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CD11c$^+$ macrophages and levels of TNF-α and MMP-3 are increased in synovial and adipose tissues of osteoarthritic mice with hyperlipidaemia

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Summary

To understand more clearly the link between osteoarthritis and hyperlipidaemia, we investigated the inflammatory macrophage subsets and macrophage-regulated matrix metalloprotease-3 (MMP-3) and A disintegrin and metalloprotease with thrombospondin motifs-4 (ADAMTS4) in synovial (ST) and adipose tissues (AT) of osteoarthritic mice with hyperlipidaemia (STR/Ort). CD11c$^+$F4/80$^+$CD11b$^+$ macrophage populations in the ST and AT of 9-month-old STR/Ort and C57BL/6J mice were characterized and compared by flow cytometry and real-time polymerase chain reaction (PCR) analyses. Expression of tumour necrosis factor (TNF)-α, MMP-3 and ADAMTS4, and the response of these factors to anionic liposomal clodronate induced-macrophage depletion were also evaluated by real-time PCR. Expression of TNF-α in CD11c$^+$ cells, which were isolated by magnetic beads, was compared to CD11c$^-$ cells. In addition, the effect of TNF-α on cultured synovial fibroblasts and adipocytes was investigated. CD11c$^+$F4/80$^+$CD11b$^+$ macrophages were increased in ST and AT of STR/Ort mice. The CD11c$^+$ cell fraction highly expressed TNF-α. Expression of TNF-α and MMP3 was increased in ST and AT, and was decreased upon macrophage depletion. TNF-α treatment of cultured synovial fibroblasts and adipocytes markedly up-regulated MMP-3. CD11c$^+$F4/80$^+$CD11b$^+$ macrophages were identified as a common inflammatory subset in the AT and ST of STR/Ort mice with hyperlipidaemia. The induction of inflammation in AT and ST may be part of a common mechanism that regulates MMP3 expression through TNF-α. Our findings suggest that increased numbers of CD11c$^+$ macrophages and elevated levels of TNF-α and MMP-3 in AT and ST may explain the relationship between hyperlipidaemia and OA.

Keywords: hyperlipidaemia, macrophage, matrix metalloprotease-3, osteoarthritis, tumour necrosis factor-α

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Introduction

Several recent studies have examined the relationship between primary osteoarthritis (OA) and metabolic factors, including cholesterol, triglyceride, glucose and adipocytokines [1–4]. For example, Sturmer *et al.* [4] reported that hypercholesterolaemia is associated with generalized OA, and Hart *et al.* [1] found that several metabolic factors, such as blood glucose and cholesterol, are linked to the development of knee OA. These findings suggest that the aetiology of OA involves important systemic and metabolic components. However, the specific effects of these factors, particularly hyperlipidaemia, on the mechanisms underlying primary OA remain unclear.

Dyslipidaemia increases the number of circulating myeloid cells [5,6] which, when recruited into adipose tissue (AT), differentiate into macrophages that contribute to inflammation and insulin resistance upon activation [7–9]. Synovial inflammation has been implicated in many of the signs and symptoms of OA, including joint swelling and effusion. Histologically, OA synovium typically exhibits hyperplasia characterized by an increased number of lining cells and macrophages in the infiltrate [10]. Because macrophages in OA synovium produce proinflammatory cytokines and destructive mediators [11,12], we speculate that an increase of activated macrophages in AT and synovial tissue (ST) may contribute to

the observed relationship between OA development and hyperlipidaemia.

Accumulating evidence suggests that matrix metalloproteases (MMPs) and disintegrin and metalloprotease with thrombospondin motifs [A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) proteins] are important co-factors and mediators of insulin resistance [13,14] and OA [15–17]. Macrophages and macrophage-produced cytokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β, modulate the function of MMP-3 and ADAMTS4 [15,18–20]. For example, the treatment of human adipocytes with macrophage-conditioned medium markedly increases the expression of MMP1 and MMP3 [20]. Several MMP-3 and ADAMTS4 proteins that are found commonly in synovial fibroblast cultures are also regulated by macrophages and the cytokines IL-1β and TNF-α [15]. In collagenase-induced OA mice, macrophage depletion reduces the expression of MMP2, -3, and -9 in ST [18]. Based on these findings, we speculated that MMP-3 and ADAMTS4 are common disease mediators, and that examining their regulation by macrophage and macrophage-produced cytokines may reveal the relationship between hyperlipidaemia and OA.

