Exp. Anim. 62(3), 255–265, 2013

—Original—

Increase of Circulating CD11b$^+$Gr1$^+$ cells and Recruitment into the Synovium in Osteoarthritic Mice with Hyperlipidemia

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Abstract: Although recent studies suggest that hyperlipidemia is a risk factor for osteoarthritis (OA), the link between OA and hyperlipidemia is not fully understood. As the number of activated, circulating myeloid cells is increased during hyperlipidemia, we speculate that myeloid cells contribute to the pathology of OA. Here, we characterized myeloid cells in STR/Ort mice, a murine osteoarthritis model, under hyperlipidemic conditions. Ratios of myeloid cells in bone marrow, the spleen, and peripheral blood were determined by flow cytometry. To examine the influence of the hematopoietic environment, including abnormal stem cells, on the hematopoietic profile of STR/Ort mice, bone marrow transplantations were performed. The relationship between hyperlipidemia and abnormal hematopoiesis was examined by evaluating biochemical parameters and spleen weight of F_2 animals (STR/Ort x C57BL/6J). In STR/Ort mice, the ratio of CD11b$^+$Gr1$^+$ cells in spleens and peripheral blood was increased, and CD11b$^+$Gr1$^+$ cells were also present in synovial tissue. Splenomegaly was observed and correlated with the ratio of CD11b$^+$Gr1$^+$ cells. When bone marrow from GFP-expressing mice was transplanted into STR/Ort mice, no difference in the percentage of CD11b$^+$Gr1$^+$ cells was observed between transplanted and age-matched STR/Ort mice. Analysis of biochemical parameters in F_2 mice showed that spleen weight correlated with serum total cholesterol. These results suggest that the increase in circulating and splenic CD11b$^+$Gr1$^+$ cells in STR/Ort mice originates from hypercholesterolemia. Further investigation of the function of CD11b$^+$Gr1$^+$ cells in synovial tissue may reveal the pathology of OA in STR/Ort mice.

Key words: hyperlipidemia, myelopoiesis, osteoarthritis, STR/Ort

Introduction

Recent studies have focused on the relationship between primary osteoarthritis (OA) and metabolic factors, including cholesterol, triglyceride, glucose, and adipocytokines [1, 3, 4, 9, 16, 17]. For example, Chaisson *et al*. [3] reported a significant association between women with hand OA and elevated serum cholesterol levels, while Hart *et al.* [9] found that metabolic factors, such as blood glucose and hypercholesterolemia, were linked with the development of knee OA. Based on that evidence, they proposed that the etiology of OA had an important systemic and metabolic component. However, the specific effects of these factors on the mechanisms

(Received 27 December 2012 / Accepted 30 March 2013)
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underlying primary OA remain unclear.

Dyslipidemia increases the number of circulating neutrophils and activates myeloid cell differentiation, which together contribute to cardiovascular disease. Notably, Swirski *et al*. [18] demonstrated that hypercholesterolemia increases circulating inflammatory monocyte counts, while more recently, Mazor *et al*. [14] found that an increased rate of superoxide release and CD11b surface expression were positively correlated with the severity of hyperlipidemia. However, in addition to contributing to cardiovascular disease, myeloid cells can also affect OA pathology. Koch *et al.* [11] reported that monocyte migration into synovial tissue contributes to OA pathology. Based on these findings, we were interested in the effects of hyperlipidemia on OA pathology and the involvement of myeloid cells in OA.

The pathology of OA is often studied in STR/Ort mice, which represent a well-characterized, spontaneous model of OA [13, 15, 25, 26]. Our recent studies have revealed that STR/Ort mice display human hyperlipidemic-like symptoms such as high serum total cholesterol, high serum triglyceride, and hyperinsulinemia [19, 22]. Therefore, investigation of myeloid cell populations in STR/Ort mice may reveal the relationship between OA pathology and hyperlipidemia.

Here, we characterized myeloid cells in peripheral blood, the spleen, bone marrow, and synovial tissue in STR/Ort mice.

Materials and Methods

Animals

Male STR/Ort mice (*H-2*^{b}) aged 8–35 weeks were examined together with age-, sex-, and MHC class II-matched C57BL/6J control mice (*H-2*^{b}) (Charles River Laboratories Japan, Yokohama, Japan). C57BL/6 *TgN (act-EGFP) OsbY01* mice (*H-2*^{b}; referred to as B6.GFP in this study) were bred in our animal facility. (B6.GFP X B6) F_1 mice were used as the source of bone marrow mononuclear cells (BMNCs), as transgenic homozygous cells emitted excessively strong fluorescence such that both negative and positive cells examined by flow cytometry could not be visualized in the identical plotted field [20]. Specific pathogen-free (SPF) colonies of STR/Ort and C57BL/6J mice were maintained at Nippon Charles River Laboratories, Japan (Kanagawa, Japan). The mice were housed in a semi-barrier system with a controlled environment (temperature, 23 ± 2°C; humidity, 55%±10%; lighting, 12-h light/dark cycle) throughout the study. All of the experimental protocols were approved by the Kitasato University School of Medicine Animal Care Committee.

Micro CT analysis

μCT (InspeXio SMX-90CT, Shimadzu Corporation, Tokyo, Japan) was used to assess bone marrow space in distal femoral metaphysis and femoral diaphysis using a 12-μm isotropic voxel size, as previously described [21, 24]. Patella dislocations were also evaluated.

Enumeration of femoral mononuclear cells

After epiphyses were removed from mice, the marrow was flushed with ice-cold Hank's balanced salt solution (HBSS) containing 0.5% bovine serum albumin (BSA) and 0.02% sodium azide. After the depletion of red blood cells by hemolysis, total numbers of BMNCs were counted using an automated cell counter (Countess®, Invitrogen, Tokyo, Japan). Collected BMNCs were then used in flow cytometric analysis.

Measurements of body and spleen weight

At 8, 10, 12, 20, and 35 weeks, C57BL/6J and STR/Ort mice were sacrificed by deep anesthesia with diethyl ether. Body and spleen weights were then measured to calculate the spleen:body weight ratio (reported as mg spleen/100 g body weight). Spleens were then used for flow cytometric analysis.

Histological examination

At 35 weeks, the spleens and knees of C57BL/6J and STR/Ort mice were harvested and fixed in a 4% paraformaldehyde phosphate buffer solution. Fixed spleen tissue was then embedded in paraffin and cut into 5-μm sections [10].

