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Increase in CD5L expression in the synovial membrane of knee osteoarthritis patients with obesity

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Abstract

Introduction: *Obesity appears to be a powerful risk factor for the development of knee osteoarthritis (KOA), but the mechanisms of this are not fully understood. CD5L is expressed in tissue macrophages and is increased in obese mice. We hypothesized that CD5L expression is increased in the synovial membrane (SM) of obese KOA patients. Here, we investigated CD5L expression in the SM of these patients.*

Material and methods: *Ninety KOA patients (26 males, 64 females) were allocated to one of three groups based on body mass index (BMI): normal weight (NW, < 25 kg/m^2), overweight (OW, 25-29.99 kg/m^2) and obese (OB, ≥ 30 kg/m^2), according to the World Health Organization BMI classification (each n = 30). Expression of CD5L in SM among the groups was compared using real-time polymerase chain reaction. To investigate CD5L-expressing cells in SM, CD14$^+$ (macrophage fraction) and CD14$^-$ (fibroblast fraction) cells were separated from the SM.*

Results: *CD5L expression was significantly higher in the OB group than in the NW and OW groups ($p < 0.001$). CD5L expression was observed in the CD14$^+$ fraction but not in the CD14$^-$ fraction.*

Conclusions: *CD5L is highly expressed in the SM of KOA patients with obesity. Further investigation is required to identify the role of CD5L in the relationship between KOA pathology and obesity.*

Key words: *CD5L, obesity, synovial membrane, osteoarthritis.*

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Introduction

Epidemiological studies suggest that obesity is a risk factor for osteoarthritis (OA), particularly knee osteoarthritis (KOA) [1-6]. Obesity results in high mechanical joint loading and elevated risk of KOA [3, 7]. Interestingly, studies have also suggested that altered metabolic conditions associated with obesity increase the production of growth factors and cytokines that are associated with KOA pathology [8, 9]. Moreover, the presence of OA in the hand joints of obese subjects [10, 11] suggests that mechanical factors are not the only type of factor in KOA progression. To date, however, the mechanisms of this effect have not been well established.

Synovial cells mainly consist of two populations, macrophages (Mϕ) and fibroblasts [12-14]. In particular, Mϕ contribute to synovial inflammation via inflammatory cytokine production in the synovial membrane (SM) [15-17]. CD5L has been identified as a Mϕ-secreted protein [18] and plays a role in modulation of the recruitment of Mϕ and inflammation [19]. CD5L is detected in both mouse and human serum [20-23] and is increased in the serum of obese mice [23]. CD5L protein in the synovial fluid (SF) of KOA patients was 3.3-fold higher than that in rheumatoid arthritis patients [24]. However, it is unclear whether cells in the SM express CD5L or whether CD5L expression is increased in the SM of KOA patients with obesity.

Here, we investigated CD5L expression in the SM of obese KOA patients.

Material and methods

Subjects

Ninety subjects who underwent primary total knee replacement (TKR) at our hospital participated in this study. SM was extracted during TKR from subjects with radiographically defined KOA. The protocol was approved by the Ethics Review Board of Kitasato University (approval number: B13-113), and participants provided written informed consent to participate in this study. The patient demographic profile is summarized in Table 1. All sub-

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Table 1. Patient demographic profiles in the NW, OW, and OB groups

Variable	NW	OW	OB	*P* value
Age	74.9 ±7.3	73.7 ±1.5	68.6 ±8.8*	0.013
Gender (F/M)	21/9	18/2	25/5	0.156
BMI	22.5 ±1.9	27.6 ±1.5	32.9 ±2.0*	< 0.001
K/L grade (2/3/4)	0/10/20	1/7/22	0/11/19	0.612

Asterisks (*) indicate a significant difference of p < 0.05 between NW and other groups.
NW – normal weight, OW – overweight, OB – obese, BMI – body mass index, K/L – Kellgren/Lawrence grade

jects were allocated to three groups based on body mass index (BMI): normal weight (NW, < 25 kg/m^2), overweight (OW, 25-29.99 kg/m^2) and obese (OB, ≥ 30 kg/m^2), according to the World Health Organization (WHO) BMI classification.