The STR/Ort mouse is a well-characterized, spontaneous model of OA [21–24]. Our recent studies have revealed that STR/Ort mice display human hyperlipidaemic-like symptoms, including high serum total cholesterol and triglyceride and hyperinsulinaemia [25,26]. Recently, we reported that myeloid cell populations are increased in the peripheral blood and spleen of STR/Ort mice, and are also recruited into ST [6]. Therefore, characterization of macrophage populations in ST and AT in STR/Ort is expected to provide insight into the relationship between OA pathology and hyperlipidaemia.

Here, we characterized the inflammatory macrophages and expression profiles of MMP-3 and ADAMTS4 in AT and ST of STR/Ort mice. In addition, we investigated whether macrophage depletion by systemic injection of clodronate-laden liposomes decreases local inflammation in AT and ST.

Materials and methods

Animals

Male STR/Ort mice aged 35 weeks were examined together with age- and sex-matched C57BL/6J control mice (Charles River Laboratories, Inc., Yokohama, Japan). Specific-pathogen free (SPF) colonies of STR/Ort and C57BL/6J mice were maintained at Nippon Charles River Laboratories (Kanagawa, Japan). The mice were housed in a semi-barrier system with a controlled environment (temperature: 23 ± 2 °C; humidity: $55 \pm 10\%$; lighting: 12-h light/dark cycle) throughout the study. All the experimental protocols were approved by the Kitasato University School of Medicine Animal Care Committee.

Isolation and staining of leucocytes from adipose and synovial tissue of STR/Ort mice

C57BL/6J and STR/Ort mice were killed by deep anaesthesia and skin was then removed for the harvesting of ST with a scalpel. The harvested ST was digested with 1 mg/ml type I collagenase for 2 h at 37 °C [6]. Perigonadal fat was also harvested from mice and was digested with collagenase D solution (2 mg/ml) (Roche Diagnostics, Indianapolis, IN) for 1–1·5 h at 37 °C [27]. The released cells were stained with antibodies against F4/80, CD11b and CD11c, and 7-amino actinomycin D (7AAD) staining was used to identify dead cells.

Real-time polymerase chain reaction (PCR)

Total RNA was extracted from harvested ST and AT using TRIzol (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions, and was used as a template for first-strand cDNA synthesis using SuperScript III RT (Invitrogen). The PCR reaction mixture consisted of 2 μl cDNA, specific primer set (0·2 μM final concentration) and 12·5 μl SYBR Premix Ex *Taq*™ (Takara, Kyoto, Japan) in a final volume of 25 μl. The PCR primer pairs sequences are listed in Table 1. Quantitative PCR was performed using a real-time PCR detection system (CFX-96; Bio-Rad, Hercules, CA, USA). The PCR cycle parameters consisted of an initial denaturation at 95 °C for 1 min followed by 40 cycles of 95 °C for 5 s, and 60 °C for 30 s mRNA expression was normalized to the levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA.

Macrophage depletion by injection of clodronate-laden liposomes

To investigate the effects of macrophages on MMP-3 and ADAMTS4 expression, anionic liposomal clodronate was administered systemically to STR/Ort mice by intraperitoneal injection. After 24 h, ST and AT were harvested as described above and the expression of TNF-α, MMP-3 and ADAMTS4 was analysed by real-time PCR. Cells in ST and AT were harvested as described above and were stained with antibodies against F4/80, CD11b and CD11c.

Isolation of CD11c-positive cells in ST and AT

At 35 weeks of age, 10 ST samples were harvested from the bilateral knees of five STR/Ort mice. AT samples were harvested from the perigonadal fat of one STR/Ort mouse. Mononuclear cells were isolated from ST and AT by digestion with type I and D collagenase, respectively. Prior to the isolation of CD11c-positive cells, MACS® columns (25 LD columns; Miltenyi Biotec, Bergisch Gladbach, Germany) were filled with alpha-minimum essential medium (α-MEM) warmed to 37 °C. ST-derived mononuclear cells

Table 1. Sequences of primers used in this study

Gene	Direction	Primer sequence (5′–3′)	Product size (bp)
CD11c	F	TTC TTC TGC TGT TGG GGT TTG	132
	R	CAA CCA CCA CCC AGG AAC TAT	
TNF-α	F	CTG AAC TTC GGG GTG ATC GG	122
	R	GGC TTG TCA CTC GAA TTT TGA GA	
MMP-3	F	GTC CTC CAC AGA CTT GTC CC	102
	R	AGG ACA TCA GGG GAT GCT GT	
ADAMTS4	F	CTG GGT ATG GCT GAT GTG GG	165
	R	CCC CTG CCC ATT CAA GTT AGT	
GAPDH	F	AAC TTT GGC ATT GTG GAA GG	223
	R	ACA CAT TGG GGG TAG GAA CA	

