14,15], so a preliminary experiment was carried out. We collected iliac bone marrow aspirates in 5 ml fractions up to 30 ml and measured the number of myeloid cells to determine the level of contamination. When the volume aspirated from the iliac bone marrow exceeded 20 ml, the number of myeloid cells began to decrease and a significant difference was recognized due to contamination by peripheral blood (data not shown). Thus, the first 5 ml of bone marrow aspirate could be considered to most closely reflect the number of cells in the iliac bone marrow. So in our study, the first 5 ml of bone marrow aspirate was used for analysis.

Five ml of heparinized bone marrow aspirate was obtained from the iliac crest and the tibial proximal epiphysis by needle puncture at the time of operation. A 5 ml sample of heparinized venous blood was obtained simultaneously. MNC fraction, from bone marrow aspirate and venous blood, were separated by Ficoll-Hypaque density gradient (1.077 g/ml) centrifugation. In MNC fraction of the bone marrow aspirates, a lot of premature cells were contained depending on their density.

*Cell staining.* For FACS analysis, the cells were washed twice with phosphate buffered saline (PBS) and adjusted to $10^6$/ml in RPMI 1640. Then 100 $\mu$l of this cell suspension was exposed to 5 $\mu$l of antibody (T4, T8, MY4, Leu11c, B1-RD1), or 10 $\mu$l of antibody (MX-GA, anti-HLA-DR) for 30 min at 4°C. The cells were then washed 3 times in PBS and fixed with 1% formaldehyde in PBS.

*Monoclonal antibodies.* The monoclonal antibodies (Mab) used were fluorescein-conjugated T4 (CD4), T8 (CD8), B1-RD1 (CD20) (all from Coulter Immunology Hialeah, FL) and MX-GA (CD15, clone HL5) (from Kyowa Medix, Japan), and phycoerythrin conjugated Leu11c (CD16), anti-HLA-DR (both from Becton Dickinson Mountain View, CA) and MY4 (CD14) (from Coulter Immunology Hialeah, FL).

*2-Color flow cytometry.* 2-Color flow cytometric analysis was performed using a FACScan (Becton Dickinson Mountain View, CA) equipped with an argon laser at 488 nm. All specimens were analyzed on the day of collection. To exclude debris or dead cells, the cells were gated on the basis of forward and right angle scatter. Each test employed 20,000 MNC and the number of positive cells was expressed as a percentage of the total cell count.

*Morphologic studies.* For light microscopy, sorted cells suspensions at $10^6$/ml in RPMI 1640 were cytospun in 50 $\mu$l aliquots onto glass slides for 5 min at 6000 rpm (Shandon Cheshire, England) and stained by the May-Giemza, peroxidase and specific esterase method.

*Statistical analysis.* Results are expressed as the mean ± standard error of the mean. Data were analyzed by the Mann-Whitney U test. P values less than 0.05 were considered significant.

## RESULTS

*Number of mononuclear cells.* The numbers of MNC fraction were measured and the data are shown in Table 1. There was a marked increase in the absolute number of MNC in the iliac bone marrow from patients with RA compared with the non-RA controls. The means (± SEM) of the number of MNC were 3122 ± 225 for patients with RA and 1245 ± 311 for the non-RA controls ($p < 0.01$). The number of MNC was increased according to the severity of RA[12] ($p < 0.05$ for LES and $p < 0.01$ for MES and MUD vs non-RA controls) (Figure 1). In contrast, the absolute number of MNC in the tibial bone marrow and peripheral blood did not differ significantly between the patients with RA and the non-RA controls.

*Lymphocyte subsets.* We analyzed the ratio of the lymphocyte population by FACScan using CD4 and CD8 Mab as

Table 1. *Cell marker studies on bone marrow aspirates and peripheral blood from 56 patients with RA and 7 non-RA controls*

| | Peripheral Blood | | Iliac Bone Marrow | | Tibial Bone Marrow | |
|---|---|---|---|---|---|---|
| | Controls | RA | Controls | RA | Controls | RA |
| No. of MNC/mm³ | 974±167 | 1401±162 | 1245±311 | 3122±225* | 952±138 | 1625±212 |
| Myeloid cells | | | | | | |
| % CD15+CD16− | 2.9±0.5 | 6.5±1.5 | 19.0±3.5 | 24.4±1.7 | 6.8±2.0 | 5.8±0.9 |
| T cells | | | | | | |
| % CD4 | 43.2±3.3 | 29.5±1.8** | 17.4±3.3 | 17.6±1.1 | 15.7±6.9 | 28.0±1.7 |
| % CD8 | 25.2±2.4 | 21.2±1.6 | 20.9±4.4 | 15.1±0.9 | 15.1±3.6 | 23.3±1.5 |
| % DR+CD4+/CD4 | 10.9±1.8 | 14.1±1.5 | 12.7±3.4 | 18.7±1.6 | 15.4±2.1 | 15.3±1.4 |
| % DR+CD8+/CD8 | 13.7±2.8 | 27.7±2.1** | 14.1±4.1 | 33.2±2.3** | 13.1±2.4 | 28.8±2.7** |
| CD4/CD8 ratio | 1.84±0.34 | 1.71±0.17 | 0.91±0.1 | 1.2±0.1 | 0.95±0.20 | 1.30±0.08 |
| B cells | | | | | | |
| % CD20 | 10.4±2.7 | 10.3±1.0 | 10.8±2.4 | 9.8±0.8 | 9.3±3.2 | 13.4±1.8 |
| NK cells | | | | | | |
| %CD16 | 17.3±2.2 | 15.7±1.6 | 10.8±2.3 | 8.2±0.7 | 8.9±2.0 | 16.3±1.5 |

Results are expressed as mean ± SEM.

* $p < 0.01$ as compared with the non-RA controls.

** $p < 0.05$ as compared with the non-RA controls.

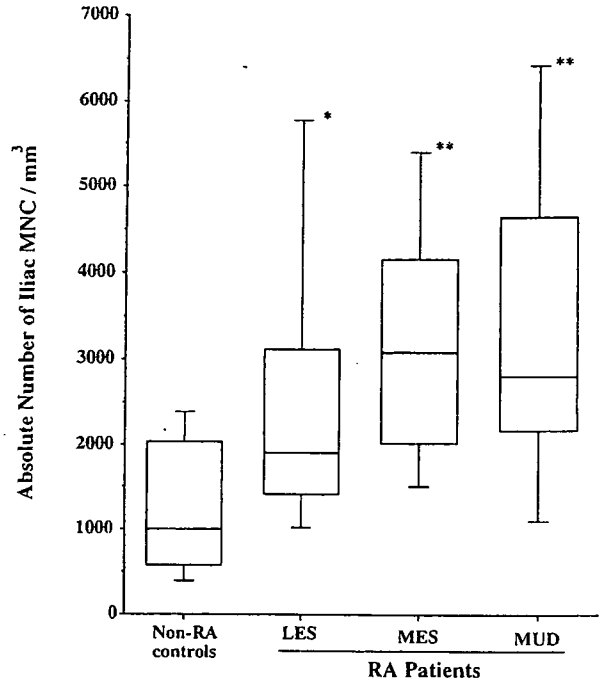


*Fig. 1.* The absolute number of iliac bone marrow mononuclear cells in non-RA controls and patients with RA. Patients with RA are classified into 3 disease subsets: the subset with least erosive disease (LES), the subset with more erosive disease (MES), and the subset with mutilating disease (MUD). The line in the middle of the box represents the median. The top of the box represents the 75th percentile, and the bottom of the box the 25th percentile. *p <0.05 and **p <0.01 as compared with the non-RA controls.

T cell markers, CD20 as a B cell marker and CD16 as a natural killer cell marker (Table 1). In the iliac bone marrow of patients with RA, the percentages of lymphocytes subsets did not differ significantly from the non-RA controls, but the number of MNC was increased 3-fold compared with the non-RA controls as mentioned above. The epiphyseal bone marrow, on the other hand, showed the similar number and percentage of lymphocytes subsets to those of peripheral blood, and are thought to reflect fundamentally the peripheral circulation. The peripheral blood of patients with RA demonstrated characteristic decrease of CD4 cells compared with the non-RA controls ($p < 0.05$). As to CD4/CD8 ratio there was no significant difference between patients with RA and the non-RA controls in the iliac bone marrow, the tibial bone marrow and the peripheral blood. In general, there were no significant differences in the percentage of lymphocytes in the iliac bone marrow between the patients with RA and the non-RA controls.

*HLA-DR positive lymphocytes.* We analyzed the percentage of HLA-DR+CD4 and CD8 cells among the total population of CD4 or CD8 cells (Table 1). The percentage of HLA-DR+CD4 cells with respect to all CD4 cells did not differ significantly between patients with RA and the non-RA controls in the iliac bone marrow and peripheral blood. In contrast, the percentage of HLA-DR+CD8 cells to all CD8 cells differed significantly between patients with RA and the non-RA controls in the iliac and tibial bone marrow and peripheral blood ($p < 0.05$). To investigate HLA-DR positive lymphocytes in more detail, we classified the patients with RA into 3 disease subsets[12] and compared each subset with the non-RA controls. The percentage of HLA-DR+CD8 cells to all CD8 cells in the iliac bone marrow was increased significantly in LES and MES when compared with the non-RA controls ($p < 0.05$), whereas the percentage of HLA-DR+CD4 cells to all CD4 cells did not differ significantly between each RA subset and the non-RA controls (Figure 2). Similar differences were observed in the peripheral blood and the tibial bone marrow. In the most severely affected

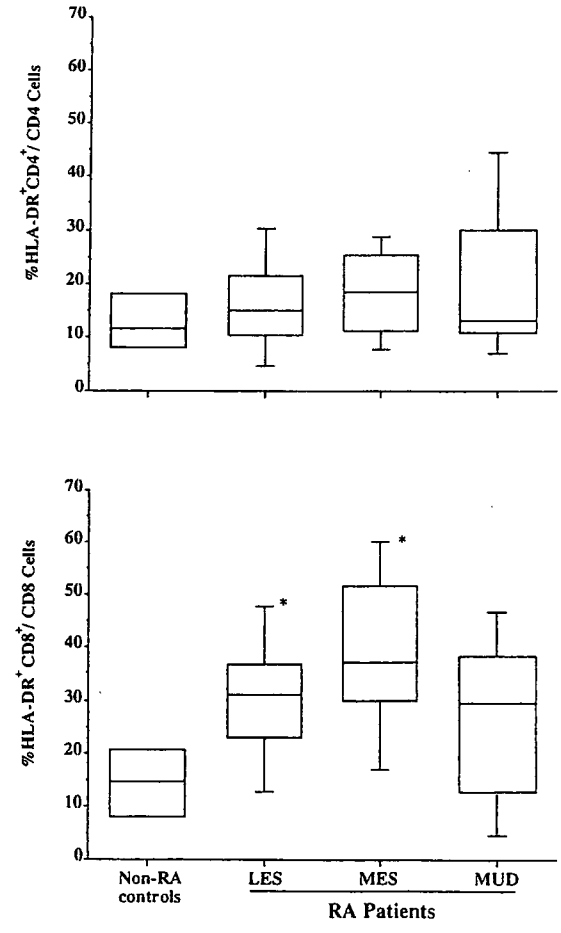


*Fig. 2.* HLA-DR antigen expression by CD4 and CD8 cells from the iliac bone marrow of the non-RA controls and patients with RA. Patients with RA were as classified in Figure 1. The line in the middle of the box represents the median. The top of the box represents the 75th percentile, and the bottom of the box the 25th percentile. *p <0.05 as compared with the non-RA controls.

patients, MUD, the percentage of HLA-DR+CD8 cells to all CD8 cells varied so greatly that no significant differences could be determined when compared with the non-RA controls.

