Owaki H, Yukawa K, Ochi T, Shimaoka Y, Ono K.	Facs analysis of myeloid differentiation stages in epiphyseal bone marrow, adjacent to joints affected with rheumatoid arthritis.	Scand J Rheumatol.	20(2)	91-7	1991

Ⅲ.研究成果の刊行物・別刷

New Aspects of RA
- Bone Marrow and CD14+ Cells -

Reports of Studies supported in part by a grant-in-aid from the Health Science Research grant from the Ministry of Health and Welfare of Japan (until 2010)

Introduction summarizing this report; A new aspect about bone marrow and CD14 positive mononuclear cells in RA patients

Our research works relating to RA pathophysiology have been progressed supported for years by the research grants from government (Ministry of Health and Welfare, Japan). As we could believe our hypothesis were getting sure, we have summerized them as follows.

1) Hematopoietic bone marrow could be the most important site inducing and progressing the pathophysiological changes in patients with RA (RA patients).
2) CD14+ cells ,among mononuclear cells (MNCs) of RA patients, were thought to play very important roles in inducing and regulating pathophysiology of RA.

It had been a well known concept that synovial tissues is the most important pathophysiological site in RA. We, orthopaedic surgeons, could not necessarily agree those concepts because of at least two reasons.

1) At operations of RA patients, synovial tissues were not necessarily remarkable even in active and severe RA.
2) After synovectomies, localized pain and inflammation were reduced from the operated joints, but joint destructions still progressed thereafter (9). And, systemic remissions were hard to be induced by synovectomy.

Therefore, we suspected the possibility that there could exsist any pathophysiological sites other than synovial tissues which might induce and progress inflammation and destruction of various joints in RA patients.

In around 1980, we unsuspectedly observed polyarthritis in the inbred C3H/He (host) mice injected intraperitoneally (ip) the allogeneic thymocytes from the inbred BALB/c mice (1). To know how the polyarthritis was triggered, we labeled the allogeneic thymocytes for ip injection with ^{3}H-Tdr. From day 1 after the injection of ^{3}H-labelled thymocytes, ^{3}H-Tdr was observed mostly in the bone marrow, where the myelopoiesis was initiated. Three weeks after the treatments, polyarthritis appeared and continued for months resulting in contracture of various joints just like in RA. To our regret, their matings became too slow to continue more experiments. So, we just kept those in mind and in file, which were described later in this report.

Recently, DNase II-null mice were reported to induce naturally

progressing polyarthritis whose bone marrow macrophages accumulated a lot of DNA from nuclei of erythroid precursors and triggered to enhance various immunological functions (Kawane et al. PNAS, 2010). Patho-physiological mechanisms inducing polyarthritis triggered by macrophage-monocytes in bone marrow, which might be similar to the findings in our mice model and also those in RA patients as described in this report.

Then, we began to study the bone marrow cells of RA patients. Mononuclear cells (MNCs) in iliac bone marrow of RA patients significantly increased up to about 3 fold comparing to non-RA controls, and were thought to produce various pathological cellular changes as follows. Among MNCs in RA, functions of CD14+ cells were remarkable.

1) Unusual CD14+ myeloid cells were differentiated there and proiduce at least partially the pathological changes of severer disease of RA.
2) RA-nurse like cells were suspected to be differentiate there and play the very important roles in regulating pathophysiology of RA.
3) RA-specific osteoclast- like cells could be differentiated from CD14+ cells supported by NLCs differentiated in the iliac bone marrow of RA.

Recently, the analyses of gene expression profiles of MNCs showed that the abnormaly enhanced functional networks of immune responses were triggered in bone marrow of RA patients.

It was very impressive for us that those findings of MNCs in RA patients showed some relations with the prognoses of joints destruction in RA. By analyzing the iliac bone marrow MNCs, we might prospectively susupect the prognoses of RA.

Finishing the chief of our study group in March 2011, I would like to appreciate sincerely to Dr Peter Lipsky kindly collaborating with us in many studies of ours summarized in this report.

March, 2011

Professor Emeritus, Osaka University Medical School
Director, Osaka Police Hospital
Takahiro Ochi, MD and PhD

Contents

I . Experimental polyarthritis suggesting bone marrow to be an important pathophysiological site.

1) Unsuspected polyarthritis in mice induced by allogenetic thymocytes

In around 1980, we unsuspectedly observed polyarthritis (1) in inbred C3H/He mice which were injected intraperitoneally (ip) with 5×10^7 allogeneic thymocytes from inbred BALB/c mice. Three weeks after the treatments, polyarthritis appeared gradually. At 4 weeks after the initial injections, polyarthritis were observed in about 30% of the treated hosts. If the same treatments were repeated at 2 weeks, incidence of polyarthritis were enhanced up to about 80% at 4 weeks after the initial treatments.

Both of these inbred BALB/c and inbred C3H/He mice were maintained by Dr Shizuo Tanabe, Dept of Bacteriology, Osaka University Medical School, Japan. Both of those 2 strains had already been maintained more than 50 breedings by strict brother and sister mating, and their matings became too slow to continue our other additional experiments.

To know in which organ any pathological changes were triggered, we labeled with ^{3}H-Tdr those thymocytes from BALB/c mice, and injected them ip into the C3H/He mice. At day 1, the ^{3}H-Tdr labeled cells were observed to accumulate mostly in both spleen and bone marrow. So, we preliminaly removed spleens from C3H/He mice (host mice), and then administrated the thymocytes labeled with ^{3}H-Tdr into those host mice. Polyarthritis was observed just as those without splenectomy. At day 1 and thereafter, the labeled cells were microscopically found to accumulate mostly in the bone marrow of the host mice, much less in lung, kidney, skin ,but not at all in joint tissues.