F = forward; R = reverse; MMP3 = matrix metalloprotease-3; TNF = tumour necrosis factor; ADAMTS4 = A disintegrin and metalloproteinase with thrombospondin motifs 4; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

were suspended in 500 μl phosphate-buffered saline (PBS) containing biotinylated anti-CD11c antibody and then incubated for 30 min at 4 °C. The cells were washed once with PBS, resuspended in 1 ml anti-biotin microbeads, and then loaded onto columns held in a Quadro MACS® magnetic support. Warmed (37 °C) culture medium was then added to the column to collect unbound (CD11c-negative) cells. The column was removed from the magnetic support and an additional 4 ml of α-MEM was added to collect CD11c-positive cells. The collected CD11c-positive and -negative cells were centrifuged at 300 ***g*** for 10 min. The supernatants were removed and cell pellets were then used directly for RNA isolation, as described above. CD11c, TNF-α, MMP-3 and ADAMTS4 expression in both cell types was analysed by reverse transcription (RT)–PCR. The experiment was performed three times.

Effect of TNF-α on ST-derived fibroblasts and cultured adipocytes

Cells in ST were harvested as described above. ST-derived mononuclear cells were suspended in 500 μl phosphate-buffered saline (PBS) containing biotinylated anti-CD45 antibody and then incubated for 30 min at 4 °C. The cells were washed once with PBS, resuspended in 1 ml anti-biotin microbeads and then loaded onto columns (25 LD columns; Miltenyi Biotec) held in a Quadro MACS® magnetic support. Warmed (37 °C) culture medium was added to the column to collect unbound (CD45-negative) cells, which were then cultured in α-MEM in six-well plates. 3T3-L1 cells were cultured to confluence in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) calf serum. At 2 days post-confluence (designated day 0), cells were induced to differentiate with DMEM supplemented with 10% (v/v) fetal bovine serum (FBS), 0·25 μM dexamethasone (Sigma, St Louis, MO, USA), 0·5 mM isobutylmethylxanthine (Sigma) and 5 μg/ml insulin (Novo Nordisk A/S). After 2 weeks, synovial fibroblast and 3T3L1 cells were incubated with 0, 2·5 and 25 ng/ml mouse recombinant TNF-α (Biolegend, San Diego, CA, USA) for 24 h. Cells were then harvested for RNA isolation, as described above, and MMP-3 and ADAMTS4 expression in both cell types was analysed by RT–PCR. The experiment was performed four times.

Statistical analysis

All statistical analyses were performed using SPSS software version 11.0 (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) with Tukey's multiple comparison test was used to examine differences in gene expression. A *P*-value of $<0{\cdot}05$ was considered statistically significant.

Results

Expression of CD11c, TNF-α, MMP-3 and ADAMTS4 in AT and ST of STR/Ort mice

Flow cytometric analysis of macrophage populations in the ST of STR/Ort mice revealed that both $F4/80^+CD11b^+$ (Fig. 1a-1,a-2,a-5) and $CD11c^+$ macrophages (Fig. 1a-3,a-4,a-6) were increased significantly compared to the levels found in C57BL/6J mice. The percentage of $F4/80^+CD11b^+$ macrophages in the AT of STR/Ort was lower than that found in the AT of C57BL/6J (Fig. 1b-1,b-2, b-5); however, the percentage of $CD11c^+$ macrophages was significantly higher than that in C57BL/6J (Fig. 1b-3,b-4,b-6). Real-time PCR analysis also showed that the expression of CD11c was elevated significantly in AT and ST of STR/Ort mice (Fig. 2a,e). The expression of the TNF-α was also increased significantly in the ST and AT of STR/Ort mice compared to that in C57BL/6J (Fig. 2b,f, respectively). Expression of MMP-3 and ADAMTS4 was also increased significantly in ST and AT of STR/Ort mice (Fig. 2c,d,g,h).

Effect of macrophage depletion on the expression of CD11c, TNF-α, MMP-3 and ADAMTS4

The induction of macrophage depletion by the injection of liposomal clodronate tended to decrease the number of $F4/80^+CD11b^+$ cells (Fig. 3a-1,a-2,a-5) and decreased significantly the number of $F4/80^+CD11b+CD11c$ cells in ST