*Myeloid cell population.* Myeloid cells were analyzed using the MX-GA (CD15) antibody which detects a broad range of myeloid lineage cells from myeloblasts to polymorphonuclear cells[16,17], and using CD16 which detects mature granulocytes (PMN) and natural killer (NK) cells. In short CD15+CD16− cells were composed of undifferentiated pregranulocyte cells. The CD15+CD16− cells were sorted and cytospun onto glass slides, and stained by the May-Giemza, peroxidase and specific esterase methods. Light microscopy showed that the CD15+CD16− cells were positive for peroxidase and specific esterase staining, and these cells were found to be promyelocytes and metamyelocytes (data not shown). Thus, CD15+CD16− cells were considered to be panmyelocytes.

In the iliac bone marrow, the percentage of myeloid cells among the total MNC fraction showed a clear tendency to increase in patients with RA (Table 1). In addition, the absolute number (/mm³) of myeloid cells in patients with RA in the 3 disease subsets, differed significantly from the number in the non-RA controls (LES: $p < 0.05$, MES and MUD: $p < 0.01$) (Figure 3).

*Abnormal myeloid cells in bone marrow.* We tried to analyze the systemic existence of the abnormal myeloid cells found in the involved epiphyseal bone marrow of RA[6]. A preliminary study employing the various monoclonal antibodies showed that abnormal and normal myeloid cells could be well separated using the MY4 (CD14) monoclonal antibody, which reacted with abnormal myeloid cells as well as monocyte-macrophages, but not with normal myeloid cells. In the tibial epiphyseal bone marrow of severe RA (MES and MUD), myeloid cells could be sometimes observed microscopically, and all these cells were recognized as abnormal MY4+ by FACS analysis. On the other hand, no myeloid cells could be found in the tibial epiphyseal bone marrow of the normal controls or LES. It was characteristic that myeloid cells in the iliac bone marrow could be separated into MX-GA+MY4− cells and MX-GA+MY4+ (abnormal) cells in severe RA (MES and MUD). But in the non-RA controls or LES, all myeloid cells in the iliac bone marrow were MX-GA+MY4− (Figure 4). To make clear the population of MX-GA+MY4+ cells in the iliac bone marrow, 2-color flow cytometric analysis was carried out. The purified population of MX-GA+MY4+ cells was selected by sorting, and was examined using cytospin preparations and histochemical stains. As shown in Figure 5, these cells were confirmed to be of myeloid lineage, but not monocyte lineage. In FACS analysis, contamination of counting error must be taken into consideration, and in this study the average background level was 4%. As shown in Table 2, in the tibial epiphyseal bone marrow, the mean percentage (± SEM) of abnormal myeloid cells (MX-GA+MY4+ cells) was almost the same as the background level. There was no significant difference in the patients with severe RA (MES and MUD) when compared with the non-RA controls, although several individual patients in MES and MUD had up to 11.8 and 13.7% of these cells, respectively. So in such cases the abnormal myeloid cell population was recognized. In the iliac bone marrow of patients with more severe RA (MES and MUD), this abnormal myeloid cell population was observed more clearly. In particular, the mean absolute number ± SEM (/mm³) of these cells in the iliac bone marrow showed a significant difference compared with the non-RA controls ($p < 0.05$). The results are summarized in Table 2.

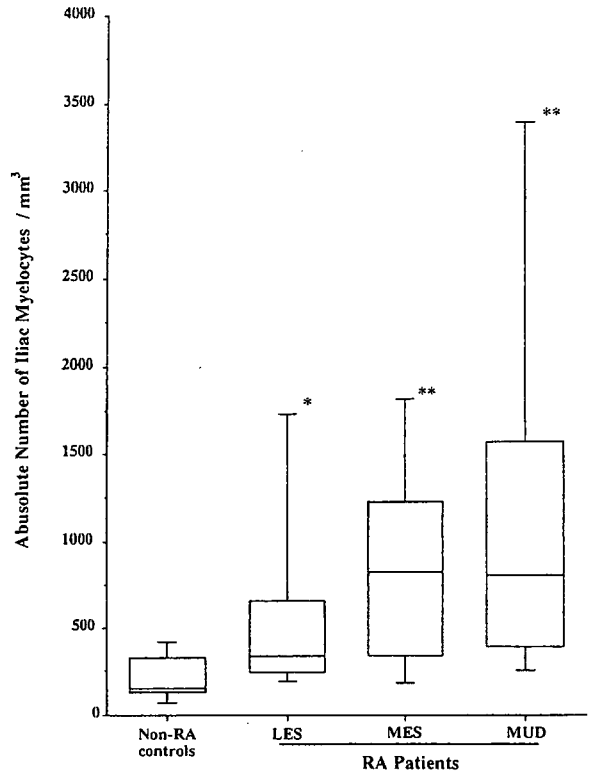


*Fig. 3.* The absolute number of iliac bone marrow myelocytes (CD15+CD16− cells) in the non-RA controls and patients with RA. Patients with RA were classified as in Figure 1. The line in the middle of the box represents the median. The top of the box represents the 75th percentile, and the bottom of the box the 25th percentile. *p <0.05 and **p <0.01 as compared with the non-RA controls.

## DISCUSSION

Our first major question was whether there were any cellular changes in the iliac bone marrow which is one of the major systemic hematopoietic organs in adults. In infants and children, active hematopoiesis takes place in most of the marrow cavities, including the distal long bones. At 5–7 years of age, fat cells begin to replace the hematopoietic marrow in the extremities; in normal adults, hematopoiesis is con-

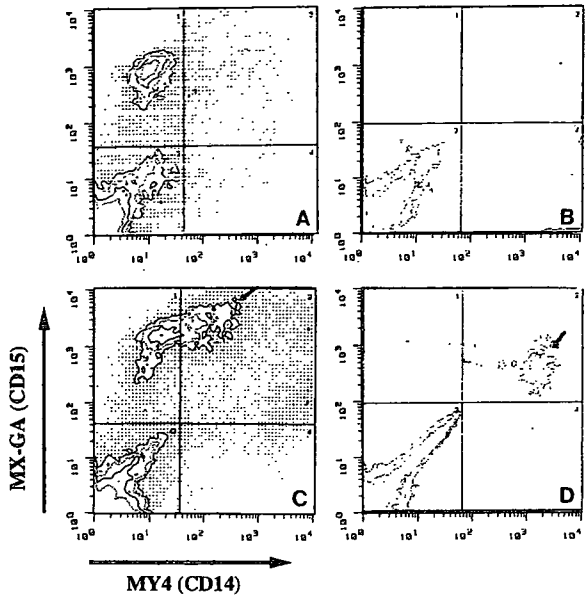


*Fig. 4.* 2-Color flow cytometric analysis of a normal donor and a patient with severe RA. Panel A: Cells in the iliac bone marrow of a normal donor. Panel B: Cells in the tibial epiphyseal bone marrow of a normal donor. Panel C: Cells in the iliac bone marrow of a patient with severe RA. Panel D: Cells in the tibial epiphyseal bone marrow of a patient with severe RA. In the iliac bone marrow of a normal donor, one myeloid cell population (MX-GA+MY4− cells) was recognized. In the tibial epiphyseal bone marrow, no myeloid cell population was recognized. In contrast, in the iliac bone marrow of a patient with severe RA, two myeloid cell populations (MX-GA+MY4− cells and MX-GA+MY4+ cells) were recognized. And in the tibial epiphyseal bone marrow, an abnormal cell population (MX-GA+MY4+ cells) was recognized. Arrows indicate the abnormal myeloid cell population (MX-GA+MY4+ cells).

fined to the vertebrae, ribs, sternum, pelvis, scapulae, skull, and the extreme proximal portions of the humeri and femora[18,19]. Thus, the tibial epiphyseal bone marrow is not hematopoietic in adults. We chose the iliac bone marrow to investigate hematopoietic marrow in the present study.

The absolute number of MNC (/mm³) in the iliac bone marrow of patients with RA was increased up to 3-fold compared with the non-RA controls, although the relative proportions of each MNC fraction in the iliac bone marrow was similar between patients with RA and non-RA controls. As there was no significant difference among 3 disease subsets, the increase of MNC was recognized as general to the iliac bone marrow of patients with RA, regardless of the severity of bone and joints destruction. Since the peripheral blood white cell count was not significantly increased in the patients with RA compared with the non-RA controls, the rapid turnover and functional enhancement of white blood cells in peripheral organs of patients with RA was suggested.

One of the characteristic findings in the iliac bone marrow of patients with RA was a significant enhancement of T cell activation. Although the percentage of CD8 cells among MNC was not statistically different between patients with RA and the non-RA controls, the percentage of HLA-DR+CD8 cells to all CD8 cells was significantly higher in both the iliac bone marrow and peripheral blood of patients with LES and MES, but not with MUD. The reason for this is unclear at present, but there could be some fundamental differences of the iliac bone marrow that are unique to MUD. Similar changes were also recognized in peripheral blood but were thought to reflect changes in the iliac bone marrow. The ratio of CD4 cells, on the other hand, was significantly decreased in the peripheral blood as also shown by

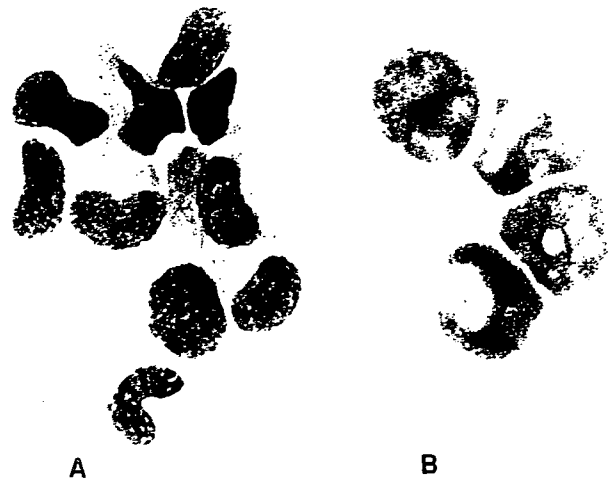


*Fig. 5.* Histochemical staining of purified MX-GA+MY4+ cells. MX-GA+MY4+ cells were stained by the esterase (A) and peroxidase (B) methods (magnification × 800). The morphological findings showed that these MX-GA+MY4+ cells were of the myeloid lineage.