To know how such polyarthritis was induced, we micropsopically observed precisely various organs including joint tissues. At day 1 in their bone marrow, myelopoiesis was suddenly induced (Fig 1).

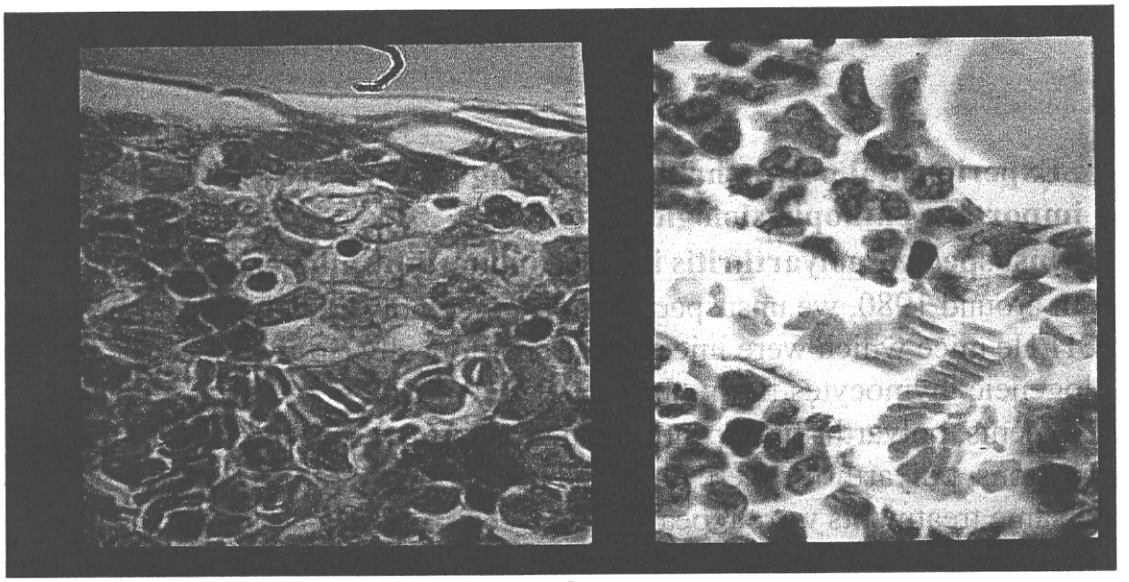


Figure 1A (left); Bone marrow of an untreated mouse
Figure 1B (right); Bone marrow of the treated mouse at day 1

At day 5, fibroblastic tissue accompanying small blood vessels (Sy in Fig 2B) were observed migrating in joint space (JS in Fig 2B) through the small canals connecting with bone marrow (BM in Fig 2B). These findings were observed in various joints in those mice at the onset of arthritis.

Fig. 2A

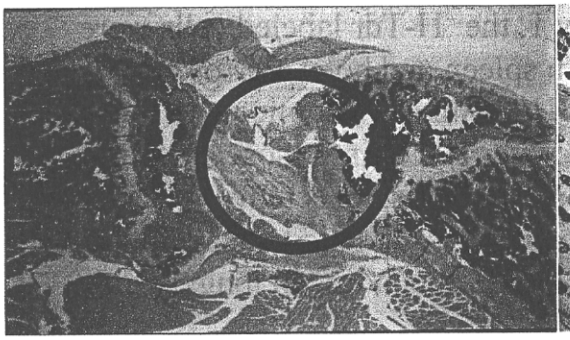


Fig.2B

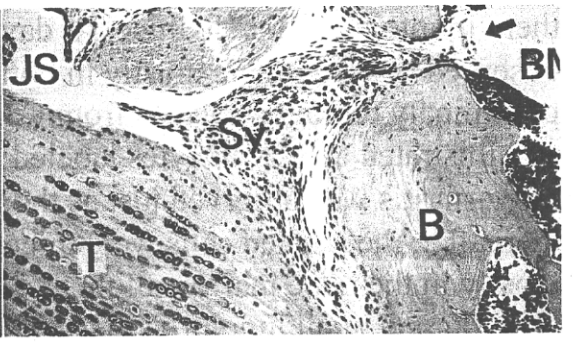


Figure 2. Migration of fibrobrast like cells from bone marrow into joint space accompanying small blood vessels at day 5 after treatments. Magnification of the circle in Fig 2A is Fig 2B..

At 3 weeks, remarkable synovitis were observed in various joints. Their characteristic findings (Fig 3) were synovial cell proliferation, vascular invasion, and inflammatory cell infiltration similar to those of RA. Observing those findings, we suspected the bone marrow could be the most important site in inducing those polyarthritis.

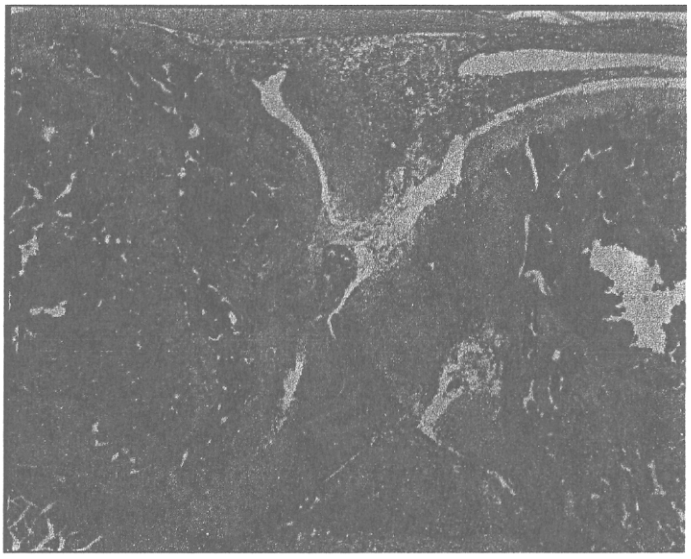


Figure 3. Remarkable synovitis observed at 3 weeks after treatments

There remained a big question. Was the synovial tissue really not important in inducing or progressing polyarthritis? We suspected the possibility that pathological inflammatory synovial tissue, but not normal synovial tissue, could accumulate the pathological cells (labeled cells) promoting polyarthritis (exacerbation).