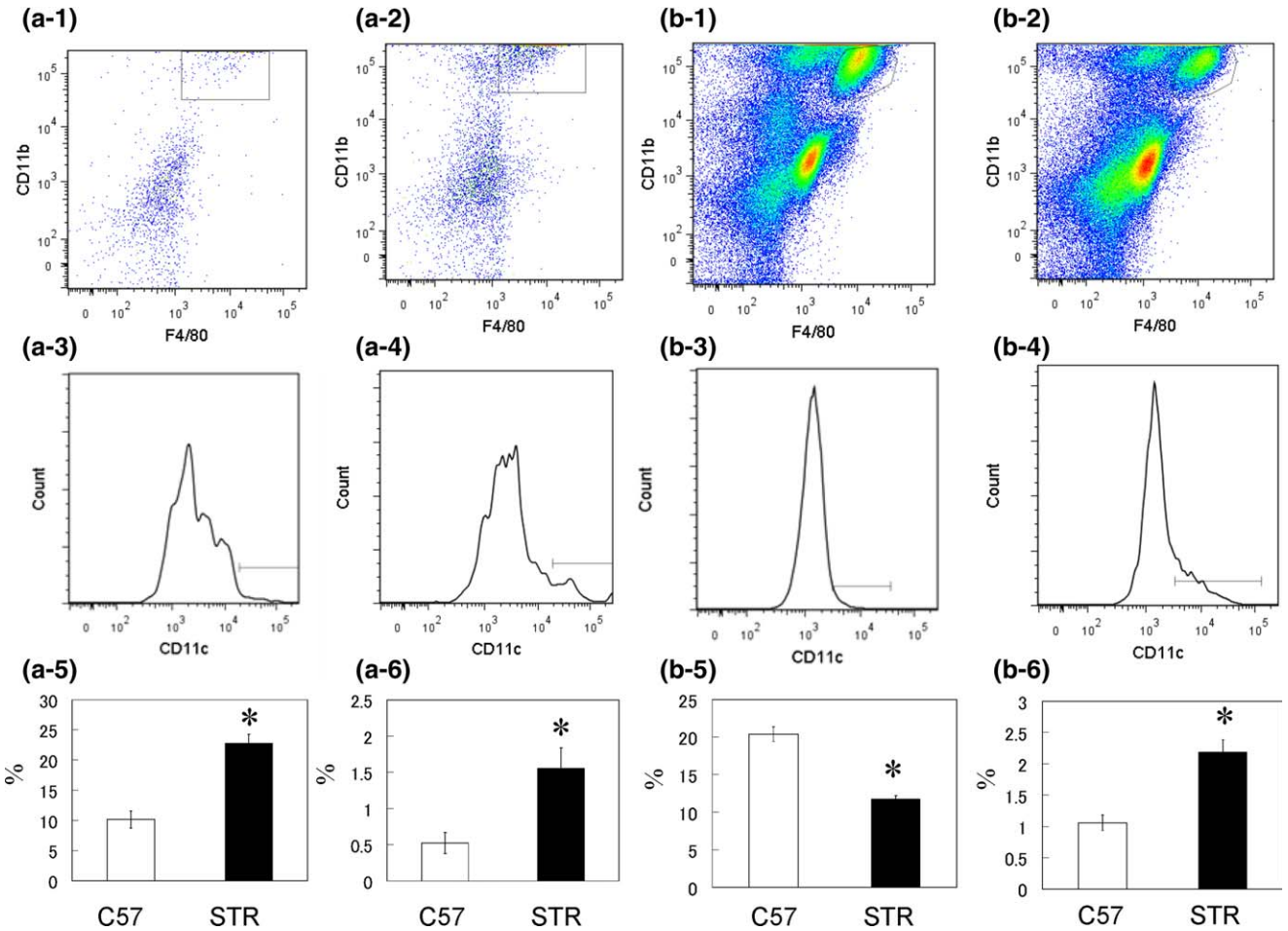

Fig. 1. Flow cytometric analysis of $CD11c^+F4/80+CD11b$ macrophage cells in the synovial tissue (ST) and adipose tissue (AT) of STR/Ort (STR) and C57BL/6J (C57) mice. (a-1,-2, b-1,-2) Dot-plot analysis of $F4/80^+CD11b^+$ cells in ST of C57 (a-1) and STR (a-2) and AT of C57 (b-1) and STR (b-2); *x*-axis, CD11; *y*-axis, F4/80. (a-3,-4, b-3,-4) Histogram analysis of $CD11c^+$ in cells in gated regions in the dot-plot in ST of C57 (a-3) and STR (a-4) and AT of C57 (b-3) and STR (b-4). Percentage of F4/80- and CD11b-positive cells in ST of C57 and STR (a-5) and AT of C57 and STR (b-5) in gated regions of the dot plot ($n = 5$). Percentage of $CD11c^+$ cells in F4/80- and CD11b-positive gated regions is shown in a-6 (AT) and b-6 (AT) ($n = 5$).

of STR/Ort mice (Fig. 3a-3,a-4,a-6). Macrophage depletion also led to a significant decrease of $F4/80^+CD11b^+$ (Fig. 3b-1,b-2,b-5) and $CD11c^+F4/80^+CD11b^+$ cells (Fig. 3b-3,b-4,b-6) in AT of STR/Ort mice, and significant down-regulation of CD11c and TNF-α expression in both ST and AT of STR/Ort mice (Fig. 4a,b,e,f). Expression of MMP-3 in ST and AT of STR/Ort was suppressed by macrophage depletion (Fig. 4c,g). Similarly, ADAMTS4