Flow cytometric analysis

BMNCs, splenocytes, and peripheral blood cells (2×10^5 cells) were stained with lineage-specific antibodies (anti-CD4, anti-CD8, anti-B-220, anti-Mac-1, or anti-Gr1; BioLegend, San Diego, CA, USA) in ice-cold HBSS containing 0.5% BSA and 0.02% sodium azide, and were then washed once with HBSS. After washing, stained cells were examined by flow cytometric analyses on a FACSCalibur (Becton Dickinson and Co., Mountain View, CA, USA). $CD11b^+Gr1^+$ cells in bone marrow, the spleen, and peripheral blood were also examined by flow

cytometric analyses.

Isolation and staining of leukocytes from synovial tissue in STR/Ort mice

At 35 weeks, the skin of STR/Ort was removed to confirm the presence of synovial hyperplasia above the patella groove. Synovial tissue above the patella was carefully harvested with a scalpel and digested with 1 mg/ml type I collagenase for 2 h at 37°C, and cells were then stained with antibodies against CD11b, CD45, and Gr1.

Mouse BMC transplantation

BMNCs were collected from B6.GFP and STR/Ort mice by flushing excised femurs and tibias with alpha-minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Eight-week-old B6, B6.GFP, and STR/Ort mice were irradiated using an MBR-1505R X-ray irradiator (Hitachi Medical Corporation, Tokyo, Japan) equipped with a filter (Cu, 0.5 mm; Al, 2 mm) for monitoring the cumulative radiation dose. After the recipient mice received 10.5 Gy irradiation, BMNCs derived from B6.GFP or STR/Ort were intravenously injected into the irradiated mice [20, 23].

Flow cytometric analysis of splenocytes after transplantation

Single-cell suspensions were obtained from the spleens of irradiated mice six months after the intravenous injection of BMNCs. Splenic $CD11b^+Gr1^+$ cells were examined by flow cytometric analyses on a FACSCalibur system (Becton Dickinson and Co.).

Correlation between spleen weight and biochemical parameters in F_2 mice

Male STR/Ort mice were crossed with female C57BL/6J mice to generate F_1 offspring. Male and female F_1 mice were then crossed to generate 58 F_2 offspring. To eliminate the influence of OA pathology on the examined biological parameters, we analyzed F_2 mice 10 weeks before the onset of OA. Spleen weights were measured, and serum total cholesterol, triglyceride, and glucose levels were then assayed using the LabAssay™ series of test kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan) [22].

Distribution of GFP-positive cells in GFP-labeled bone marrow-transplanted STR/Ort mice

Left knees were obtained from five irradiated STR/Ort mice six months after the intravenous injection of B6.GFP-BMNCs. Collected knees were immersed in a carboxymethyl cellulose (CMC) gel, transferred to hexane, and completely frozen using solid CO_2. The frozen femur samples were cut into 6-μm-thick sections using a CM3050 S IV cryomicrotome (Leica Microsystems, Wetzlar, Germany). After the sections were dried, the distribution of GFP-positive cells in the synovium was examined by fluorescence microscopy. The serial sections were also stained with hematoxylin and eosin (HE) using standard procedures (Kureha Special Laboratory Co., Ltd., Tokyo, Japan).

Statistical analysis

All statistical analyses were performed using SPSS software (Version 11.0; SPSS, Inc., Chicago, IL, USA). The unpaired *t*-test was used to examine differences between age-matched C57BL/6J and STR/Ort mice. Pearson's correlation was used to evaluate the correlations between spleen weight and biochemical parameters or the ratio of the $CD11b^+Gr1^+$ population. One-way ANOVA with Tukey's multiple comparison test was used to examine differences in the $CD11b^+Gr1^+$ population ratio after bone marrow transplantation. A P value of <0.05 was considered statistically significant.

Results

Hematopoietic parameters in STR/Ort and C57BL/6J mice

We observed a fourfold reduction in the bone marrow cellularity of STR/Ort mice compared with that of C57BL/6J mice (Fig. 1A), a finding that was most likely due to decreased marrow space in the femur (Fig. 1B). The ratio of $CD11b^+$ and $Gr1^+$ cells was higher and that of $CD4^+$ and $CD8^+$ cells was lower in STR/Ort mice compared with C57BL/6J mice (Table 1). However, a significant decrease in the number of both myeloid and lymphoid cells in bone marrow was detected in STR/Ort mice.

We next attempted to determine if the spleen was functioning as a compensatory site of hematopoiesis in STR/Ort mice. The corrected spleen weight in STR/Ort mice was significantly higher than that in C57BL/6J mice at all examined ages (Fig. 2A). In addition, a pronounced

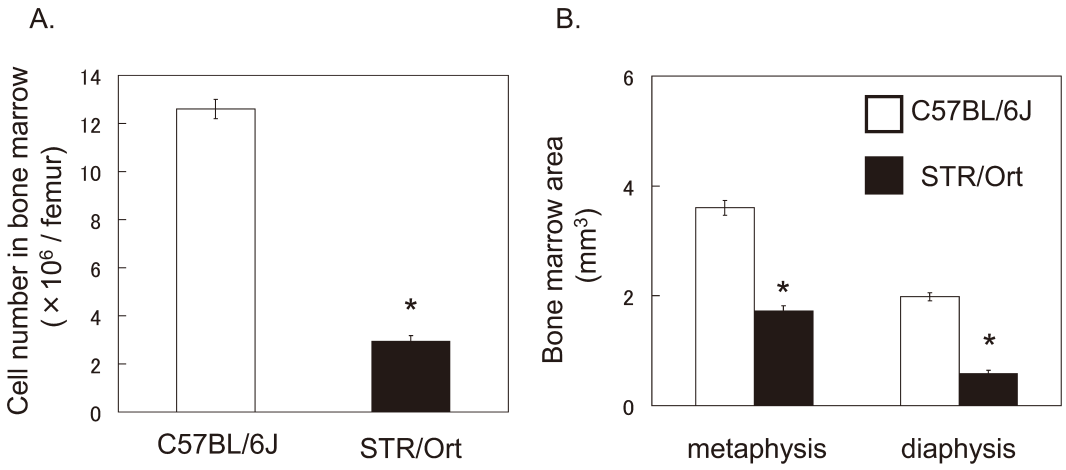

Fig. 1. Reduced bone marrow volume and cell number in the femurs of STR/Ort mice. A. Total cell number in femurs. B. Bone marrow volume in femoral metaphysis and diaphysis. Values are presented as the mean ± SE. *Indicates a significant difference (P<0.05) compared with C57BL/6J mice (n=5).