To make clear these aspects, we preliminaly induced polyarthritis by injecting ip the unlabeled allogeneic thymocyted into the host mice. After the swelling of their feet appeared at 4 weeks, we injected ip the ^{3}H-Tdr labeled thymocytes to the host mice having arthritis, and continued to observe microscopically. On the following day, ^{3}H-Tdr labeled cells were observed aroud the small blood vessels (Fig.4b) in the synovial tissue (Fig 4a), as well as in the bone marrow.

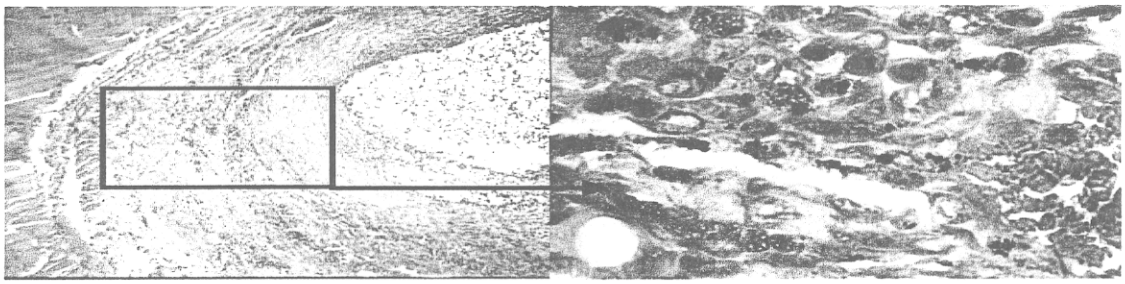


Figure 4. Microscopical findings of synovial tissue 1 day after the ip injection of ^{3}H-Tdr labeled allogeneic thymocytes into host mouse having polyarthritis. Magnification of squared part of Fig 4A is Fig 4B

Thereafter, the polyarthritis became very active, and aggressively progressed (Fig 5). Their articular cartilage was observed to be invaded not only from the joint surface, but also from inside the bone marrow space (in the circle of Fig 5).

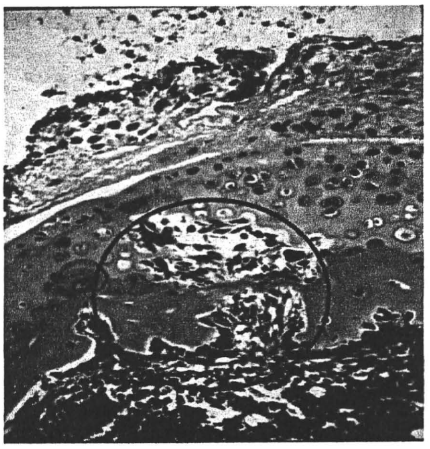
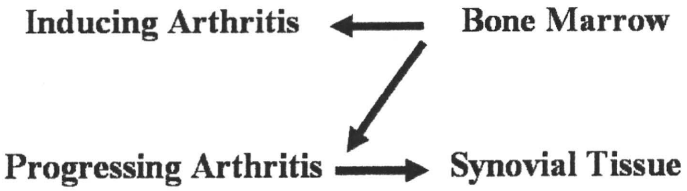


Figure 5. Invasion of joint tissue after exacerbation with additional treatments. See text for details

Observing all those findings, the bone marrow could be supposed an important site in both inducing and progressing arthritis, although the synovial tissue in progressing, but not in inducing those murine polyarthritis (Fig 6).

Fig. 6



2) Bone marrow changes in conventional experimental arthritis rats

We intended to obtain any further evidences supporting such previously unbelievable hypotheses that the bone marrow might be an important site to induce polyarthritis. Pathohistological changes and fluctuation of cytokines in bone marrow were studied using the conventional experimental arthritis; collagen-induced arthritis (CIA) (6,10,12) and adjuvand- induced arthritis (AIA) in rats (10).

At day 4, enhanced myelopoiesis were clearly found in bone marrow **(Fig 7)** of both CIA (Fig 7D) and AIA (Fig 7B), before the synovial proliferations were observed at day 10 in CIA and at day 7 in AIA rats. Swelling of hindfoot appeared at day 14 in CIA rats and at day 10 in AIA rats and persisted. Three months after the treatments, no more remarkable swelling in joints were observed but stiffness of involved joints persisted in both experimental arthritis rats.