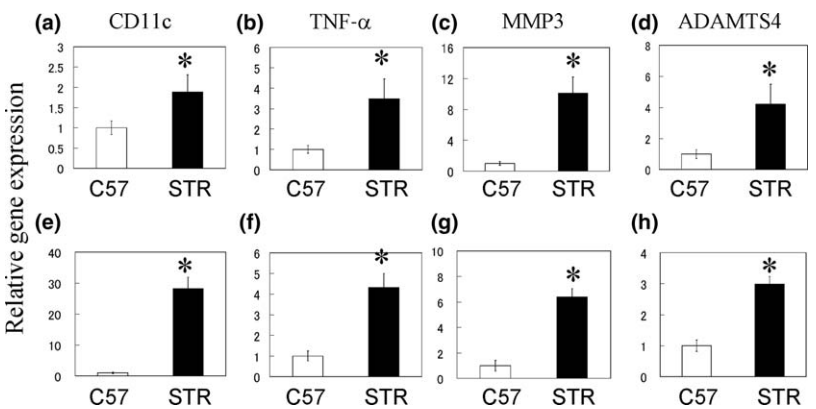

Fig. 2. Real-time polymerase chain reaction (PCR) analysis for the expression of the CD11c, tumour necrosis factor (TNF)-α, matrix metalloprotease-3 (MMP-3) and A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) in synovial (ST; a–d) and adipose tissues (AT; e–h) of C57BL/6J (C57) and STR/Ort (STR) mice. *Statistically significant difference between C57 and STR mice. All data are presented as the mean ± standard error (s.e.) ($n = 10$).

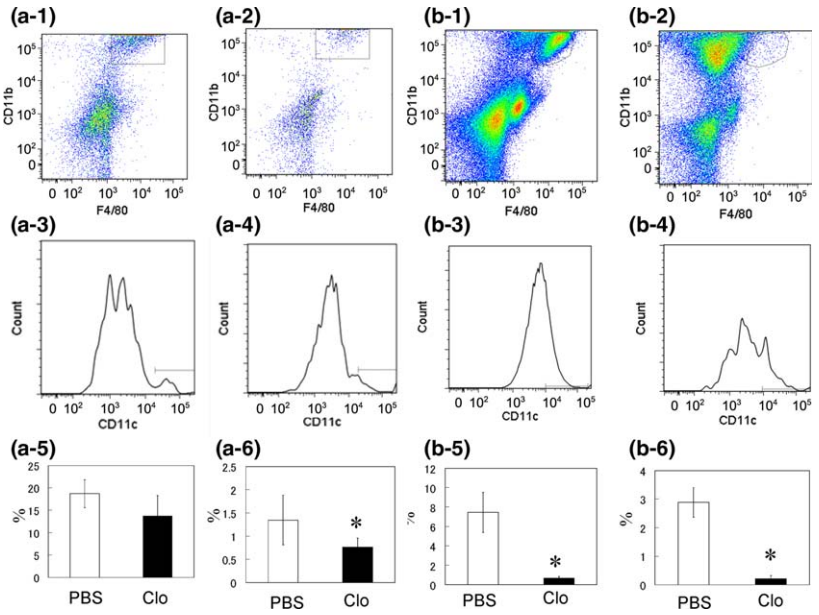

Fig. 3. Flow cytometric analysis of $CD11c^+F4/80^+CD11b$ macrophage cells in synovial tissue (ST) and adipose tissue (AT) of phosphate-buffered saline (PBS)-injected STR/Ort mice [phosphate-buffered saline (PBS)] and clodronate-injected STR/Ort mice (Clo). (a-1,-2, b-1,-2) Dot-plot analysis of $F4/80^+CD11b^+$ cells in ST of PBS (a-1) and Clo (a-2) and AT of PBS (b-1) and Clo (b-2); *x*-axis, CD11; *y*-axis, F4/80. (a-3,-4, b-3,-4) Histogram analysis of $CD11c^+$ in cells in gated regions in the dot-plot in ST of PBS (a-3) and Clo (a-4) and AT of PBS (b-3) and Clo (b-4). Percentage of F4/80- and CD11b-positive cells in ST of PBS and Clo (a-5) and AT of PBS and Clo (b-5) in gated regions of the dot-plot ($n = 5$). Percentage of $CD11c^+$ cells in F480- and CD11b-positive gated regions is shown in a-6 (ST) and b-6 (AT) ($n = 5$).

expression in ST of STR/Ort was suppressed significantly by macrophage depletion (Fig. 4d). However, macrophage depletion had no significant effect on the expression of ADAMTS4 in AT (Fig. 4h).