Table 1. Ratio of hematopoietic lineage cells at 15 weeks in the bone marrow, spleen, and peripheral blood of C57BL6J and STR/Ort mice

		B220	CD4	CD8	CD11b	Gr1	$CD11b^+Gr1^+$
BM	C57BL/6J	42.2 ± 1.2	1.3 ± 0.2	1.4 ± 0.1	48.9 ± 1.1	51.0 ± 0.6	44.99 ± 0.94
	STR/Ort	38.7 ± 0.9*	0.6 ± 0.0*	0.18 ± 0.0*	55.8 ± 0.9*	56.6 ± 1.0*	52.32 ± 1.12*
Spleen	C57BL/6J	51.8 ± 1.6	26.7 ± 1.2	14.0 ± 0.7	8.9 ± 0.4	17.8 ± 1.1	2.09 ± 0.19
	STR/Ort	51.6 ± 3.4	25.9 ± 0.7	10.9 ± 0.7*	10.7 ± 0.9	22.3 ± 0.7*	5.07 ± 0.72*
PB	C57BL/6J	48.5 ± 2.3	13.1 ± 1.3	9.7 ± 0.3	22.7 ± 1.5	18.5 ± 1.4	10.75 ± 1.47
	STR/Ort	35.0 ± 3.6*	10.0 ± 0.8	5.8 ± 0.3*	33.9 ± 2.6*	29.6 ± 3.3*	21.11 ± 2.42*

Ratios of hematopoietic lineage marker-positive cells in bone marrow (BM), spleen, and peripheral blood (PB) of C57BL6J and STR/Ort mice at 15 weeks were determined by flow cytometry (n=5). Values are presented as the mean ± SE. *Indicates a significant difference at P <0.05 compared with C57BL/6J mice.

accumulation of megakaryocytes was observed in the splenic red pulp of STR/Ort mice, a finding that is indicative of extramedullary hematopoiesis (Fig. 2C). The frequency of $CD11b^+Gr1^+$ cells in STR/Ort mice was significantly higher than that in C57BL/6J mice, whereas the number of $CD8^+$ cells was significantly lower. The spleens of STR/Ort mice increased by approximately twofold in size at all examined ages (Fig. 2A), an increase that was likely sufficient to compensate for the loss of bone marrow hematopoiesis. Hematopoiesis in the spleen was reflected in the peripheral blood (PB) of STR/Ort mice, which showed increased numbers of $CD11b^+Gr1^+$ cells and decreased numbers of $CD8^+$ cells compared with C57BL/6J mice (Table 1). In addition, no significant decreases in white and red blood cells, or platelet counts in PB were detected (data not shown).

Increase in $CD11b^+Gr1^+$ cells in STR/Ort mice

We next investigated the population of $CD11b^+Gr1^+$ cells in the bone marrow, spleen, and PB of STR/Ort mice. The ratio of $CD11b^+Gr1^+$ cells in STR/Ort mice was higher than that in C57BL/6J mice in all three hematopoietic tissues analyzed (Table 1). The ratios of splenic $CD11b^+Gr1^+$ cells in STR/Ort mice were also higher than those in C57BL/6J mice at all examined ages (Fig. 2B). In addition, a positive correlation between the ratio of $CD11b^+Gr1^+$ cells and spleen weight was found (Fig. 2B).

Bone marrow transplantation

To determine whether the increase in $CD11b^+Gr1^+$ cells could be reversed by bone marrow transplantation, bone marrow from transgenic GFP mice was transplanted into irradiated C57BL/6J or STR/Ort recipient mice, and the reconstitution efficiency was then monitored.