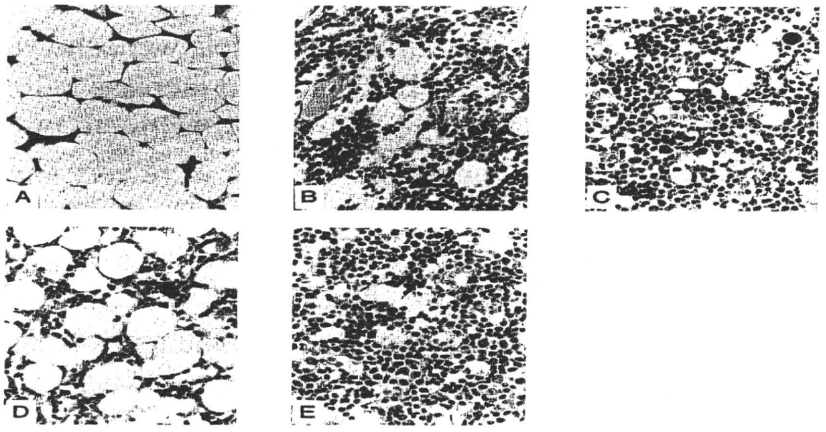
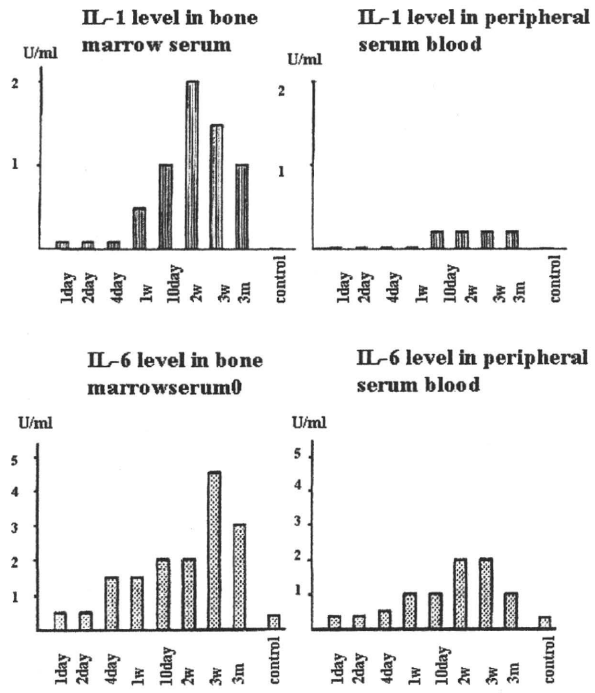


Fig 7; Hematoxylin Eosin-stained sections of distal tibial bone marrow from A, normal rat; B, a rat with AIA at day 4; C, a rat with AIA at day 21; D, a rat with CIA at day 4; E, a rat with CIA at day 21.

Fig. 8



Fluctuation of cytokine activity(U/ml) in the bone marrow serum and peripheral blood serum of both experimental models were shown in **Fig 8.** In the bone marrow serum, IL-1 and IL-6 began to increase before the onset of arthritis in relation to the progression of arthritis (10,12). The fluctuation of IL-6 in their peripheral blood serum were very similar to, but much less levels than those in bone marrow serum. The levels of IL-1 activity in the peripheral blood serum did not increased, except in some CIA cases. In both animal models, we could realize to occur myelopoiesis in bone marrow primarily, cytokine elevation in bone marrow serum, and their synovial prolifiration accompanying apparent foot swelling.

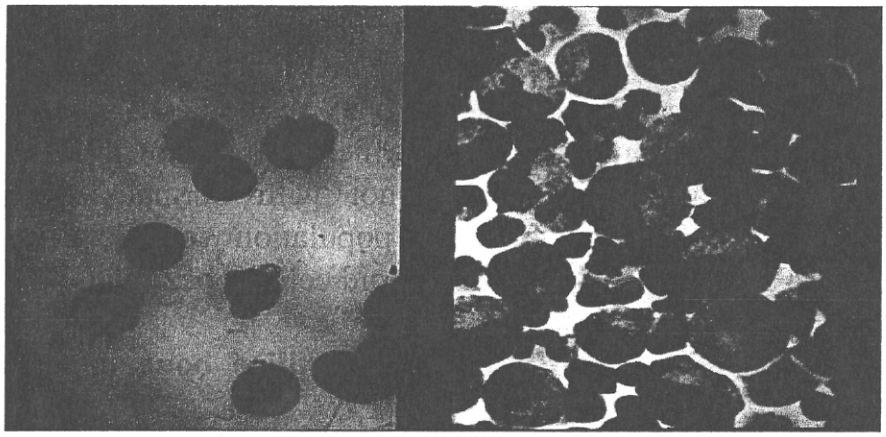
Ⅱ. Characteristic changes of blood cell lineages in RA bone marrow

1) An abnormal myeloid cell population found in epiphyseal bone marrow of RA patients.

i) A CD14(+) myeloid cell population with oncofetal membrane structure

We tried to find out any unknown pathological cells in the involved epiphyseal bone marrow of RA patients relating to the active severe inflammation and joint destruction, which were uncontrollable with conventional medications just like malignant diseases. So, we surveyed any cells expressing undifferentiated malignancy, using monoclonal antibodies to aberrant carbohydrate structures; mono- or di-fucosyl type 2 chain (mono- or dimeric Lex: FH2 or FH4 antigen) (4) kindly given by Dr Hakomori, Seattle, USA.

In the epiphyseal bone marrow blood from active severe RA patients, some (between 2.3 and 14.4%; mean 7.8%) of MNCs showed a strong reaction with FH4 antibody under fluorometric microscopy, but not at all in non-RA subjects. By immunoelectron microscopy, those cells reacting with FH4 monoclonal antibody were identified as myeloid cell population. In those patients, we confirmed microscopically by Giemsa stain the existence of myeloid cells population containing very immature staged cells (4) **(Fig 9)**.



Non RA,LES MES,MUD

Fig 9 Giemsa stain of MNCs in bone marrow Left; severer RA (MES and MUD) Right; non RA and mild RA

Two color cyto-fluorometric pattern with FH2 and FH4 antibodies showed the FH2+FH4+ cells (between 1.5 and 12.3%; mean 5.7%) in bone marrow MNCs from patients with severe disease of RA (MES or MUD; Fig 10-C), and those with burned-out RA (Fig 10-D) although a small number. They were virtually absent in those from patients with mild disease of RA (LES; Fig 10-B), osteoarthritis(Fig 10-E), infectious arthritis (Fig 10-F), and from normal adult subjects (Fig 10-A). In the epiphyseal bone marrow blood containing FH2+FH4+ cells in active severe RA patients, various differentiation stages of myeloid cells (8) and the elevated activity of myeloid growth factor were found (7). In those areas, especially high levels of IL-1 (17) or PMN factor (5) were also found, which were thought to induce the connective tissue degeneration and/or destruction.