Table 2. *Abnormal myelocytes (MX-GA+MY4+ myelocytes) in iliac and tibial bone marrow*

| | n | Percentage in Tibial BM | Percentage in Iliac BM | Absolute Number in Tibial BM (/mm³) | Absolute Number in Iliac BM (/mm³) |
|---|---|---|---|---|---|
| Non-RA controls | 7 | 3.7±0.9 (1.5–7.0) | 4.4±0.6 (1.8–7.3) | 41 ± 13 | 86 ± 26 |
| LES | 17 | 2.7±0.5 (0.6–7.0) | 3.6±0.5 (0.4–8.0) | 33 ± 3 | 96 ± 18 |
| MES | 26 | 4.0±0.6 (0.9–11.8) | 6.4±1.3 (1.5–26.0) | 102 ± 37 | 246 ± 79* |
| MUD | 13 | 4.3±2.0 (1.0–13.7) | 7.9±2.3 (1.8–29.5) | 94 ± 74 | 219 ± 65** |

Values are the mean ± SEM. Values in parentheses indicate the range. BM: bone marrow.
* $p < 0.05$ vs MES and the non-RA controls. ** $p < 0.05$ vs MUD and the non-RA controls.

prior studies[20,21]. However, the ratio of these cells in the iliac bone marrow was not significantly different to that in the non-RA controls. This could be a result of rapid turnover, and is probably related to the activation of CD4 cells in the peripheral blood. Although the precise pathological mechanisms are not yet known, the activation of T cell subsets could induce various immunologic enhancements such as the elevation of cytokines in the iliac bone marrow. For example, the elevated levels of IL-6 and IL-8 in the iliac bone marrow serum may be related to the remarkable synovial proliferation in multiple joints which is frequently seen in LES and MES[22].

Another characteristic finding in the iliac bone marrow of patients with RA was the induction of abnormal myeloid cells, which were previously reported in epiphyseal bone marrow[6]. In our preliminary study, these cells were clearly distinguished from normal myeloid cells by their antigenic reactivities (MX-GA+MY4+ as well as mono-Lex+di-Lex+ as reported[6]). Because of availability of Mab, in our study these abnormal myeloid cells were defined by staining with MX-GA and MY4. MX-GA+MY4+ cells showed a significant increase in the iliac bone marrow of patients in MES and MUD compared with baseline value found in the non-RA controls. In the tibial epiphyseal bone marrow, there was no significant difference in the percentage or cell number of MX-GA+MY4+ cells between severe RA and the normal controls. This is because the number of these abnormal cells in the epiphyseal bone marrow were reduced during the remission period of RA. In several active cases, however, abnormal myeloid cells were present at up to 11.8% in MES, and 13.7% in MUD, respectively. Thus, abnormal myeloid cells occurred to a greater extent in the iliac bone marrow than in the tibial epiphyseal bone marrow, and was a characteristic feature of MES and MUD. Although the precise physiology and mechanisms involved are still under study, we have reported that polymorphonuclear neutrophils (PMN; the final differentiation stage of myeloids) with a special activity for tissue injury such as PMN factor activity[23,24] and high IL-1 levels[25] were detected in the bone marrow of patients with severe RA (MES and MUD) where those abnormal myeloid cells accumulated.

We have previously analyzed the natural course of RA based on the extent of joint destruction, and reported the existence of 3 disease subsets of RA; LES, MES and MUD[12,13]. We classified these disease subsets according to the number of joints with erosion, serum Clq levels and the annual reduction of the ratio of carpal height[26]. In rough analysis, we found inflammatory variables [C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and rheumatoid factor (RF)] were persistently remarkably high for at least 5 years after the onset of disease in MES and MUD. Among these inflammatory parameters, CRP levels were persistently above 4 mg/dl within 5 years of onset in MES and MUD, but were usually less than 2 mg/dl in LES. The present study suggested that these clinically defined 3 disease subsets showed significantly different tendencies in the iliac bone marrow cells. That is to say, the increase of HLA-DR+CD8+ T cells in the iliac bone marrow occurs in LES and MES, while the increase of MX-GA+MY4+ cells is characteristic of more severe disease subsets of RA (MES or MUD) (Figure 6). These results suggest that differences at the cellular level affect the natural courses of RA.

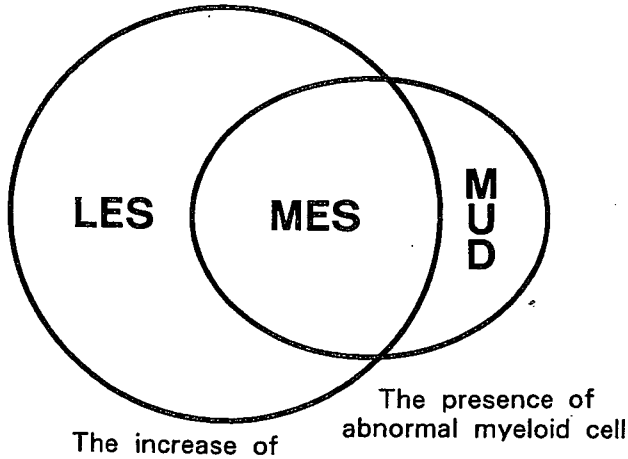


*Fig. 6.* Characteristic changes of iliac bone marrow cells in each RA disease subset. The increase of the percentage of HLA-DR+CD8 cells to all CD8 cells was recognized in LES and MES, and the presence of abnormal myeloid cells was characteristic to MES and MUD.

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# Remarkable Elevation of Interleukin 6 and Interleukin 8 Levels in the Bone Marrow Serum of Patients with Rheumatoid Arthritis

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*ABSTRACT.* ***Objective.*** Characteristic cellular changes have previously been reported in the bone marrow of patients with rheumatoid arthritis (RA). We investigated the levels of various cytokines in RA bone marrow.
***Methods.*** We studied 25 patients with RA (22 women and 3 men) and 10 trauma patients (7 women and 3 men) as non-RA controls. Twelve kinds of cytokines [interleukin (IL)-1α, IL-1ß, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, granulocyte colony stimulating factor, granulocyte/macrophage colony stimulating factor, tumor necrosis factor (TNF)-α, and TNF-ß] were assayed by ELISA in iliac bone marrow serum (BMS), tibial BMS, and peripheral blood serum.
***Results.*** Markedly elevated levels of IL-6 and IL-8 were detected in iliac BMS, and much lower levels were found in tibial bone marrow and peripheral blood serum. The levels of IL-6 and IL-8 in iliac BMS showed a close relationship to the extent of synovial proliferation.
***Conclusion.*** Iliac bone marrow may be an important site for the production or accumulation of IL-6 and IL-8 in RA, and these cytokines may influence synovial proliferation in patients with polyarthritis.
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*Key Indexing Terms:*
BONE MARROW SERUM CYTOKINE RHEUMATOID ARTHRITIS

Earlier we investigated the epiphyseal bone marrow of patients with rheumatoid arthritis (RA) and found abnormal myeloid cells that are possibly related to tissue injury in severe destructive RA[1]. More recently, other characteristic cellular changes of the bone marrow have been reported[2-5]. These findings suggest that the bone marrow cells may play an important role in inducing and/or promoting polyarthritis in patients with RA. In the bone marrow of rats with collagen induced arthritis and adjuvant arthritis, we found that the levels of interleukin (IL)-1α and IL-6 were elevated along with cellular changes[6]. An increase of these cytokines to high levels preceded the induction of arthritis and persisted while the arthritis lasted. Thus, the bone marrow of patients with RA may show changes in soluble factors as well as cellular components.

Many investigators have described the immunological effects of various soluble factors in patients with RA, including IL-1ß, IL-6, IL-8, and granulocyte/macrophage colony stimulating factor (GM-CSF). These cytokines are believed to be involved in pathogenesis of RA by stimulating synovial proliferation[7], inflammatory cell infiltration[8,9], acute phase protein synthesis[10], and articular cartilage destruction[11-13]. Cellular changes and elevated cytokine levels have been detected in the inflamed synovial tissue and synovial fluid (SF) of RA[14-17]. However, during operations on the joints of patients with severe RA, we sometimes observe that there is little SF accumulation and/or synovial tissue proliferation. Accordingly, we considered the possibility that the bone marrow of patients with RA may also be a site of cytokine production, and thus we investigated the bone marrow levels of various soluble factors.

## MATERIALS AND METHODS

*Patients.* The subjects were 25 patients with RA who satisfied the diagnostic criteria for RA of the American College of Rheumatology[18], and underwent reconstructive surgery at Osaka University Hospital or related hospitals. At the time of surgery, both iliac bone marrow blood and peripheral blood were sampled from all 25 patients and the cytokine levels were measured. Cytokine levels of tibial bone marrow blood could be measured in 15 of these 25 patients. There were 22 women and 3 men with a mean age of 54.7 years (range: 40–70 years) and a mean disease duration of RA of 9.2 years (range: 1–30 years). All the patients had been treated with disease modifying antirheumatic drugs (minimum; for 1 to 9 years), but they still required reconstructive operations for joint destruction. Bone marrow blood was obtained only after the patients gave informed consent. The operated joints were 20 knees, 3 elbows, 4 hands, 1 shoulder, and 1 hip. As

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non-RA controls, we also studied 10 trauma patients who had no systemic inflammatory disease or immune abnormalities. They included 7 women and 3 men with a mean age of 56.7 years (range: 42–75 years).

*Serum samples.* For bone marrow blood samples, we collected 5 ml of nonheparinized blood from the iliac crest and from the proximal tibial metaphysis by needle puncture at the time of operation. At the same time, we collected nonheparinized peripheral venous blood by venipuncture. To obtain the maximum volume of serum, samples were let stand at 37°C for 60 min and then at 4°C for 60 min until the blood was thoroughly clotted. Centrifugation was then done at 1600 × G for 30 min to separate the supernatant, which was stored at −80°C until measurement. A preliminary study confirmed that the levels of cytokines showed no significant difference between blood samples that were processed rapidly or let stand at 37°C for 60 min.