CD11c and TNF-α expression of in CD11c-positive fractions of ST and AT

CD11c-positive macrophages in AT produce markedly higher levels of TNF-α compared to CD11c-negative cells [28]. To evaluate whether CD11c-positive cells in ST also produce TNF-α, the expression of TNF-α and CD11c in CD11c-positive cells isolated from ST and AT of STR/Ort mice was examined by real-time PCR. CD11c expression in CD11c-positive cell fractions of ST and AT was 6.9- and 3.5-fold higher, respectively, than in CD11c-negative cells (Fig. 5a,e). Similarly, TNF-α expression in CD11c-positive cells was 8·1- and 7·1-fold higher, respectively, than in CD11c-negative cell fractions (Fig. 5b,f). In contrast, expression of MMP-3 and ADAMTS4 in the CD11c-positive cell fraction was lower than that in the CD11c-negative cell fraction (Fig. 5c,d,g,h).

Effect of TNF-α on expression of ADAMTS4 and MMP-3 in synovial fibroblasts and adipocytes

Expression of MMP-3 increased significantly in synovial fibroblasts and adipocytes in the presence of both low and high concentrations of exogenously added TNF-α (Fig. 6a,c). ADAMTS4 expression also increased significantly in synovial fibroblasts in the presence of low and high concentrations of TNF-α (Fig. 6b). In contrast, expression of ADAMTS4 in adipocytes was increased only at the higher TNF-α concentration, and was not affected at the lower concentration (Fig. 6d).

Discussion

In our study of the mechanisms underlying primary OA, we found that $CD11c^+$ populations of macrophages are increased in the ST and AT of STR/Ort mice. Higher

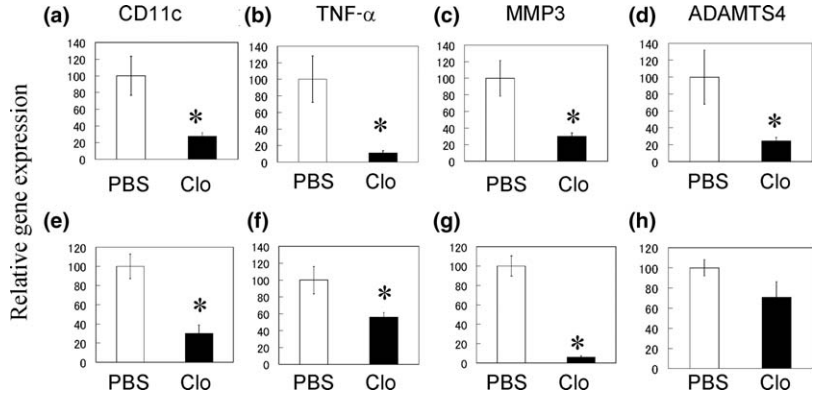

Fig. 4. Real-time polymerase chain reaction (PCR) analysis for the expression of the CD11c, tumour necrosis factor (TNF)-α, matrix metalloprotease-3 (MMP-3) and A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) in synovial (ST; a–d) and adipose tissue (AT; e–h) of phosphate-buffered saline (PBS)-injected STR/Ort (PBS) and clodronate-injected STR/Ort (Clo) mice. *Statistically significant difference between PBS and Clo mice. All data are presented as the mean ± standard error (s.e.) ($n = 10$).

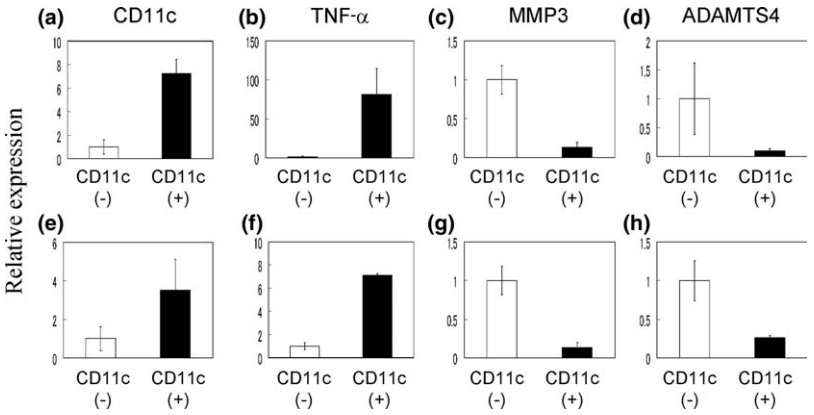

Fig. 5. Expression of CD11c, tumour necrosis factor (TNF)-α, matrix metalloprotease-3 (MMP-3) and A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) in CD11c-negative and -positive cell fractions of synovial (ST) and adipose tissues (AT) of STR/Ort mice. All data are presented as the mean standard error (s.e.) of three experiments ($n = 3$).