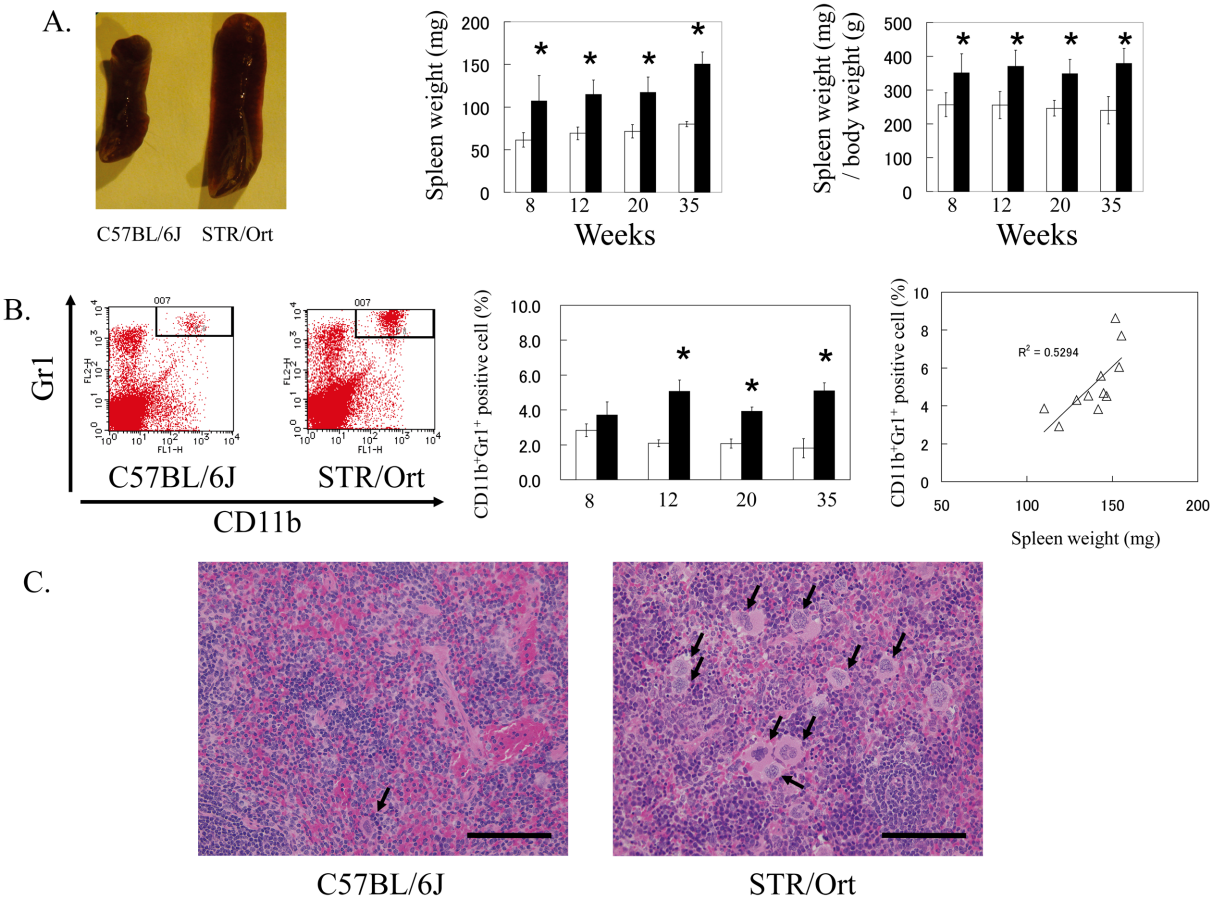

Fig. 2. Phenotypic analysis of the spleen in STR/Ort mice. A. Gross spleen morphology (upper left), spleen weight (upper middle), and spleen weight (mg)/100 g body weight for C57BL/6J (white bars) and STR/Ort mice (black bars) (n=10). B. Dot plot analysis of $CD11b^+Gr1^+$ cells (left), ratio of $CD11b^+Gr1^+$ cells (middle), and correlation between spleen weight and the ratio of $CD11b^+Gr1^+$ cells (left) (n=10). C. Histology of the spleen of C57BL/6J (left) and STR/Ort mice (right). Arrows indicate megakaryocytic cells. The scale bar indicates 100 μm. Values are presented as the mean ± SE. *Indicates a significant difference (P <0.05) compared with age-matched C57BL/6J mice .

Table 2. Percentage of donor-derived splenocytes and $CD11b^+Gr1^+$ cells after bone marrow transplantation

Donor → Recipient	Donor-derived splenocytes	$CD11b^+Gr1^+$ cells
GFP.B6→C57BL/6J	97.1 ± 0.4	1.42 ± 0.13[b]
GFP.B6→STR/Ort	97.3 ± 0.4	6.04 ± 1.21[a]
STR/Ort→ GFP.B6	96.1 ± 0.8	3.08 ± 0.40
Age-matched C57BL/6J	–	1.81 ± 0.17[b]
Age-matched STR/Ort	–	5.10 ± 0.48[a]

Data are presented as the mean ± SE (n=7–8). [a]Significantly differenent compared with age-matched C57BL/6J mice (P<0.05). and [b]Significantly different compared with age-matched STR/Ort mice (P <0.05).

FACS analyses showed that 97% of the spleen cells were GFP positive (Table 2), and that all hematopoietic lineage cells were reconstituted from donor-derived cells (data not shown). No differrences were detected in the ratio of $CD11b^+Gr1^+$ cells between the spleens of GFP bone marrow-transplanted and age-matched C57BL/6J mice (Table 2). $CD11b^+Gr1^+$ cells in the spleens of GFP bone marrow-transplanted STR/Ort mice were signifi-

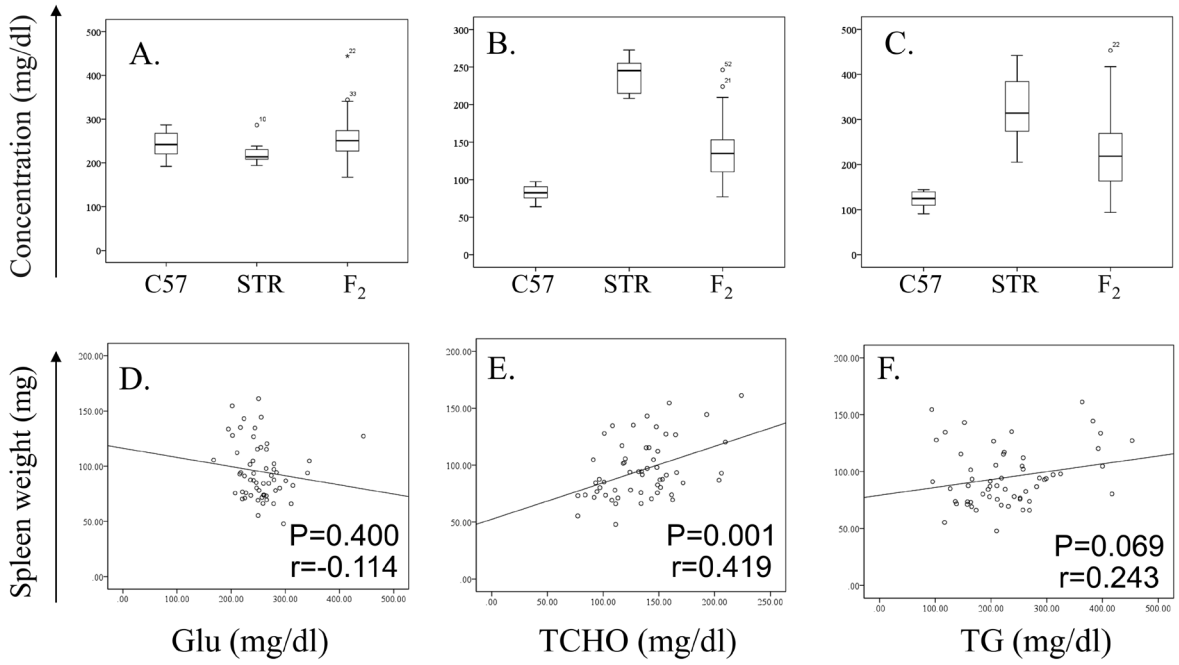

Fig. 3. Correlation between spleen weight and select biochemical parameters in F_2 mice at 10 weeks. A. Serum concentrations of glucose (left), total cholesterol (middle), and triglyceride (right). B. Correlation between spleen weight and glucose (Glu; left), total cholesterol (TCHO; middle), and triglyceride levels (TG; right).

cantly higher than in age-matched and GFP bone marrow-transplanted C57BL/6J mice (P<0.05), whereas no differences were detected between GFP bone marrow-transplanted and age-matched STR/Ort mice (Table 2). For the reverse transplantation, in which STR/Ort bone marrow was transplanted into GFP mice recipients, 96% of the spleen cells were GFP negative (Table 2) and all myeloid and lymphoid lineage cells were reconstituted from donor STR/Ort-derived BMNCs (data not shown). In addition, no differences in the ratio of $CD11b^+Gr1^+$ cells were detected between age-matched C57BL/6J mice and STR/Ort bone marrow-transplanted GFP mice (Table 2).