*Quantitation of cytokines.* The serum levels of 12 different cytokines [IL-1α, IL-1ß, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, granulocyte colony stimulating factor (G-CSF), GM-CSF, tumor necrosis factor (TNF)-α, and TNF-ß] were measured by ELISA (ELISA kit: Quantikine, R&D System), and the concentrations were determined in pg/ml.

*Activity of synovitis.* At the time of operation, we observed the operated joint and the extent of synovial proliferation was roughly evaluated and classified from grade 0 to 3. Grade 0 indicated no synovial proliferation, while Grade 1 was mild synovitis limited to less than 20% of the joint cavity. Grade 2 indicated moderate synovitis affecting less than half of the joint cavity, and Grade 3 indicated severe synovitis affecting more than half of the joint cavity. There were 6 grade 3 cases, 9 grade 2 cases, 11 grade 1 cases and no grade 0 cases. In addition, 6 patients were unclassified. All of the non-RA controls had grade 0 synovitis.

*Laboratory variables.* Blood was collected just before the operation and the red blood cell (RBC) count, hemoglobin (Hb) level, white blood cell (WBC) count, % neutrophils (%Neu), and platelet (Plt) count were measured. In addition, the erythrocyte sedimentation rate (ESR), and the levels of rheumatoid factor (RF), immunoglobulin G (IgG), IgA, IgM, and C-reactive protein (CRP) were measured, and the relationships of all these variables with each of the cytokines were studied.

*Statistical analysis.* Spearman's rank test was used for determining the correlations between the extent of synovitis and the various laboratory variables, while the Wilcoxon signed rank test was used to assess the significance of differences.

## RESULTS

*Cytokine levels in bone marrow serum.* As shown in Figure 1, only the IL-6, IL-8, and G-CSF levels were elevated in bone marrow serum (BMS), except in 3 patients who also had raised IL-1α levels. The other cytokines were close to or below the assay detection limit. Iliac BMS contained IL-6 in 8 of the 25 patients with RA (range: 14–820 pg/ml; median: 46.5 pg/ml), IL-8 in 23 patients (range: 45–7890 pg/ml; median: 200 pg/ml) and G-CSF in 8 patients (range: 17–4200 pg/ml; median: 161 pg/ml) (Figure 1, Table 1). Among the 10 non-RA controls one patient showed a low level of IL-6 (23 pg/ml), and another showed a low level of IL-8 (30 pg/ml). Tibial BMS contained IL-6 in 8 of the 15 patients with RA tested (range: 14–45 pg/ml; median: 18.25 pg/ml). In addition, IL-8 was detected in 9 patients (range 35.5–2300 pg/ml; median: 140.5 pg/ml), and G-CSF

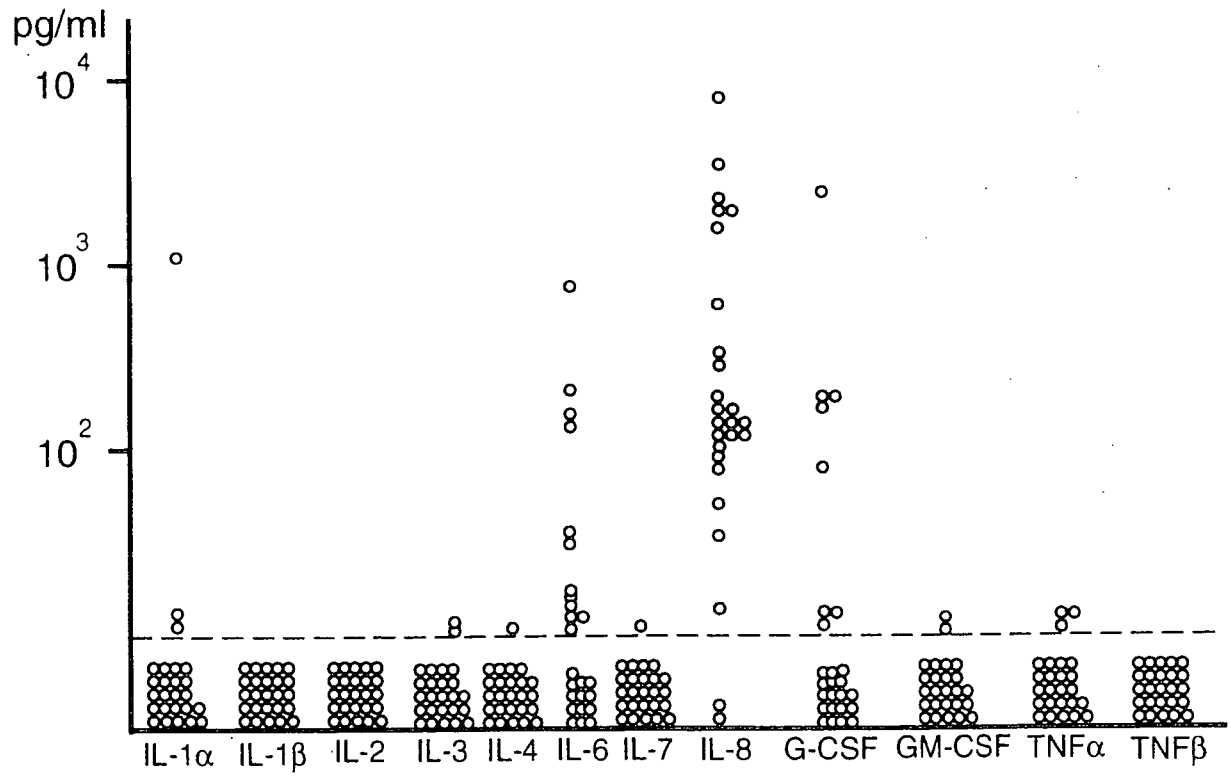


*Fig. 1.* Levels of IL-1α, IL-1ß, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, G-CSF, GM-CSF, TNF-α, and TNF-ß in bone marrow supernatant from patients with RA. Bone marrow supernatant was obtained as described in Materials and Methods. Cytokine levels were determined with ELISA kits. Values less than the lower limit of detection for each assay are shown beneath the broken line.

Table 1. *Cytokine levels of IL-6, IL-8, and G-CSF in the patients with RA and controls*

| | IL-6 | | | IL-8 | | | G-CSF | | |
|---|---|---|---|---|---|---|---|---|---|
| | Tibial BMS | Iliac BMS | PBS | Tibial BMS | Iliac BMS | PBS | Tibial BMS | Iliac BMS | PBS |
| RA | | | | | | | | | |
| Number of patients | 8/15 | 8/25 | 5/25 | 9/15 | 23/25 | 15/25 | 4/15 | 8/25 | 8/25 |
| Range (pg/ml) | 14–45 | 14–820 | 12.5–75 | 35.5–2300 | 45–7890 | 55–1150 | 53.5–10,200 | 17–4200 | 34–2370 |
| Median (pg/ml) | 18.25 | 46.5 | 28.5 | 140.5 | 200 | 270 | 455 | 161 | 113.5 |
| Mean (pg/ml) | 24.3 | 158.9 | 34.6 | 629.5 | 1167.6 | 458.2 | 2790.9 | 642.4 | 654.4 |
| | | *# | | | **## | | | | |
| | | ** | | | | | | | |
| Control | | | | | | | | | |
| Number of patients | | 0/10 | 1/10 | | 1/10 | 1/10 | | 0/10 | 0/10 |
| Range (pg/ml) | | | 23 | | 42 | 42 | | | |
| Median (pg/ml) | | | 23 | | 42 | 42 | | | |

*$p < 0.05$ and ** $p < 0.01$ (Spearman's rank correlation coefficient).
# $p < 0.05$ and ## $p < 0.01$ (Wilcoxon signed rank test).

was found in 4 patients (range: 53.5–10200 pg/ml; median: 455 pg/ml).

*Relationship between IL-6, IL-8, and G-CSF in bone marrow serum.* We examined whether there were any statistical correlations between the levels of IL-6, IL-8, and G-CSF. The IL-6 level in iliac BMS was closely correlated with the IL-8 level ($p < 0.01$) (Table 1), but the G-CSF was not correlated with either the IL-6 or IL-8 level. In tibial bone marrow, there was no correlation between the levels of IL-6, IL-8, and G-CSF. In addition, there was no correlation between the IL-6, IL-8, and G-CSF levels in iliac bone marrow and those in tibial bone marrow.

*Relationships between IL-6, IL-8, and G-CSF in BMS and peripheral blood.* The cytokine levels in iliac bone marrow were compared with those in peripheral blood (Figure 2, Table 1). IL-6 was detected in the iliac BMS of 8 patients with RA, the tibial BMS of 8 patients, and the peripheral blood serum (PBS) of 5 patients, with the maximum levels being 820 pg/ml, 45 pg/ml, and 75 pg/ml, respectively. In 6 of 8 patients, the iliac BMS levels were the highest among these 3 samples. The median IL-6 levels were 46.5, 18.25 and 28.5 pg/ml in iliac BMS, tibial BMS, and PBS, respectively, and the iliac BMS level was significantly higher than the PBS level. IL-8 was detected in iliac serum from 23 patients, tibial serum from 9 patients, and peripheral serum from 15 patients, with the highest levels being 7890, 2300, and 1150 pg/ml respectively. In 18 patients the IL-8 levels were highest in iliac BMS. The median IL-8 level was 200 pg/ml in iliac BMS, 140.5 pg/ml in tibial BMS, and 270 pg/ml in PBS, respectively. The median level of iliac BMS included 23 samples which contained 14 cases more than tibial BMS and 8 more than PBS. These additional cases usually showed lower levels and made the median level lower than that of PBS. Although statistical analysis was performed we measured cytokine levels in all 3 lesions, and the levels of iliac BMS were higher than that of PBS.

There were no significant correlations between the IL-6 and IL-8 levels in tibial bone marrow and in peripheral blood. In the case of G-CSF, 2 patients had higher levels and another 2 had lower levels in iliac bone marrow than in peripheral blood (Figure 2).

*Relationship of cytokine levels to synovitis.* We analyzed the relationship between synovitis and the levels of each cytokine detected in iliac BMS (IL-6, IL-8, and G-CSF). As shown in Figure 3, the levels of both IL-6 and IL-8 increased as synovial proliferation became greater (IL-6: $p < 0.05$; IL-8: $p < 0.005$). In contrast, no correlation was observed between the extent of synovial proliferation and the G-CSF level.

*Relationship of cytokines and laboratory variables.* To clarify the relationship between bone marrow cytokines and systemic inflammation, we analyzed the correlations with various hematologic variables (CRP, ESR, WBC, %Neu, RBC, Hb, and Plt) and immunologic variables (RF, IgG, IgM, and IgA). No correlations were observed between the levels of IL-6, IL-8, and G-CSF and the laboratory variables. Thus, the elevation of cytokine levels in the bone marrow was not related to systemic inflammation.