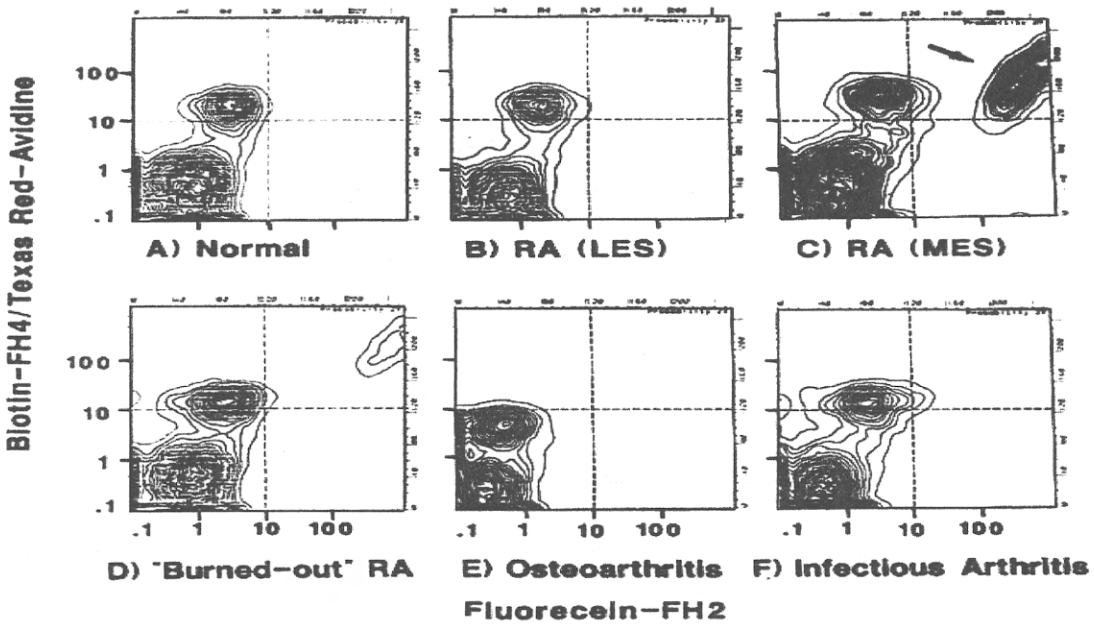


Fig 10 Two color cyto-fluorometric pattern with FH2 and FH4 antibodies in bone marrow MNCs from RA patients and non-RA controls. See text for details.

In other additional studies, we knew that those unusual myeloid cells reacted with FH2 and/or FH4 monoclonal antibodies also reacted with CD14 (MY4) monoclonal antibody, which were known to react with monocyte-macrophage lineages, but not with normal myeloid cells. Thereafter, those abnormal myeloid cell population were identified as those bearing CD14+CD15+, and normal myeloid cells as bearing CD14-CD15+.

ii) CD14+ myeloid cells differentiated in iliac bone marrow of RA patients.

Although we suspected such unusual CD14+CD15+ myeloid cells were specifically localized in epiphyseal bone marrow of RA patients, we also surveyed those cells in their iliac bone marrow; one of the major sites with systemic hematopoietic functions (16). Two color FACS analyses of iliac bone marrow MNCs as well as epiphyseal bone marrow MNCs from RA patients and non-RA controls were done. In non-RA controls and patients with mild disease of RA, all the myeloid lineage cells in iliac bone marrow were CD14-15+ (Fig 11, above left), and no myeloid cell was observed in epiphyseal bone marrow (Fig 11, above right). In the severer diseases of RA (MES and MUD), myeloid lineage cells in the iliac bone marrow (Fig 11. below left) were separated into 2 groups; CD14-CD15+ cells and CD14+CD15+ cells (indicated by an arrow), and those in the involved tibial epiphyseal bone marrow (Fig 11, below right) were bearing CD14+CD15+ (indicated by an arrow), which were confirmed as the same cell population defined as FH2+FH4+.

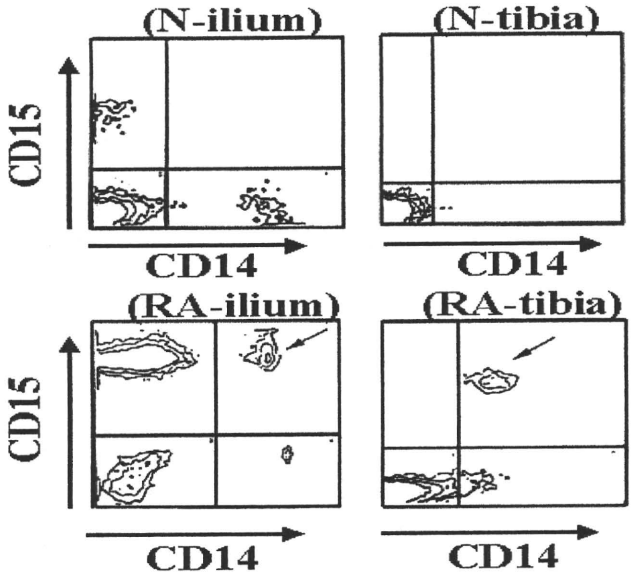
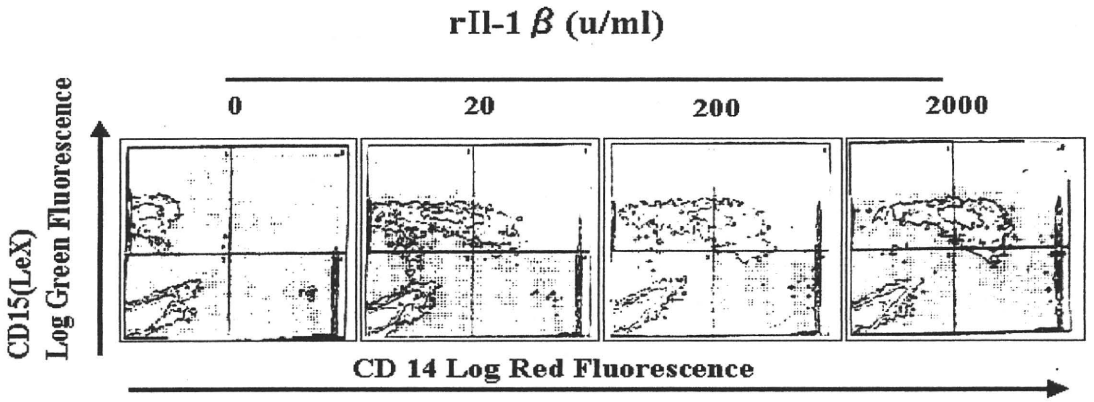