expression of TNF-α, MMP-3 and ADAMTS4 was also observed in both ST and AT. Macrophage depletion by treatment of STR/Ort mice with clodronate-laden liposomes decreased TNF-α and MMP-3 in ST and AT. In addition, TNF-α stimulated MMP-3 expression in cultured synovial fibroblasts and adipocytes. Taken together, our findings suggest that $CD11c^+F4/80^+CD11b^+$ macrophages are a common inflammatory subset and regulate TNF-α and MMP-3 expression in ST and AT. Increased numbers of macrophages and elevated levels of TNF-α and MMP-3 may explain the relationship between hyperlipidaemia and OA.

Activated macrophages are observed frequently in the AT and ST of OA patients and obese murine models. Adipose tissue macrophages (ATMs), which have been well characterized in previous studies, consist of at least two different phenotypes: classically activated M1 macrophages and alternatively activated M2 macrophages, both of which display increased activation in hyperlipidaemic mice [[28]]. M1 ATMs, which express CD11c, produce a number of proinflammatory cytokines, including TNF-α, IL-6 and monocyte chemoattractant protein (MCP)-1, and therefore contribute to the induction of insulin resistance [29–31]. In human and animal OA models macrophages are also activated in ST; however, the inflammatory macrophage subset in these tissues has not been fully defined. A recent study reported that the levels of M2 macrophages are elevated in OA patients compared to those found in rheumatoid arthritis patients, although the relative levels in healthy controls were not compared

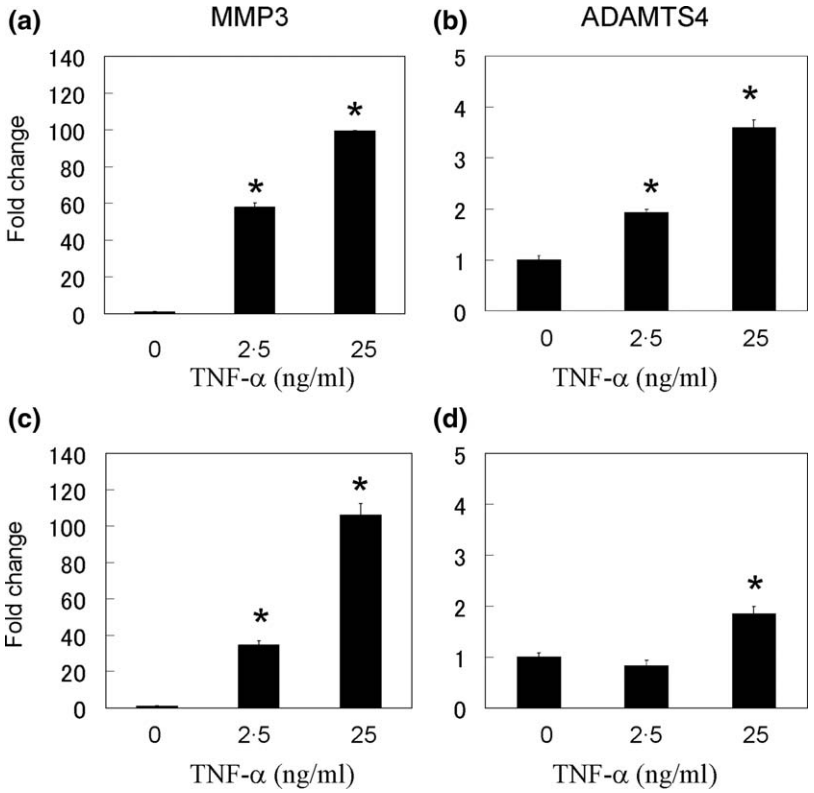

Fig. 6. Effect of TNF-α on matrix metalloprotease-3 (MMP-3) and A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) expression in cultured synovial fibroblasts and adipocytes. Expression of MMP-3 in synovial fibroblasts (a) and adipocytes (c); expression of ADAMST4 in synovial fibroblasts (b) and adipocytes (d). All data are presented as the mean ± standard error (s.e.) of four experiments ($n = 4$).