## DISCUSSION

Our study showed that the IL-6, IL-8, and G-CSF levels were markedly elevated in the iliac bone marrow serum and the peripheral blood of some patients with RA. The levels of IL-6 and IL-8 in many patients were higher in the iliac bone marrow than in the peripheral blood, but G-CSF was more variable and the level was sometimes higher and sometimes lower in the bone marrow than in the peripheral blood. Accordingly we concentrated our investigation on IL-6 and IL-8.

Two possible problems with our experimental procedures need to be considered. One is the dilution of bone marrow blood with peripheral blood at the time of aspiration. In

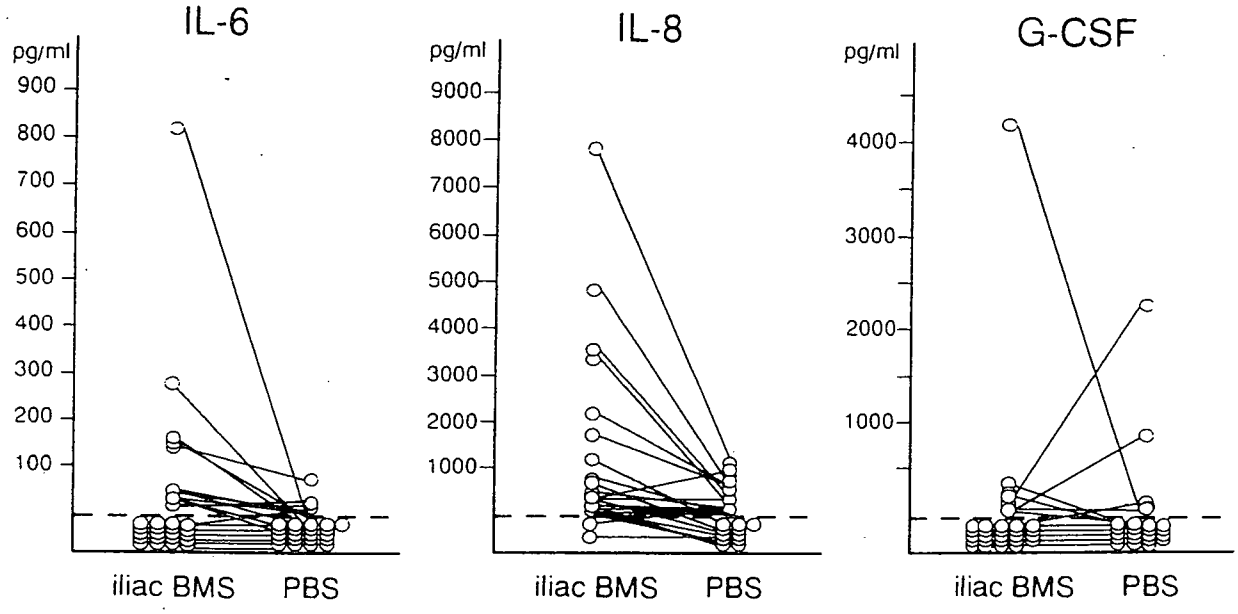


*Fig. 2.* Comparison of IL-6, IL8, and G-CSF levels between iliac BMS and PBS. Both IL-6 and IL-8 levels in iliac bone marrow were higher than those in peripheral blood (IL-6: $p < 0.05$; IL-8: $p < 0.005$), and the peripheral blood levels increased in proportion with those in iliac bone marrow (IL-6: $p < 0.05$; IL-8: $p < 0.001$). In the case of G-CSF, showed 2 patients had higher levels and another 2 had lower levels in iliac bone marrow than in peripheral blood.

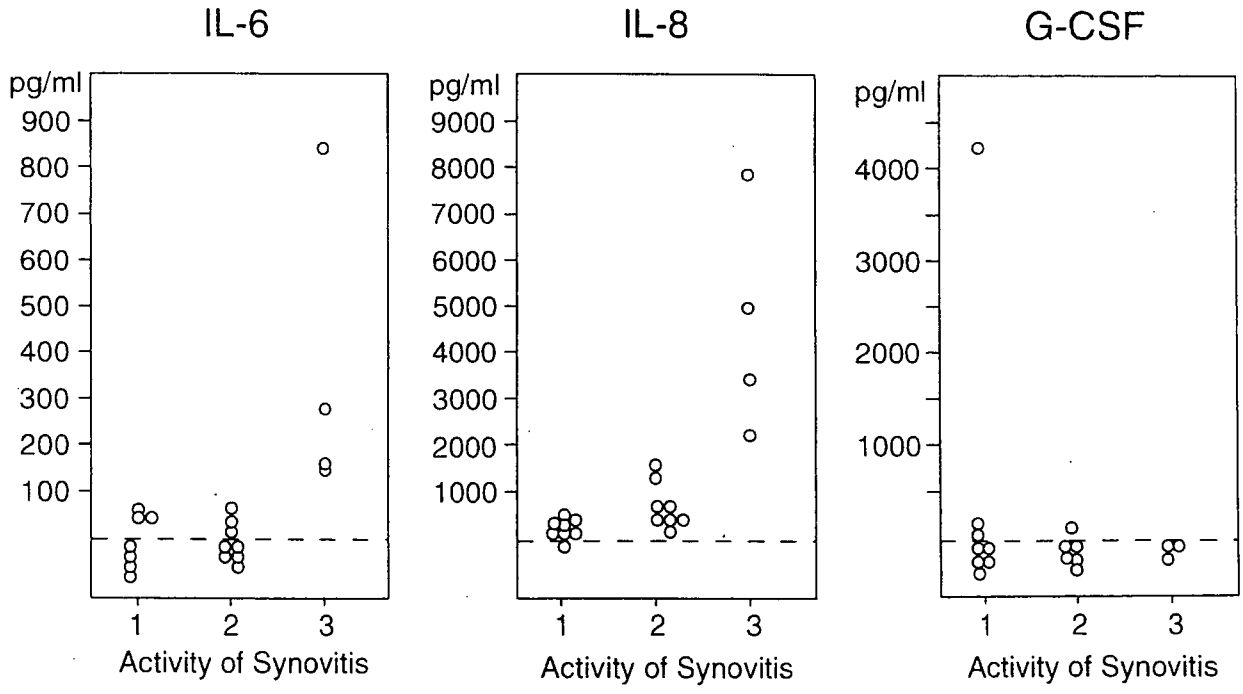


*Fig. 3.* Relationship between the grade of synovitis and the levels of IL-6, IL-8 and G-CSF. As the extent of synovial proliferation increased, the levels of both IL-6 and IL-8 became higher (IL-6: $p < 0.05$; IL-8: $p < 0.005$). However, no correlation was observed between the extent of synovial proliferation and the G-CSF level.

preliminary experiments, we confirmed that the initial 5 ml of bone marrow blood was minimally diluted with peripheral blood. Thus, the actual IL-6 and IL-8 levels in BMS would be only slightly higher than those reported here. The 2nd potential problem is related to our procedure of leaving the samples at 37°C for 60 min, and at 4°C for 60 min. However, we compared the cytokine levels in serum samples obtained by procedure with those of serum obtained from fresh bone marrow blood, and found no difference.

Elevated levels of IL-6 and IL-8 have been detected in the synovial tissue, SF and peripheral blood of patients with RA in many studies[8,14-17]. In our study, we also measured the levels of IL-6 and IL-8 in the SF of 6 patients. The IL-6 level ranged between 290 pg/ml and 400 pg/ml, while the IL-8 level ranged from 390 pg/ml to 16600 pg/ml. These values were comparable with those reported by other authors[8,16,17]. The cytokine levels in SF were generally higher than those in iliac BMS. However the levels of IL-6 and IL-8 in SF did not show a significant correlation with those in iliac BMS, suggesting that these cytokines were produced independently in the synovial tissue and the iliac bone marrow. Thus, the elevation of these cytokines in synovial tissue reported by other authors could represent a localized mechanism promoting synovial proliferation, while the production of cytokines in iliac bone marrow may represent a systemic mechanism. As the accumulation of SF is not always observed in RA, systemic production of these cytokines could sometimes be important in the exacerbation of synovitis.

The IL-6 and IL-8 levels in PBS of our patients with RA were significantly higher than those in the non-RA controls, but these levels were much lower than those found in iliac BMS or SF, possibly because of dilution or degradation in the peripheral blood.

The epiphyseal bone marrow is a hemopoietic organ in children, but not in adults. However, as myeloid cells including immature cells are found in epiphyseal bone marrow lesions adjacent to the involved joints of adults with severe active RA[2], this bone marrow might regain its infantile hemopoietic function in RA and behave more like iliac bone marrow. In our study, we found that the levels of IL-6 and IL-8 in tibial BMS were not significantly different from those in the peripheral blood. Although higher levels of cytokines occasionally could be found in the tibial BMS, there was no statistical difference in the cytokine levels of epiphyseal bone marrow obtained from bone marrow adjacent to joints with and without synovitis. Our findings suggest that the epiphyseal bone marrow is not a major source of IL-6 and IL-8 like the iliac bone marrow. As there were no significant differences between the cytokine levels in tibial BMS and the peripheral blood, the tibial bone marrow appears to behave differently from the iliac marrow with respect to cytokine production.

The main pathological changes of the synovial villi in RA are known to be synovial cell proliferation, invasion of small blood vessels, and perivascular inflammatory cell infiltration. Elevation of IL-6 and IL-8 levels in the iliac bone marrow and in peripheral lesions could induce the systemic exacerbation of proliferative synovitis in patients with RA, since IL-6 is reported to induce synovial proliferation[7], and IL-8 induces inflammatory cell accumulation[8,9]. IL-6 has also been found to induce osteoporosis by stimulating the proliferation of osteoclasts[19], and IL-8 may play a role in neutrophil mediated cartilage degradation[11]. Although the relationship of these cytokines to bone destruction was not clarified in our study, one of the important mechanisms promoting the exacerbation of synovitis could be elevated levels of IL-6 and IL-8 in iliac bone marrow. Accordingly the disease activity may be controlled systemically by some mechanism in bone marrow.

Despite the correlation between elevated levels of IL-6 or IL-8 and the exacerbation of synovitis in our patients with RA, there was no correlation between these cytokines and laboratory variables of systemic inflammation. This was probably because the levels of acute phase reactants are also influenced by many other factors in RA.