Figure 11 Two color flow cytometric analysis of MNCs of iliac bone marrow of a normal donor (upper left), of tibial epiphyseal bone marrow of a normal donor (upper right), of iliac bone marrow of a severe RA (MES) patient (lower left) and of tibial epiphyseal bone marrow of a severe RA (MES) patient (lower right). See text for details.

Then, we isolated T-depleted non-phagocytic CD14-CD15+ cells from

the iliac bone marrow MNCs of the patients with severer diseases of RA. We cultured those cells with various doses (0, 20, 200, 2000 μ g/ml) of recombinant human Interleukin-1 β (rIl-1 β). As shown in **Fig. 12**, CD14+CD15+ cells differentiated from CD14-CD15+ cells by adding rIl-1 β dose-dependently, and were also observed by adding with GM-CSF (26). It was noteworthy that the differentiation to CD14+CD15+ myeloid cells was inhibited by adding T lymphocytes of donor patients (25).

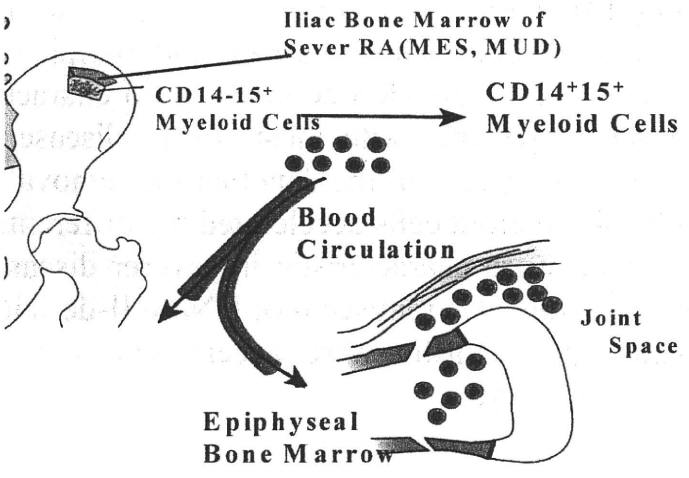
Fig 12 Differentiation of CD14+CD15+ myeloid cells from CD14-CD15+ cells



CD 14Antigen Expression on CD15 Cells from RA Patient's T-Depletd Non-Phagocytic Iliac Density Cells Cultured with rIL-1 β

As such unusual differentiation of CD14+CD15+ cells (16, 25) were specifically observed in iliac bone marrow of the active severe RA, we supposed those CD14+CD15+ cells could be differentiated in iliac bone marrow, transferred by peripheral blood, and accumulated in the epiphyseal bone marrow. Then they could play roles in making pathological changes in and around the involved joints.

Figure 13. Supposed differentiation and migration of CD14+CD15; myeloid cells in RA



12

2) Characteristic changes of MNCs in iliac bone marrow of RA

We suspected any other changes of MNCs in bone marrow cells of RA patients, and analysed MNCs in iliac bone marrow blood, proximal tibial epiphyseal bone marrow blood, and peripheral blood of RA patients and non-RA controls (16) **(Table 1)**. Although the absolute numbers of MNCs in the iliac bone marrow were increased by about 3-fold in RA patients compared with the non-RA controls, the ratio of each MNCs population to total MNCs in iliac bone marrow blood was not significantly different from those of non-RA controls. Each MNC population of the epiphyseal bone marrow blood showed similar numbers and ratios to those of peripheral blood, and were thought to reflect fundamentally the peripheral circulation, although the epiphyseal bone marrow could accumulate the unusual CD14+CD15+ myeloid cells. These data suggested the enhanced proliferation of MNCs in the iliac bone marrow and the rapid turnover expressing various immunological enhancement in peripheral blood (16).