[32]. In the present study, the levels of $CD11c^+F4/80^+CD11b^+$ macrophages and TNF-α expression in STR/Ort mice were higher than those in C57BL/6J mice in both AT and ST. Notably, macrophage depletion reduced the expression of both CD11c and TNF-α. TNF-α was also elevated significantly in $CD11c^+$ cells in ST and AT compared to the levels detected in $CD11c^-$ cells. The $CD11c^+$ macrophage subset exhibited an M1 phenotype and was increased in ST and AT. Together, these findings suggest that $CD11c^+$ macrophages are a common TNF-α-producing subset in both AT and ST of OA mice with hyperlipidaemia.

MMP-3 has degradative effects on the extracellular matrix, and has been suggested to function as an important mediator in metabolic diseases and OA [16,18,29,33–36]. In OA patients, plasma MMP-3 levels correlate closely with joint narrowing [35], and MMP-3 expression is increased in the AT of high-fat diet mice [33,37] and in the ST of collagenase-induced OA model mice in response to cartilage damage [18]. Here, we found that MMP-3 is up-regulated in both the AT and ST of STR/Ort mice. Our *in-vivo* experiments demonstrated that macrophage depletion reduces TNF-α and MMP-3 expression in both AT and ST. In addition, MMP-3 expression was also increased markedly by TNF-α stimulation of cultured synovial fibroblasts and adipocytes. These findings, taken together with our present results, indicate that the inflammation of AT and ST that is associated with OA and hyperlipidaemia is induced by a common mechanism that regulates MMP-3 expression through TNF-α.

ADAMTS4 also has degradative effects on the extracellular matrix, and has been suggested to function as an inflammatory mediator in metabolic diseases [38,39] and OA [15,16]. Miller *et al.* [39] showed that ADAMTS4 expression is increased in the adipocytes of obese mice. Bondeson *et al.* [15] demonstrated that specific neutralization of TNF-α suppresses ADAMTS4 expression in synovial fibroblasts *in vitro*. Here, we found that ADAMTS4 is up-regulated in both the AT and ST of STR/Ort mice. However, in *in-vivo* experiments, macrophage depletion reduced ADAMTS4 expression significantly in ST, but did not affect expression markedly in AT. Consistent with the results of the *in-vivo* experiments, TNF-α stimulation at both low and high concentrations increased ADAMTS4 expression in cultured synovial fibroblasts. In contrast, the expression of ADAMTS4 in adipocytes was increased only by high concentrations of TNF-α. A recent study showed that adipocytokines and leptin regulate ADAMTS4 expression in chondrocytes [40]. Taken together, these findings suggest that TNF-α modulates the expression of ADAMTS4 in ST, but another factor may also regulate ADAMTS4 expression in AT.

Several recent studies suggest that TNF-α is a key factor and drug target for OA [41–44]. In a patient with inflammatory knee OA, an anti-TNF drug had marked benefits on pain and walking distance, as well as synovitis, synovial effusion and bone marrow oedema [42]. In a recent pilot study, involving intra-articular injections of the anti-TNF antibody infliximab, significant symptomatic relief was observed compared with placebo [41]. Infliximab has also been shown to slow the progression of OA [43]. Increased TNF-α production in response to hyperlipidaemia leads to decreased insulin sensitivity [45,46]. Notably, recent studies reported that anti-TNF treatment in RA patients with insulin resistance improved not only RA symptoms, but also insulin resistance [47–49]. In addition to decreased TNF-α expression in ST, macrophage depletion in STR/Ort mice led to decreased serum glucose levels, a result that is consistent with the finding that macrophage depletion in high-fat diet mice improves insulin sensitivity and reduces plasma glucose levels [50]. These observations and our present results of high TNF-α expression in ST and AT macrophage of OA mice with hyperlipidaemia corroborate the link between OA and hyperlipidemia.

Several limitations of the study warrant mention. First, the mechanism proposed in this study was based on the results of cross-sectional analysis. Secondly, although we demonstrated that TNF-α and MMP-3 are elevated in ST and AT, it remains to be determined whether these factors contribute to OA pathology. Finally, it is also unclear whether OA onset and/or progression are inhibited by continuous macrophage depletion.

In conclusion, $CD11c^+$ macrophages were identified as a common inflammatory subset in OA mice with hyperlipidaemia, and the depletion of macrophages with clodronate-laden liposomes reduced the levels of TNF-α and MMP3 in AT and ST. The induction of inflammation in AT and ST may be part of a common mechanism that regulates MMP3 expression through TNF-α. Our findings suggest that increased numbers of macrophages and elevated levels of TNF-α and MMP-3 may explain the relationship between hyperlipidaemia and OA.

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Author contributions

All authors read the manuscript and had input into revising it for intellectual content and style. For experimental design: K. U. and M. T.; for acquisition of data: K. U., M. S., G. I., K. O., K. I. and M. M.; for analysis and interpretation of data: K. U., M. S. and K. I. For drafting the manuscript: K. U. and M. T.

Disclosure

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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