Although IL-1 levels are elevated in the BMS of rats with adjuvant arthritis or with collagen induced arthritis from the early period of the disease[6], the IL-1 levels were not markedly raised in BMS of our patients, except in 3 cases. One patient with an elevated IL-1 level was a 56-year-old man with arthritis of the knee for 6 months, who underwent synovectomy for both diagnostic and therapeutic purposes. After observation for more than 6 months, we diagnosed him as having RA. The other 2 patients were women aged 64 and 63 years with RA for over 5 years. We obtained their iliac BMS at the beginning of an exacerbation of the disease, and found elevated IL-1 levels. At subsequent operations, we were able to obtain their iliac BMS twice more, and found that the IL-1 level was not elevated. Thus, the increase of IL-1 in bone marrow serum could be a phenomenon of short duration occurring with the initiation or exacerbation of RA. In the future, we will continue to investigate how the production and accumulation of cytokines in bone marrow related to intraarticular synovial proliferation and the clinical features of RA.

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## 教育研修講演

# 慢性関節リウマチの自然経過と治療計画

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**Key words** : Rheumatoid arthritis, Natural course, A concept for therapy, Disease subsets, Pathology of bone marrow

慢性関節リウマチ(RA)は，何年にもわたり多くの関節の炎症性破壊が進行する慢性進行性疾患である．発症後の経過は軽症に終始する例も，どんどん重症に陥っていく例もあり，さまざまである．このようなRAが発病後10年以上経てどのようになるかの予後はさまざまで，手足の末梢関節のみが侵され日常生活上支障の少ない例から，大関節も侵されて高度の機能障害に陥る例までいろいろある．

しかし，患者さんはRAの診断がつけられると，自分が将来に重症RAに進んで重篤な機能障害に陥ってしまう恐怖感に陥る．またわれわれ臨床家は，目前の患者さんが将来どのようになるかの予測がつかずに困惑するのが実状である．RA患者の長期の経過と予後像を予測することは，患者の長期の治療計画を立てるうえで，また患者に長期の生活設計を立てさせるためにも重要なことであり，われわれはその解析を試みた．その結果，われわれはRAの罹病早期から軽症例，重症例それぞれが別の自然経過をとる，すなわち，病型に分かれていると考えるに至った．そんな疾病であるのに，RAという診断がつくと1つのパターンで金療法を初めとする抗リウマチ剤が考えられるのは不十分ではないか．病態的な差異をも検討し，治療に関しても考察するのが本稿の目的である．

第66回 日本整形外科学会学術集会(神戸)において，教育研修講演として発表した．

## I．RAの自然経過

### A．早期RA

早期RAの症状は，発熱や全身倦怠感を伴いながら，手のこわばりや，手関節，手指または足趾の関節の明らかな腫れが数週間以上にわたって続く．早期リウマチの診断基準を明確にしようとする活動は，厚生省リウマチ調査研究事業としての研究班[6]や日本リウマチ学会の委員会などで精力的に進められているので，近年中に一定のコンセンサスを得られたものが作られるであろう．

われわれは，手関節や手足指関節などの数関節に慢性に明らかな腫れが続き，赤沈値やCRP値などの炎症反応が明らかな亢進を示し，膠原病や慢性感染症(結核など)などの類似疾患が臨床検査値で否定的なときには，早期RAの可能性が大きいと考えて治療している．

RAが進行してくると，多くは関節にX線的な破壊を認めるようになってくる．やがて誰が診てもRAの状態になり，診断基準[1]を満たして診断が確定されてくる．関節の破壊をX線で認めない程度の例もあり，診断基準でもX線的な関節破壊の画像は必ずしも必要条件ではない．事実，X線的なerosionを認めなくても，手術目的で開けてみると著明な滑膜増殖を認めることはよくある．X線的なerosionもあくまで診断基準の1項目として考えるべきである．当然

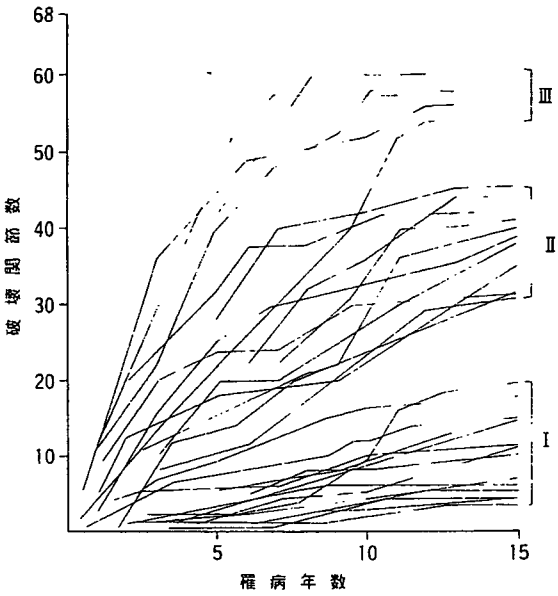


図1 RA患者の罹病年数に伴う関節破壊の進行. Ⅰ群は少関節破壊型, Ⅱ群は多関節破壊型, Ⅲ群はムチランス型病型.

のことであるが, 診断基準は感度と危険率を前提にしたものであり, 絶対的なものではないので, 観察は続けなくてはならない.

診断基準が満たされた後に, 患者の不自由度が長期経過でどのようになっていくかを調べようとしたのが以下の研究である. 患者の不自由度あるいは, 整形外科医が重症だなあと感じる指標を種々検討して, 結局は全身の関節の単純X線像で破壊を認める関節数(破壊関節数; 後述)が最も的確と結論して研究を進めた.

B. RA患者の関節破壊の広がりと病型

RAと診断された患者の関節破壊が将来どのように進行していくのか, 10年以上の経過でどのようになっていくのかを調べた. 大阪大学附属病院および関連施設において罹病早期より10年以上の経過を観察できたRA患者を対象に, 関節破壊進行の自然経過を解析した[7),9)]. 代表的な30例の全身の68関節中の破壊関節数(number of joints with erosion; NJE)の経年的な変化を図1に示す. 縦軸の破壊関節数は全身68関節のX線写真よりerosion以上の変化を示す関節数で, RAによる関節破壊の広がりを示す. 横軸はRAの罹病年数を示す. 罹病5年ぐらいまでは徐々にNJEが増すが10年以後はほとんど変わらないⅠ群, 罹病早期よりNJEは急激に増し10年以内にほとんどの関節が侵され, それ以後はほとんど変わらないⅢ群, およびⅠ群とⅢ群の間のⅡ群である. ⅡおよびⅢ群を重症RAと呼べるが, われわれの調べた母集団全体の中では約30%を占めた. 図1に示されるように, NJEの群間の相違は10年以上で明瞭になるので, 罹病10-15年の240例の成人発症RA患者を無作意に集め, 全身関節の単純X線像よりNJEを評価してその分布を調べた.

その結果, 独立したポアッソン分布を示す3つのピークが見られた. 罹病10-15年でのRA患者は関節破壊の広がりの程度によって3群に分かれたわけである. それぞれのグループが10-15年でどの程度の関節破壊に陥っているかの平均NJEは10.9(SD=0.5), 32.2(SD=4.8), 53.5(SD=4.8)であった. 統計学的に3群が示されおのおのをとりあえず少関節破壊型病型(least erosive subset; LES), 多関節破壊型病型(more erosive subset; MES), ムチランス型病型(mutilating disease; MUD)とよぶ[19)].

C. 関節破壊の病型別特徴

1. 破壊関節部位の予後の概略

各病型を関節破壊の広がりで分類したが, 侵される関節にも特徴がないかを調べた. 図2は各病型の罹病10年以上での関節破壊部位を, X線上erosion以上に侵された関節の頻度で調べた[7),9)]ものである. LESでは手関節(72%), 手足の指MP関節(22-29%)などの末梢小関節の破壊が主体で機能障害も軽度である. MESでは末梢小関節はもとより, さらに膝関節(76%)や股関節(37%)などの大関節も侵され機能障害は重度であるが, 合併症が軽度で再建手術で十分の機能を回復できる. MUDではほぼ全関節が高度に破壊され, 合併症も重度で再建手術も効果は少なく, 寝たきりになることも多い.

2. 各関節破壊進行の病型別特徴

関節破壊の広がりと部位の予後は上記のようなものである. 同じ関節の破壊でも, その程度は病型によって大きく異なる. 概して言えば, LESは増殖性滑膜炎による関節のerosionが主体である. MUDでは靱帯の高度な変性による弛みに起因する不安定な動きにより, 高度な骨粗鬆症に陥った骨が圧壊されるのが主体である. MESではLESとMUDの移行型で, 炎症の程度によってLES寄り, またはMUD寄りになっている. 以下に各関節に見られる破壊の概略を述べる.

a. 手の症状

ほとんどの例で手の腫脹, 疼痛, 可動域制限が認められるが, 予後は病型により異なる. LES症例の場合には70%以上の頻度で手関節のX線的変化を認め, しばしば橈尺関節の亜脱臼も見られる. しかし, 罹病10

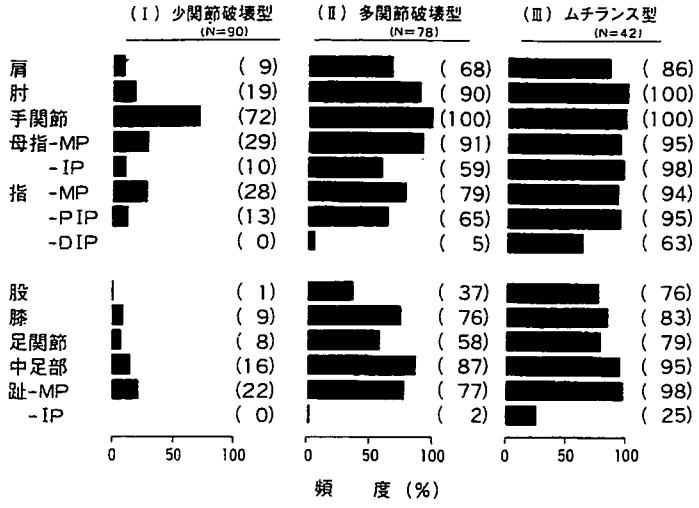


図2　罹病 10-15 年の各病型の RA 患者の破壊関節部位．破壊の確率を%表示した．

年以上経っても X 線像(図 3)では近位手根骨列の靡爛(erosion)による関節裂隙の狭小化(図 3-A)，または橈骨と手根骨の一部が癒合する(partial fusion：図 3-B)程度である．亜脱臼変形は少なく，握力もよく保たれる．MES や MUD の重症 RA の場合には，ほぼ全例で進行した手関節破壊が認められる(図 4)．罹病 10 年経った MUD の場合には，手関節は掌尺側へ高度な亜脱臼を起こす(図 4-C)か，比較的炎症反応の軽度の症例では，関節が骨化により固定(図 4-A)されてしまう．MES 症例では経過中の炎症反応値と関連して，上記の LES に認められる軽度の破壊に終わるものから，MUD に認められる高度な破壊までさまざまなものが認められる．しかし通常は MES に認められる関節裂隙の狭小化(図 4-B)は，手根骨の近位手根骨列の中程度以上の破壊を伴う．手関節の掌尺側への亜脱臼が進み，変形と脱力が進行する．