Analysis of biochemical parameters and spleen weight in F_2 mice

To determine whether hyperlipidemic conditions in STR/Ort mice contributed to abnormal hematopoiesis, we next analyzed several biochemical parameters in F_2 mice. To evaluate large numbers of F_2 mice, spleen weight was measured as an index of extramedullary hematopoiesis and increased $CD11b^+Gr1^+$ cells, because these parameters correlated with spleen weight (Fig. 2B). Serum total cholesterol and triglyceride levels in STR/Ort mice were markedly higher than those in C57BL/6J mice. F_2 mice exhibited a number of phenotypes, which included the presence or absence of hyperlipidemia. The spleen weight of F_2 mice correlated with total serum cholesterol (r=0.419, Pearson's correlation coefficient, P<0.001), but not with triglyceride or glucose levels (Fig. 3).

Analysis of $CD11b^+Gr1^+$ cells in synovial tissue

At 35 weeks of age, patella dislocations were observed in 26% of STR/Ort mice. Synovial hyperplasia above the patella groove was also detected in the dislocated patella of STR/Ort mice (Fig. 4A). Flow cytometric analysis revealed the presence of $CD45^+CD11b^+Gr1^+$ cells in the synovium (Fig. 4B).

Distribution of GFP-positive cells in GFP bone marrow-transplanted STR/Ort mice

To determine whether patella dislocation in STR/Ort mice contributed to the recruitment of circulating cells into the synovium above the patella groove, the distribution of GFP-positive cells in GFP bone marrow-transplanted STR/Ort mice was analyzed. Patella dislocation and synovial hyperplasia in the patellofemoral joint was

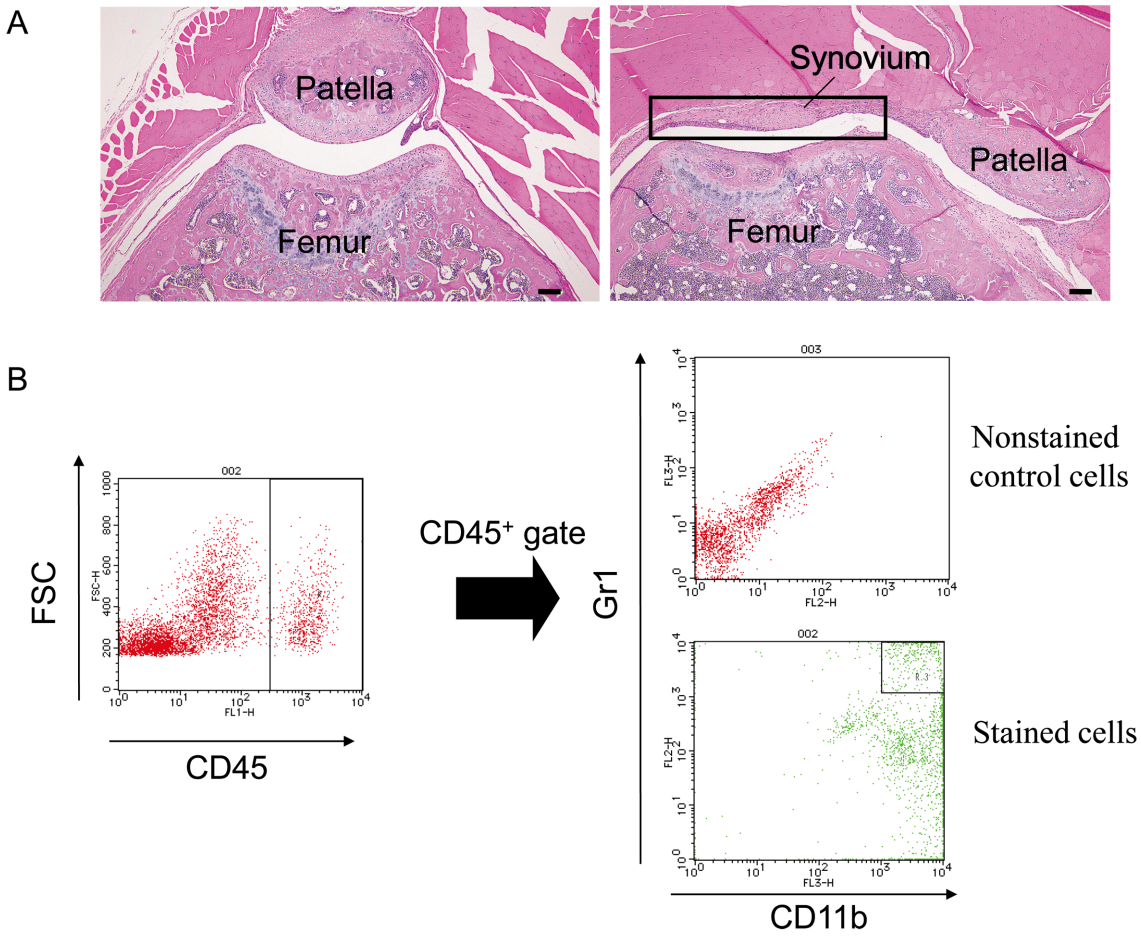

Fig. 4. Analysis of $CD11b^+Gr1^+$ cells in the hyperplastic synovium of STR/Ort mice. A. Histological appearance of the patellofemoral joint, showing a section of the synovium without (left) and with hyperplasia (right). The boxed area indicates synovial hyperplasia above the patella groove. B. Flow cytometry analysis to detect $CD11b^+Gr1^+$ cells in the synovial tissue of STR/Ort mice (n=3). The scale bar indicates 100 μm.

observed in 20% of GFP bone marrow-transplanted STR/Ort mice (Figs. 5A and 5C). In these mice, cartilage destruction was also observed in mice with patella dislocation, in which GFP-positive cells were predominantly located in the synovium above the patella groove (Figs. 5B and 5D). In the remaining eight GFP bone marrow-transplanted STR/Ort mice, patella dislocation, recruitment of GFP cells in the patellofemoral joint, and cartilage destruction were not observed (Figs. 5E–5H). In addition, no marked difference in the ratio of splenic $CD11b^+Gr1^+$ cells was detected between mice with and without patella dislocation (data not shown).