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

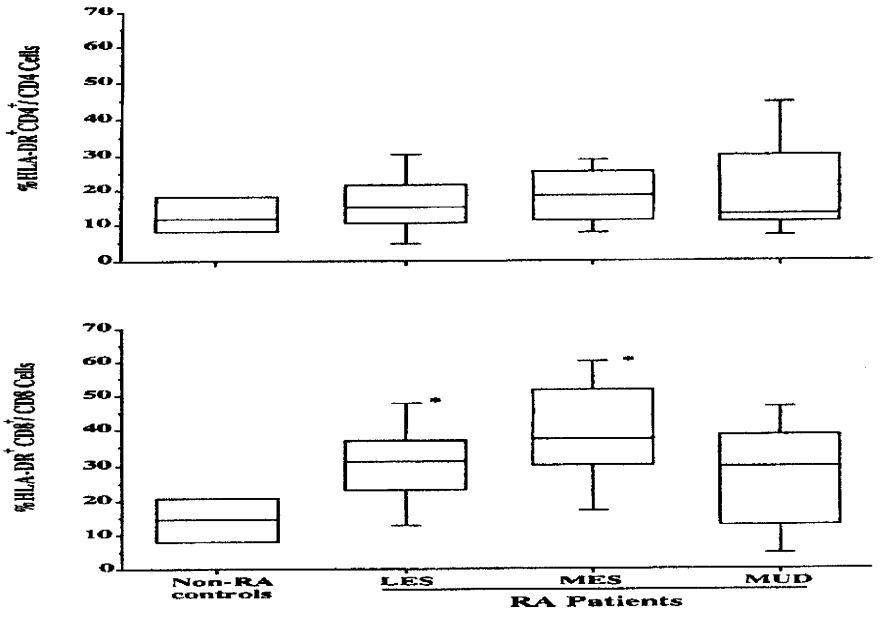
	Peripheral Blood		Iliac Bone Marrow		Tibial Bone Marrow	
	Controls	RA	Controls	RA	Controls	RA
No. of MNC/mm³	974±167	1401±162	1245±311	3122±225*	952±138	1625±212
Myeloid cells						
% CD15+CD16−	2.9±0.5	6.5±1.5	19.0±3.5	24.4±1.7	6.8±2.0	5.8±0.9
T cells						
% CD4	43.2±3.3	29.5±1.8**	17.4±3.3	17.6±1.1	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.

One characteristic changes of RA patients were found in the lymphocyte population (16). The ratio of HLA-DR+CD8+ cells to total CD8+ cells **(Fig 14)** increased significantly in the iliac bone marrow as well as in tibial epiphyseal bome marrow and peripheral blood of RA patients compared with the non-RA controls. Such characteristic increases were not observed in patients with most severe disease of RA (MUD). These results were reminiscent of the data that the removal of lymphocytes in cultures of CD14- myeloid cells accelerated the differentiation to CD14+ myeloid cells. which were characteristic in severer diseases of RA (MES and MUD). These results also remiscent of DNase II-deficient mice (Kawase et al, 2010) whose polyarthritis were accelerated joint damages under genetical lack of lymphocytes.

In combination with the data about the appearances of CD14+ myeloid cells in the iliac bone marrow of severer diseases of RA (MES & MUD), we realized that the cellular changes in iliac bone marrow as shown in **Fig 15** reflected the natural courses of joint destruction in RA (3) described later.

Fig14; HLA-DR antigen expression by CD4 and CD8 cells from the iliac bone marrow of the non-RA controls and patients with RA. RA patients were classified into 3 disease subsets (LES, MES and MUD) as described in the last chapter of this report.



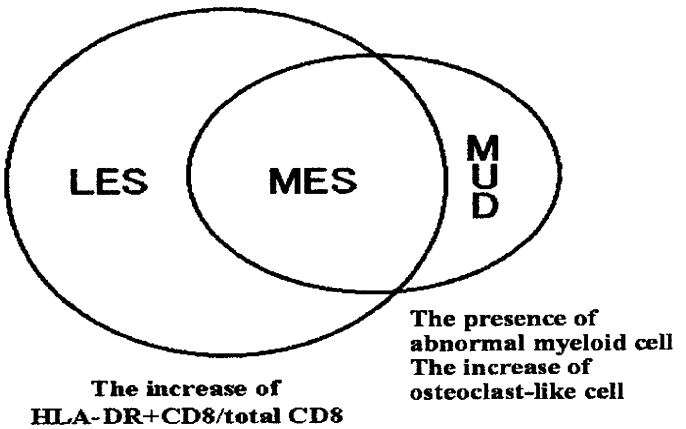


Fig 15 Characteristic changes of iliac bone marrow cells in each RA disease subset. The increase of the percentage of HLA-DR+CD8 cells to all CD8 cells was recognized in LES and MES, and the presence of abnormal myeloid cells was characteristic to MES and MUD(16). LES; **subset with least erosive disease, MES; the subset with more erosive disease , MUD; the subset with mutilating disease (3)**

3) Remarkable elevation of cytokine levels in iliac bone marrow blood of RA patients

We then investigated the levels of various cytokines in bone marrow of RA patients and non-RA controls. Among 12 kinds of cytokines [interleukin(IL)-1 α, -1 β, -2, -3, -4, -6, -7, -8, GMCSF, GCSF, TNF-α, and TNF-β] assayed by ELISA, markedly elevated levels of IL-6 and IL-8 were detected in iliac bone marrow serum comparing with those in tibial bone marrow serum and peripheral blood serum as shown in **Table 2** (15). The levels of IL-6 and IL-8 assayed in synovial fluid were usually higher than those in iliac bone marrows and did not show a significant correlation. Data suggested that these cytokines were produced independently in the synovial tissue in addition to those in the iliac bone marrow.

Table 2 Cytokine levels of IL-6, IL-8,and G-CSF in iliac bone marrow serum of the patients with RA and controls (15)

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/m)	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)

III. Synovial fibroblastic stromal cells (FSCs) migrated from epiphyseal bone marrow

We studied the relation between the epiphyseal bone marrow and synovial tissue by using collagen induced arthritis (CIA) in rats (22). We prepared Lewis rats whose fibroblastic stromal cells (FSCs) in bone marrow were partially labeled with fluorescence (FITC) or ^{3}HTdr. After inducing CIA in those rats, the migration of bone marrow FSCs was observed. As shown in **Fig 16**, a large number of labeled FSCs migrated from bone marrow into the joint cavity through canals in the bare zone, and synovial tissue was formed in joint cavity (**Fig 17**).