LES，MES ともに通常は指関節の罹患も伴うが，MES の方が高度である．中手指節間(MP)関節の慢性的腫脹が続き屈曲拘縮が起きると，いわゆるスワンネック変形になりやすい．また近位指節間(PIP)関節の場合にはボタンホール変形になりやすい．MUD では通常は手指関節部の骨の壊滅により，関節は緊張を失いブラブラになり，いわゆるオペラグラス変形に陥る．総指伸筋腱や長母指伸筋腱などの腱断裂もしばしば見られる．

b. 足の症状

いずれの病型でも，足趾の MP 関節炎に伴う足底(MP 部の)痛，前足部の開張，外反母趾，足趾変形拘縮(hammer toe)などの変形を起こす．中足部距舟関節炎は高頻度で見られ，特にでこぼこ道での足痛を訴える．LES では距舟関節の狭小化にとどまる．

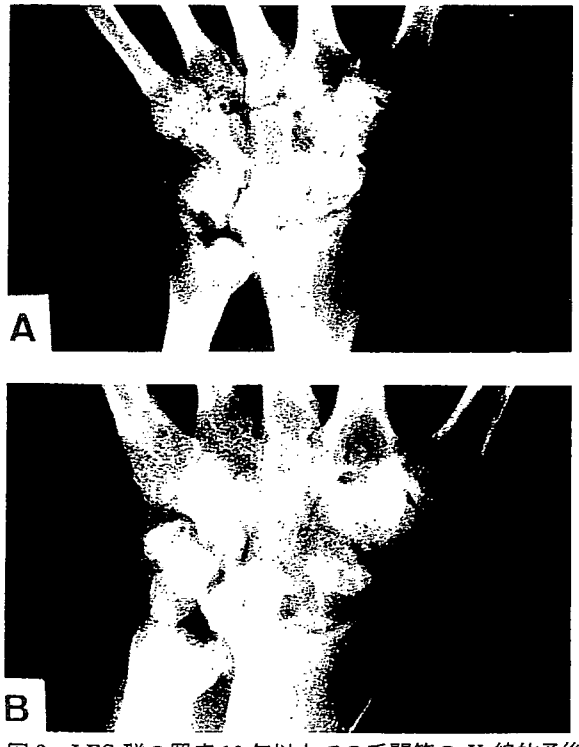


図3　LES 群の罹病 10 年以上での手関節の X 線的予後像．

MES や MUD の重症 RA の中足部では，距舟関節の亜脱臼が進むことが多い．その場合には足の縦軸アーチが崩れて扁平足変形が進むとともに，後足部では距

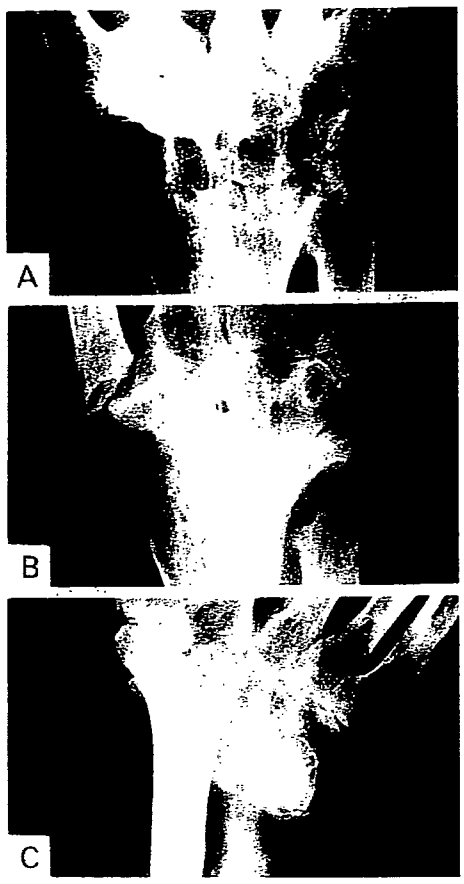


図4 MESおよびMUD群の罹病10年以上での手関節のX線的予後像.

踵関節での外反変形が進行して典型的な外反扁平足になり，強い疼痛を伴う歩行障害に陥る.

c. 脊椎の症状

頚椎変化も高頻度に認められる．読書やテレビを見たりの頚椎の前屈位を続けていて後頭部痛や頚肩痛を訴えるときには，頚椎の罹患を考えて頚椎側面の運動像のX線を撮るべきである．環軸椎(C 1/2)亜脱臼は，LESでも高頻度で見られるが，5 mmまでの程度でとどまる.

MESやMUD例になると環軸椎亜脱臼も高度になり，10 mmを越える例もしばしばであり，固定手術の適応となる．特にMUDでは，進行とともに軸椎が頭蓋内に陥入(vertical invagination)していく．高度な変形が進むと四肢麻痺に陥るので，それ以前の固定手術が必要になる．また頚椎に限らず胸腰椎にも，高度の骨粗鬆症と靱帯の弛みにより，椎体が力学的に耐ええず圧壊が進行し，脊髄圧迫症状を呈することもある.

d. 肩関節の症状

肩に起きるRA症状は肩関節周囲炎症状に始まり，関節破壊へと進んでいく．LES症例では関節面の靡爛を伴いながら滑液嚢癒着による拘縮の程度で止まる．挙上運動は肩甲骨での可動域が主になる.

MESやMUDの場合には，関節軟骨の変性による関節裂隙狭小化が進む．さらに腱板変性あるいは断裂に起因する上方亜脱臼，関節支持組織全般の弛みによる下方亜脱臼などを伴う．特にMUDでは，亜脱臼位での接触面で骨の圧壊が起き，本来の関節構造を失って不安定で高度な関節破壊に陥っていく.

e. 肘関節の症状

肘の罹患の初期には，運動痛と屈曲拘縮がまず起きる．橈尺関節部に炎症が及ぶと，前腕の回内外運動の制限が起きる．LESの例の場合にはこの段階でとどまるのが通常である.

MESやMUDの場合には，肘関節は関節面の圧壊により前後方向への亜脱臼が進み，強い運動時痛とともに筋力や可動域は著減する．不安定肘に伴って尺骨神経への緊張がかかり，種々の程度の尺骨神経麻痺がしばしば見られる.

f. 膝関節の症状

LESの場合には，膝の関節炎は起きてもX線的な病変にまで進むことは少ない．しばしば関節周囲の滑液嚢炎(ベーカー嚢腫など)を伴い，破裂すると下腿の著明な腫れと痛みが持続する.

膝に非可逆的な破壊が起きるのはMESとMUDである．MUDでは膝は靱帯の著明な弛みにより不安定になり，脛骨が後方，側方に亜脱臼し，高度な骨粗鬆症に陥った骨が接触面で圧壊されながら屈曲内(外)反拘縮に陥っていく(図11)．MESでは顕著な滑膜炎による関節面のerosionと，MUDより軽度であるが靱帯の弛みと骨の圧壊も起きて，屈曲または内外反拘縮が進行して歩行困難に陥る.

g. 股関節の症状

LESでは股関節破壊は少ない．しかし，ステロイドを連用した例では，大腿骨頭壊死様の機序が加わって起きたのではないかと疑われる症例にしばしば遭遇する.

股関節破壊は通常はMES，MUDなどの重症RAで見られる．関節痛と可動域制限で歩行障害に陥り気づく．MUDではしだいに大腿骨頭とともに臼蓋側も高度な骨破壊が進行して，いわゆる中心性脱臼に陥っていく.

表1　各病型の患者の早期診断の指標

| 指　標 | 各病型の特徴 | | |
|---|---|---|---|
| | LES | MES | MUD |
| 破壊関節数<br>(罹病10年以上) | 10.9(SD=0.5) | 32.2(SD=4.8) | 53.5(SD=4.8) |
| 血中C1q値(μg/ml)<br>(罹病5年以内) | <200 μg/ml | 250 μg/ml< | 250 μg/ml< |
| CHRの減少(×$10^{-2}$/年)<br>(罹病3年以内) | 0.6(SD=0.3) | 2.3(SD=0.7) | 9.3(SD=4.8) |
| 血中CRP値(mg/dl)<br>(罹病5年以内) | 低値の持続 | (卄)または<br>4 mg/dl以上の持続 | (卄)または<br>4 mg/dl以上の持続 |
| 腸骨骨髄中の<br>HLADR(+)CD8(+)T細胞 | 有意の増加 | 有意の増加 | 増加傾向(有意差なし) |
| 腸骨骨髄中の<br>CD15(+)CD16(−)細胞 | 正常範囲 | 有意の増加 | 有意の増加 |
| 罹患関節部骨髄中の<br>MY4(+)MXGA(+) | 認めず | 存在 | 存在 |

h. 関節外症状

炎症関節近傍のリンパ腺腫大，手根管症候群に見られる正中神経麻痺症状のように絞扼性神経疾患，瀰漫性間質性肺線維症による呼吸容積の減少，結膜や角膜の障害によりSjögren syndromeや自己限局性の上強膜炎，そしてリウマチ結節などはLESにでも認められる関節外症状である．

MESやMUDではさらに重篤な症状が加わる．軽度の内膜肥厚から壊死性動脈炎まで種々の血管炎，無症状に進行していることが多いが，心膜炎は剖検例の約40%に認められると言われている．リウマチ結節が肺内にできて肺癌と区別の困難なこともある．眼症状でもMUDでは，強膜炎が進行して穿孔性強膜軟化症に至ることもある．

## II．各病型の早期診断の手がかり[7),9)]

罹病早期に病型診断を可能にする指標を並べる(表1)．RAの進行を止める治療法がない現在において，早期の病型診断はそのまま早期の予後診断となる．以下に病型診断の指標を述べる．

A．血清中のC1q蛋白量

補体の第一亜成分であるC1q値はLESと重症病型(MESとMUD)とを早期に鑑別する指標となる．測定方法は特異抗血清を用いてのsingle radial immunodiffusion法によった．ポリクロナル抗体ではうまくいくがモノクロナル抗体ではうまくいかないことがしばしばなので，注意していただきたい．健常人の平均値である136.5 μg/mlに比べRA患者は211.9 μg/mlと統計的にも明らかな高値をとる．C1q蛋白量は罹病5年以内ではRA患者おのおのの個人で安定した一定値を示し，病型診断の有力な指標になる．図5に示すように，LES症例では全期間を通じてC1q値が250 μg/mlを越えることはない．MESおよびMUD症例のC1q値はいずれも罹病5年以内では250 μg/ml以上を示し，MUD症例では，とりわけ高値を示した．いずれも罹病10年以上になると徐々に正常化傾向を示す．この値の変動を見ていると軽症に経過するLESと，重症に経過するMESまたはMUDとは罹病早期より明確に一定の自然経過が決まっていることを感じる．