Discussion

In the present study, we showed that STR/Ort mice display extramedullary hematopoiesis and have increased $CD11b^+Gr1^+$ cell populations in the spleen and PB. Bone marrow transplantation experiments and analysis of F_2 mice suggest that these hematopoietic abnormalities in STR/Ort mice are induced by hyperlipidemia. $CD11b^+Gr1^+$ cells were also observed the synovial tissue of STR/Ort mice with patella dislocation. Further functional analysis of $CD11b^+Gr1^+$ cells in the synovium may reveal the relationship between hyperlipidemia and the pathology of OA.

The abnormalities observed in STR/Ort mice, which included larger spleens, increased numbers of megakaryocytic cells, and reduced bone marrow, were remarkably similar to those found in Tie2-Cre-mediated Pparg knockout mice and Pparg $^{hyp/hyp}$ mice. A recent study reported that PPAR-γ signaling is downregulated in male

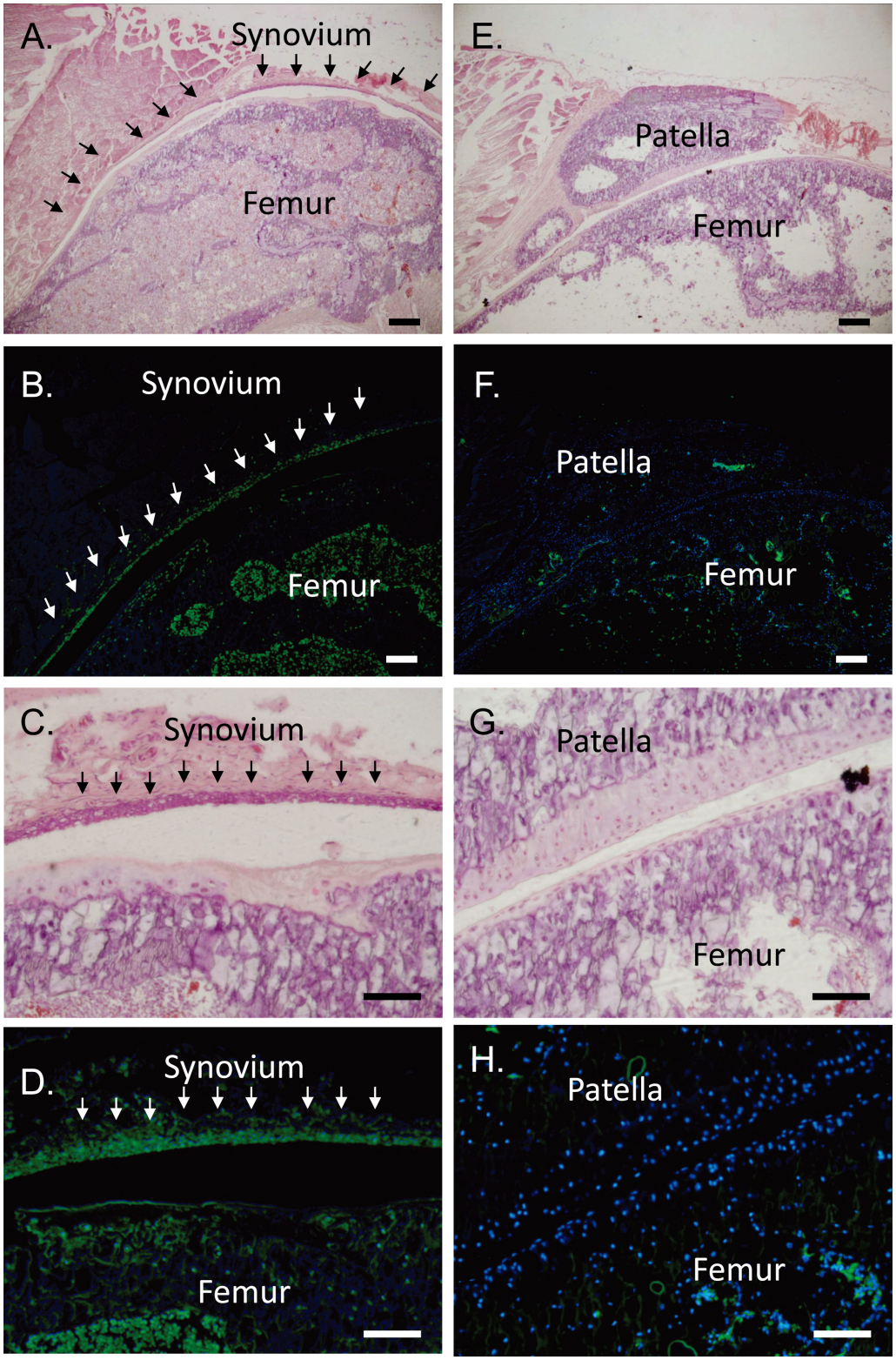

Fig. 5. Histological analysis of GFP-positive cells in the patellofemoral joint in GFP-labeled bone marrow-transplanted STR/Ort mice. Histological appearance of the patellofemoral joint, showing a section of synovium with (left, A-D) and without hyperplasia (right, E–H). (A, C, E, and G) Hematoxylin and eosin-stained sections. (B, D, F, and H) Fluorescence micrographs. Arrowheads indicate synovial hyperplasia above the patella groove. The scale bar indicates 100 μm.

STR/Ort mice [28]. In addition, Pparg$^{hyp/hyp}$ mice, which congenitally lack PPAR-γ in white adipose tissue, and Tie2-Cre-mediated Pparg knockout mice, which lack PPAR-γ in hematopoietic cells, have been found to exhibit splenomegaly and increased megakaryocytic cells and extramedullary hematopoiesis in the spleen [5, 27]. Abnormal lipid signaling in STR/Ort mice may lead to splenomegaly and extramedullary hematopoiesis.