Fig. 16

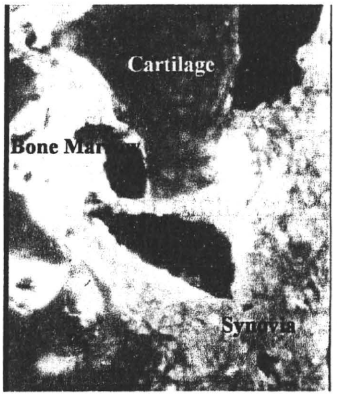


Figure 16. After inducing CIA, FSCs labeled with FITC in bone marrow migrated into synovial space.

Fig 17

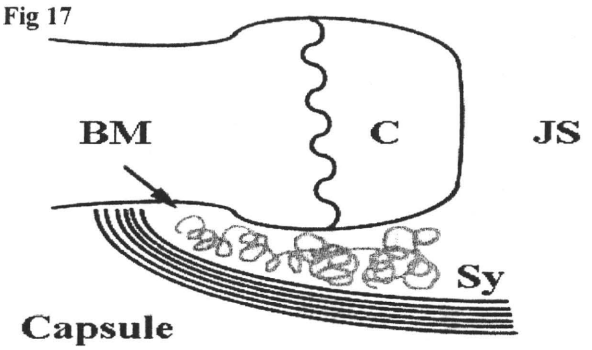


Figure 17. Diagram of Figure 16

Thus, we could have a hypothesis **Fig 18**) that pathophysiological cells of RA differentiated in iliac bone marrow could be transfered by the peripheral circulation to the epiphyseal bone marrow, and then into joint space forming the synovial tissue. Considering these results, it could be reasonable that multipotent mesenchymal stromal cells from bone marrow were found to exsist in synovial membrane.

De Bari et al, Arthritis Rheum, 2001;44:1928
De Bari et al, Arthritis Rheum, 2001;54:209
Jones EA et al, Arthritis Rheum, 2004;50:817
Ogawa M, et al. Exp Hematol. 2010;38:540

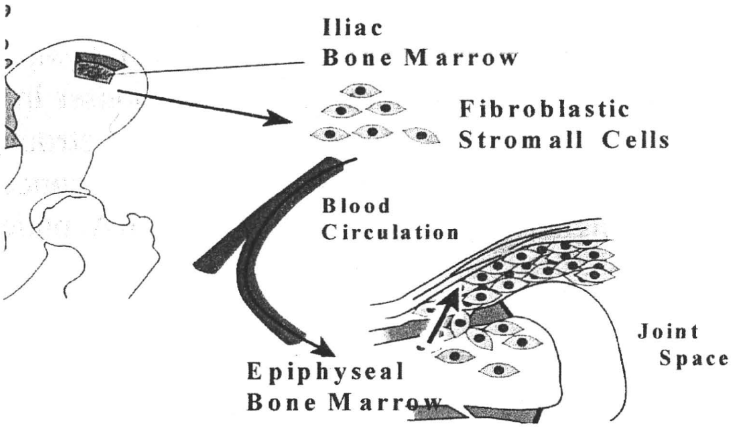


Fig. 18 Suspected transfer of FSCs from iliac bone marrow to synovial tissue

There remained a question why proliferation of FSC was remarkable in synovial tissue, but not in bone marrow. In this regard, soluble FasL (sFasL) was found to inhibit competitively the Fas-FasL mediated

apoptosis (30) of FSC bearing Fas. The levels of human sFasL in synovial fluid from RA patients were found to be significantly higher than those from osteoarthritis (OA) patients. In contrast, sFasL was not detected in the peripheral blood, nor in bone marrow blood in RA patients (31). This mechanism, therefore, could at least partially up-regulate the FSC growth in synovial tissue, but not in bone marrow.

For the persistent severe inflammation in synovial tissue of RA, accumulation and differentiation CD14+ cells were necessary in synovial tissue supported by the chemokines monocyte chemotactic protein-1 and IL-8 were clarified (45). For forming the ectopic lymphoid aggregation with germinal center-like structures, B cell attracting chemokine-1 (BXCL13) was clarified (42).

Ⅳ. Specific changes found in bone marrow of RA patients

1) A pre-B cell growth factor (BST-1/CD157) detected in RA bone marrow fibroblast-like cells

Considering that hematopoietic iliac bone marrow of RA patients could be an important pathological organ, fibroblastic bone marrow stromal cell lines were established by transecting the plasmid for expression of SV40 large T antigen and examined their ability to support the fibroblastic stromal cell dependent growth of pre-B cell line, DW34. The new growth factor was found having ability to enhance DW34 cell growth, and named it bone marrow fibroblastic stromal cell antigen 1 (BST-1) (11, 14) and BST-2 (19) . BST-1/CD157 was expressed in a wide range of tissues and in umbilical vein endothelial cells, whereas it was scarcely expressed in a variety of hematopoietic cell lines. The gene for BST-1 was assigned to chromosome 14q32.3 regulating humoral immune responses in vivo. BST-1 expression was enhanced in bone marrow fibroblastic stromal cell lines from RA patients. By investigating with ELISA system, concentrations of serum sBST-1 were higher (30-50-fold) in 7% of RA patients than in non-RA samples (24).

2) Nurse- like cells found in bone marrow and synovia of RA patients

In studying the pathophysiology of MNCs maintaining longer and/or activating more the inflammation of RA, we found that some FSCs from bone marrow (12) as well as from synovial tissue of RA patients supporting the growth and activation of MNCs co-cultured. Such function of FSCs was reminiscent of thymic nurse cells (Wekerle H et al. 1980) having the capacity to interact with thymocytes in a process known as pseudoemperipolesis (adhesion and transmigration), and support the differentiation and expansion of thymocytes of mice and rats themselves. A