B．手根骨破壊の速さ

手関節破壊の程度を第3中手骨長に対する手根骨長(carpal height ratio ; CHR ; Youm, et al.[19]; 図6)で評価し，手関節破壊の速さはその1年当りの減少率(△CHR)で評価した[9]．罹病3年以内で手関節破壊が始まっている罹患手関節を対象に比較検討した．骨破壊の速さは罹病早期から各病型で明らかに異なり，△CHRは各病型により高度の有意差を示した．この評価は約1年の経過を見ての判断指標である．

C．CRP反応

ルーチンで行われている臨床検査値としてはCRP反応はRAの自然経過を最も反映している．罹病5年以内のCRP反応はMES，MUDとも何度調べても強陽性(2+または4 mg/dl以上)を示す．それに対してLESでは88%の例で常時またはときどきにでも陰性を示している．低い値でのCRP値の変動はLESである確率が高く，予後診断の指標になる．いずれの病型の症例

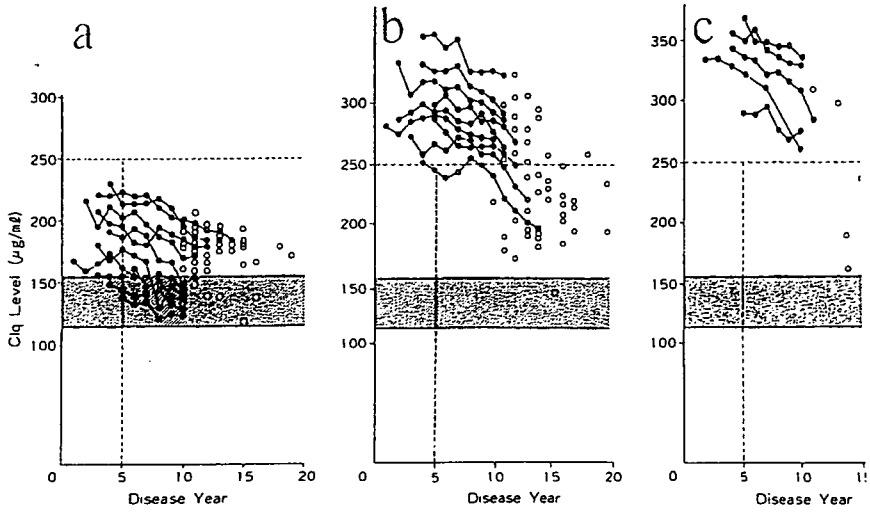


図5 RA患者の各病型別にみた血中Clq値の経年的変動．aはLES, bはMES, cはMUD．横軸は罹病年数，縦軸は血中Clq値(μg/ml)を示す．斜線部位は正常値の範囲を示す．

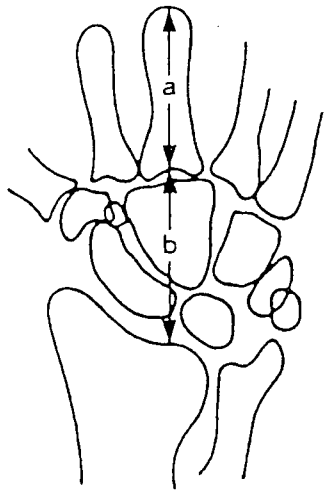


図6 Calpal height ratio (CHR)；Youmら[19]．第3中手骨長(a)に対する手根骨長(b)の比を言う(b/a)．

も罹病年数が10年を越すと陰性の率が増し，炎症の鎮静化の傾向を示した．

D．関節破壊の特徴

高齢発症RAでは，軽症の経過をとる例でも，あらかじめ変形性関節症変化のあった膝や股関節に骨破壊が進行することはよく認められる[11]．また成人発症RAでは，軽症例でもステロイド使用例などで大腿骨頭無腐性壊死が起きることがある．しかし，成人発症RAで軽症病型(LES)では一般に，膝や股関節にはX線で認めるほどの骨変化を認めることは少ない．成人発症RAで多関節のerosionが進み，膝や股関節にもerosionが認められ始めるとMESかMUDの可能性が強い．特に膝関節などerosionというよりも，顕著な靱帯の弛緩と骨粗鬆症による関節の不安定性と骨の圧壊が認められるとMUDの可能性が強い．

E．骨髄単核球分画の病態的相違

動物実験系において多発関節炎発症の病巣が滑膜のみでなく，骨髄にもあるらしいことが示唆された[3)-5),11)]．そこで，RA患者でも骨髄に異常が起きていないかを検討した．特に前記の3病型が本当に"病型"と言えるものなら，病態的相違が明確でなければならない．これを滑膜病態では明らかにできなかったので，病態的検討を骨髄細胞のレベルで検討した．骨髄としては，全身性の骨髄細胞を示す腸骨骨髄細胞と関節部の長骨骨端部骨髄として脛骨骨髄細胞[8),14),15)]を対象とした．厚生省の慢性関節リウマチの調査研究事業の病態解明に関する研究班において，RA患者の骨髄が調べられているので報告[12)]をご参照いただきたいが，本稿ではわれわれの研究室での研究結果のなかの本論旨に必要部分として，腸骨骨髄細胞単核球分画の変化についてのみ述べる．

健常成人において造血系の細胞は胸骨，腸骨，椎骨などの骨髄中に存在することが知られている．造血系組織における細胞の変化を知るためには，検体採取の比較的容易な腸骨から手術時に骨髄血を採取し調べた．RA患者の腸骨骨髄血より単核球分画の細胞数の変化を調べた．平均細胞数(SD)/mm³は健常人では1245(823)に対してLES：2640(1649)，MES：

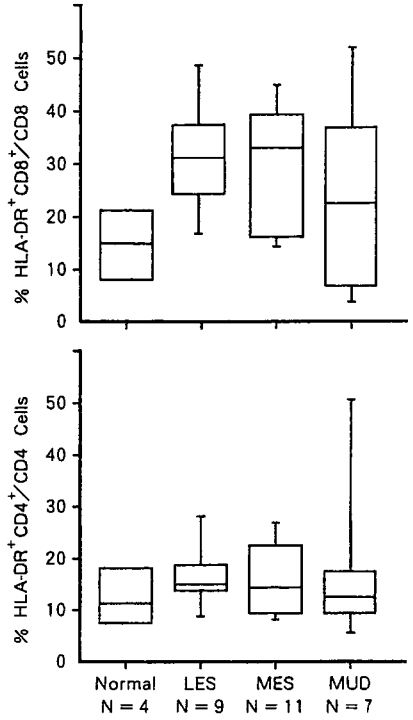


図7 腸骨骨髄単核細胞中のCD 8(+)細胞の総数に対する活性型のHLA-DR(+)のCD 8(+)細胞の比率(上)とCD 4(+)細胞の総数に対する活性型のHLA-DR(+)のCD 4(+)細胞の比率(下). 左から非RA対照, LES, MES, MUD.

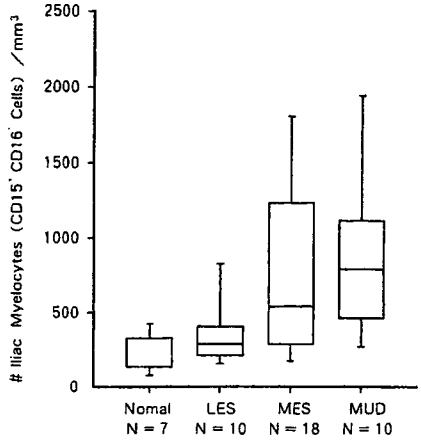


図8 腸骨骨髄中の骨髄球系細胞[CD 15+CD 16−]の絶対数[(SD)/mm³]. 左から非RA対照, LES, MES, MUD.

3843(3340), MUD : 3465(1721)とRA全般に顕著な増加を認めた. しかし, 脛骨骨髄での単核球分画の平均細胞数(SD)/mm³は健常人で952(276)に対してRA患者では1625(1322)と有意の増加を示さなかった. 末梢血の単核細胞数でも健常人とRA患者の間に有意差は認めなかったが, このことは単核細胞が盛んに造られて急速に破壊されていることを示唆している.

腸骨での細胞の表面抗原マーカーのモノクロナル抗体を細胞膜と反応させ, フローサイトメトリーにより解析した. 腸骨骨髄単核細胞中で有意差が認められたのは, CD 8(+)細胞の総数に対する活性型のHLA-DR(+)のCD 8(+)細胞の比率の上昇(図7上)と, 骨髄球系細胞の絶対数の上昇(図8)とであった. HLA-DR(+)CD 8(+)細胞の総CD 8(+)細胞に対する平均構成比率(SD)は健常人の14.1(8.3)%に対して, 末梢小関節破壊に終わる予後良好軽症RA(LES)では30.5(12.4)%, 大関節にも破壊が及ぶ病型(MES)では39.1(16.4)%と, いずれも有意の高値を示した. しかもこの2病型間には差を認めず, 滑膜増殖を伴うRAとしては基本的病態と思われた. 全身性に高度の組織破壊が進むムチランス病型(MUD)では, なぜか低値の例が含まれ27.4(16.3)%で健常人との有意差を認めえなかった.

骨髄球系細胞[CD 15+CD 16−]の腸骨骨髄中での絶対数(SD)/mm³(図8)は健常例 : 208(137)に比べ, LESでは631(773)と有意差($p<0.05$)は少ないが, 重症RAの2病型群ではMES; 1171(1835), MUD; 1230(1179)と有意に増加し($p<0.01$), 他の細胞分画と同様に増殖の亢進が認められた. 当然のことながら, 末梢血では骨髄球を認めず, facsの値はbackgroundの値を示した.

もう少し詳細に重症RA患者の腸骨の骨髄球分画を調べてみると, 図9-BのようにMXGA(+)MY 4(+)とMXGA(+)MY 4(−)の2つの細胞集団が見られた. MXGA(+)MY 4(−)は図9-Cに示される正常の骨髄球系細胞の膜抗原の特徴を示している. しかし, MXGA(+)MY 4(+)は通常は骨髄球系細胞に認められない膜抗原であり, またこの細胞群は悪性腫瘍細胞膜の特異抗原と考えられている糖鎖膜抗原(difucosylated type 2 chain)を持つことでも特異的であった. 驚いたことに, 重症RA患者の腸骨においてMY 4(−)の骨髄球からMY 4(+)の骨髄球が産生されていることが分かった. MXGA(+)MY 4(+)の細胞をsortingして, やはり骨髄球系細胞であることは確認した. 意外にも図9-Aに示されるように, 異常なMXGA(+)MY 4(+)の骨髄球が罹患関節部骨髄に集積していたのであった.

HLA-DR(+)CD 8(+)細胞の結果と併せて考えると,