It is well known that hyperlipidemic conditions can alter the hematopoietic cell population and/or function. In our present study, STR/Ort mice also displayed hypercholesterolemia-hypertriglyceridemia properties, similar to the findings of our previous reports [19, 22]. In addition, we found that the $CD11b^+Gr1^+$ cell population increased in the PB and spleen of STR/Ort mice. $CD11b^+Gr1^+$ cells are morphologically heterogeneous, and include neutrophils, macrophages, and early myeloid progenitor cells. Previous studies have reported that $CD11b^+Gr1^+$ cells produce reactive oxygen species and contribute to several diseases, including cardiovascular disease and rheumatoid arthritis [2, 8]. De Guijter *et al.* [7] reported that patients with combined hypercholesterolemia-hypertriglyceridemia have abnormally high numbers of morphologically irregular monocyte-macrophages in the bloodstream. In addition, Apoe knockout mice fed a high-fat diet exhibit hypercholesterolemia and increased $CD45^+CD11b^+Gr1^+$ cells in PB [8]. Here, the spleen weight of F_2 mice (STR/Ort × C57BL6J intercross) at 10 weeks before the onset of OA was also positively correlated with total serum cholesterol levels. Together, our results suggest that the increase in splenic $CD11b^+Gr1^+$ cells in STR/Ort mice was directly related to the observed hyperlipidemic phenotype, rather than the pathology of OA.

To rule out the existence of abnormal hematopoietic stem cells in STR/Ort mice, we performed bone marrow transplantations with GFP transgenic mice and STR/Ort mice. However, no marked differences in the percentage of $CD11b^+Gr1^+$ cells were found between recipients and controls for both groups of mice. These findings indicate that the increase in splenic $CD11b^+Gr1^+$ cells results from hyperlipidemic conditions rather than the existence of abnormal hematopoietic stem cells.

Several types of immune cells, including T cells, B cells, and macrophages, have been observed in the synovium of OA patients with hyperplasia. Based on histological analyses, Das-Gupta *et al*. [6] also reported that greater than 40% of STR/ORT mice exhibit synovial inflammation, with chronic inflammation appearing as diffuse infiltrates of lymphocytes and macrophages scattered throughout the synovia of the affected joint. Using an immunological approach, we found that $CD11b^+Gr1^+$ cells were also present in the synovium above the patella groove of STR/Ort mice with patella dislocation. In addition, histological analysis showed that GFP-positive cells were recruited into the synovium and that cartilage destruction occurred in patellofemoral joints in GFP bone marrow-transplanted STR/Ort mice with patella dislocation. In contrast, synovial hyperplasia, recruitment of GFP-positive cells, and cartilage destruction were not observed in GFP bone marrow-transplanted STR/Ort mice without patella dislocation. Our results suggested that the increase in circulating $CD11b^+Gr1^+$ cells is dependent on hyperlipidemia, while the recruitment of these cells into the synovium and hyperplasia may depend on patella dislocation.

Higher superoxide production by CD11b-positive cells has been observed in hyperlipidemic patients [14]. Markers of low-grade inflammatory and oxidative stress are strongly elevated in the hyperplastic synovium of STR/Ort mice [12]. In addition, Bruhl *et al*. [2] reported that $CD11b^+Gr1^+$ cells are increased in a collagen-induced arthritis model and that these cell populations are depleted in the PB, spleen, and synovial tissue, leading to marked improvements in arthritic symptoms. Further functional analysis of $CD11b^+Gr1^+$ cells in the synovium may reveal the relationship between hyperlipidemia and the pathology of OA.

In conclusion, we have shown the first evidence that circulating and splenic $CD11b^+Gr1^+$ cells are increased in STR/Ort mice with hyperlipidemia. $CD11b^+Gr1^+$ cells were also observed in synovial tissue with hyperplasia. Further investigation of synovial $CD11b^+Gr1^+$ cells may help shed light on the relationship between hyperlipidemia and OA.

Acknowledgments

This investigation was supported in part by Grants-in-Aid from the Ministry of Education, Sports, Culture, Science and Technology of Japan to K. U; by a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan for Research on Human Genome, Tissue Engineering and Food Biotechnology to M. I.; and by research grants from the Parents' Association of Kitasato University School of Medicine. Part of this study was

supported by a grant from Kitasato University Graduate School of Medical Sciences (Integrative Research Program 2012) and a Research Grant for Young Physicians and Health Professionals from SRL, Inc.

References

1. Al-Arfaj, A.S. 2003. Radiographic osteoarthritis and serum cholesterol. *Saudi. Med. J.* 24: 745–747. [Medline]
2. Bruhl, H., Cihak, J., Plachy, J., Kunz-Schughart, L., Niedermeier, M., Denzel, A., Rodriguez, G.M., Talke, Y., Luckow, B., Stangassinger, M., and Mack, M. 2007. Targeting of Gr-1+,CCR2+ monocytes in collagen-induced arthritis. *Arthritis Rheum.* 56: 2975–2985. [Medline] [CrossRef]
3. Chaisson, C.E., Zhang, Y., Sharma, L., Kannel, W., and Felson, D.T. 1999. Grip strength and the risk of developing radiographic hand osteoarthritis: results from the Framingham Study. *Arthritis Rheum.* 42: 33–38. [Medline] [CrossRef]
4. Chen, T.H., Chen, L., Hsieh, M.S., Chang, C.P., Chou, D.T., and Tsai, S.H. 2006. Evidence for a protective role for adiponectin in osteoarthritis. *Biochim. Biophys. Acta.* 1762: 711–718. [Medline] [CrossRef]
5. Cock, T.A., Back, J., Elefteriou, F., Karsenty, G., Kastner, P., Chan, S., and Auwerx, J. 2004. Enhanced bone formation in lipodystrophic PPARgamma(hyp/hyp) mice relocates haematopoiesis to the spleen. *EMBO Rep.* 5: 1007–1012. [Medline] [CrossRef]
6. Das-Gupta, E.P., Lyons, T.J., Hoyland, J.A., Lawton, D.M., and Freemont, A.J. 1993. New histological observations in spontaneously developing osteoarthritis in the STR/ORT mouse questioning its acceptability as a model of human osteoarthritis. *Int. J. Exp. Pathol.* 74: 627–634. [Medline]
7. de Gruijter, M., Hoogerbrugge, N., van Rijn, M.A., Koster, J.F., Sluiter, W., and Jongkind, J.F. 1991. Patients with combined hypercholesterolemia-hypertriglyceridemia show an increased monocyte-endothelial cell adhesion in vitro: triglyceride level as a major determinant. *Metabolism* 40: 1119–1121. [Medline] [CrossRef]
8. Drechsler, M., Megens, R.T., van Zandvoort, M., Weber, C., and Soehnlein, O. 2010. Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. *Circulation* 122: 1837–1845. [Medline] [CrossRef]
9. Hart, D.J., Doyle, D.V., and Spector, T.D. 1995. Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *J. Rheumatol.* 22: 1118–1123. [Medline]
10. Katano, M., Naruse, K., Uchida, K., Mikuni-Takagaki, Y., Takaso, M., Itoman, M., and Urabe, K. 2011. Low intensity pulsed ultrasound accelerates delayed healing process by reducing the time required for the completion of endochondral ossification in the aged mouse femur fracture model. *Exp. Anim.* 60: 385–395. [Medline] [CrossRef]
11. Koch, A.E., Kunkel, S.L., Shah, M.R., Fu, R., Mazarakis, D.D., Haines, G.K., Burdick, M.D., Pope, R.M., and Strieter, R.M. 1995. Macrophage inflammatory protein-1 beta: a C-C chemokine in osteoarthritis. *Clin. Immunol. Immunopathol.* 77: 307–314. [Medline] [CrossRef]
12. Kyostio-Moore, S., Nambiar, B., Hutto, E., Ewing, P.J., Piraino, S., Berthelette, P., Sookdeo, C., Matthews, G., and Armentano, D. 2011. STR/ort mice, a model for spontaneous osteoarthritis, exhibit elevated levels of both local and systemic inflammatory markers. *Comp. Med.* 61: 346–355. [Medline]
13. Mason, R.M., Chambers, M.G., Flannelly, J., Gaffen, J.D., Dudhia, J., and Bayliss, M.T. 2001. The STR/ort mouse and its use as a model of osteoarthritis. *Osteoarthritis Cartilage* 9: 85–91. [Medline] [CrossRef]
14. Mazor, R., Shurtz-Swirski, R., Farah, R., Kristal, B., Shapiro, G., Dorlechter, F., Cohen-Mazor, M., Meilin, E., Tamara, S., and Sela, S. 2008. Primed polymorphonuclear leukocytes constitute a possible link between inflammation and oxidative stress in hyperlipidemic patients. *Atherosclerosis* 197: 937–943. [Medline] [CrossRef]
15. Naruse, K., Urabe, K., Jiang, S.X., Uchida, K., Kozai, Y., Minehara, H., Mikuni-Takagaki, Y., Kashima, I., and Itoman, M. 2009. Osteoarthritic changes of the patellofemoral joint in STR/OrtCrlj mice are the earliest detectable changes and may be caused by internal tibial torsion. *Connect. Tissue Res.* 50: 243–255. [Medline] [CrossRef]
16. Struthers, G.R., Scott, D.L., Bacon, P.A., and Walton, K.W. 1983. Musculoskeletal disorders in patients with hyperlipidaemia. *Ann. Rheum. Dis.* 42: 519–523. [Medline] [CrossRef]
17. Sturmer, T., Sun, Y., Sauerland, S., Zeissig, I., Gunther, K.P., Puhl, W., and Brenner, H. 1998. Serum cholesterol and osteoarthritis. The baseline examination of the Ulm Osteoarthritis Study. *J. Rheumatol.* 25: 1827–1832. [Medline]
18. Swirski, F.K., Libby, P., Aikawa, E., Alcaide, P., Luscinskas, F.W., Weissleder, R., and Pittet, M.J. 2007. Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *J. Clin. Invest.* 117: 195–205. [Medline] [CrossRef]
19. Uchida, K., Naruse, K., Ogawa, Z., Suto, K., Urabe, K., and Takaso, M. 2011. Elevation of pancreatic oxidative stress in STR/Ort mice. *J. Appl. Anim. Res.* 39: 149–152. [CrossRef]
20. Uchida, K., Urabe, K., Naruse, K., and Itoman, M. 2010. Umbilical cord blood-derived mesenchymal cell fate after mouse umbilical cord blood transplantation. *Transplantation* 90: 1037–1039. [Medline] [CrossRef]
21. Uchida, K., Urabe, K., Naruse, K., Kozai, Y., Onuma, K., Mikuni-Takagaki, Y., Kashima, I., Ueno, M., Sakai, R., Itoman, M., and Takaso, M. 2012. Differential Age-Related Bone Architecture Changes between Female and Male STR/Ort Mice. *Exp. Anim.* 61: 59–66. [Medline] [CrossRef]
22. Uchida, K., Urabe, K., Naruse, K., Ogawa, Z., Mabuchi, K., and Itoman, M. 2009. Hyperlipidemia and hyperinsulinemia in the spontaneous osteoarthritis mouse model, STR/Ort. *Exp. Anim.* 58: 181–187. [Medline] [CrossRef]
23. Ueno, M., Uchida, K., Takaso, M., Minehara, H., Suto, K., Takahira, N., Steck, R., Schuetz, M.A., and Itoman, M. 2011a. Distribution of Bone Marrow-Derived Cells in the Fracture Callus during Plate Fixation in a Green Fluorescent Protein-Chimeric Mouse Model. *Exp. Anim.* 60: 455–462. [Medline] [CrossRef]
24. Ueno, M., Urabe, K., Naruse, K., Uchida, K., Minehara, H.,

Yamamoto, T., Steck, R., Gregory, L., Wullschleger, M.E., Schuetz, M.A., and Itoman, M. 2011b. Influence of internal fixator stiffness on murine fracture healing: two types of fracture healing lead to two distinct cellular events and FGF-2 expressions. *Exp. Anim.* 60: 79–87. [Medline] [CrossRef]

25. Walton, M. 1977a. Degenerative joint disease in the mouse knee; histological observations. *J. Pathol.* 123: 109–122. [Medline] [CrossRef]
26. Walton, M. 1977b. Degenerative joint disease in the mouse knee; radiological and morphological observations. *J. Pathol.* 123: 97–107. [Medline] [CrossRef]
27. Wan, Y., Chong, L.W., and Evans, R.M. 2007. PPAR-gamma regulates osteoclastogenesis in mice. *Nat. Med.* 13: 1496–1503. [Medline] [CrossRef]
28. Watters, J.W., Cheng, C., Pickarski, M., Wesolowski, G.A., Zhuo, Y., Hayami, T., Wang, W., Szumiloski, J., Phillips, R.L., and Duong, T. 2007. Inverse relationship between matrix remodeling and lipid metabolism during osteoarthritis progression in the STR/Ort mouse. *Arthritis Rheum.* 56: 2999–3009. [Medline] [CrossRef]