CD5L expression in synovial membrane

RNA extraction, cDNA synthesis and quantitative RT-PCR (qRT-PCR) were conducted using previously reported methods [17]. CD5L primer pair sequences (product length: 152 bp) used in qRT-PCR analysis were: CD5L-Sense (5′-CTT GCC ATT TGC ACC AGA CC-3′) and CD5L-antisense (5′-GCC ACG TCC TTA ATG TCC CA-3′). CD14 and GAPDH primers were synthesized based on previous studies [17]. mRNA expression levels of CD5L in the SM were determined by normalization to that of GAPDH using the ΔΔCT method. We compared the expression of CD5L in the SM among the three groups. To evaluate the effect of aging on CD5L expression, patients were also grouped into two age groups, < 65 and ≥ 65 years, and CD5L expression in the two groups was compared.

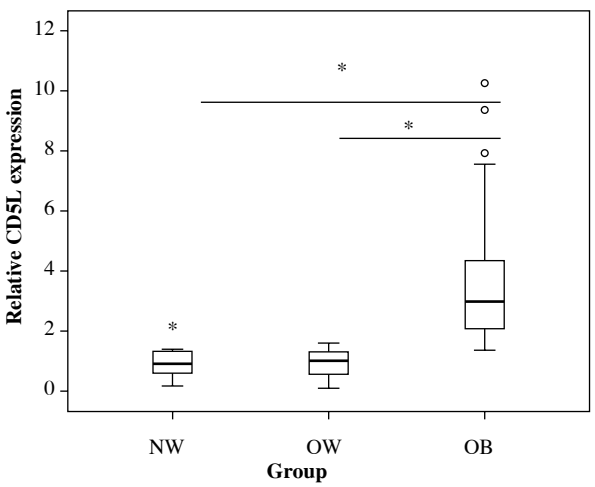

Fig. 1. Effect of obesity on synovial CD5L expression. Asterisks (*) indicate a significant difference of $p < 0.05$ between the groups. Small circles indicate mild outliers (greater than 1.5 interquartile ranges from an edge of the box). Stars indicate extreme outliers (greater than 3.0 interquartile ranges from an edge of the box)

CD5L-expressiong cells in synovial membrane

To evaluate CD5L-expressing cells, the CD14-positive (Mϕ) rich fraction was isolated from 3 SM samples. Following collagenase digestion, SM-derived cells were reacted with biotinylated anti-CD14 antibody, washed twice with PBS, streptavidin-labelled with magnetic particles (BD Biosciences, Tokyo, Japan), and reacted for 45 minutes at 4°C in a magnetic separation device (IMag, BD Biosciences). RPMI medium was added to the cell suspension to collect CD14$^-$ fractions, and an additional 3 ml of RPMI was added to collect the CD14$^+$ fraction by removing the tube from the magnetic field. The CD14$^+$ and CD14$^-$ fractions were obtained by centrifugation at 270 × g for 5 minutes, and used to evaluate CD14 and CD5L expression using qRT-PCR. To confirm the CD5L expression, qRT-PCR products were subjected to electrophoresis and stained with ethidium bromide.

Statistics

All statistical analyses were done using SPSS 25.0. Categorical variables were analyzed using the χ^2 test (two groups) and Fisher's exact test (three groups). Continuous variables were compared using the *t*-test (two groups) and Bonferroni post hoc test (three groups). Statistical significance was defined by $p < 0.05$.

Results

Patient demographic profiles in NW, OW, and OB groups

Following patient allocation to the three WHO BMI classification groups, those in the OB group were significantly younger than those in the NW group ($p = 0.016$, Table 1). Kellgren/Lawrence (K/L) grade 2/3/4 ratio and male/female ratio and were both similar among the groups (Table 1).

CD5L expression in SM of NW, OW, and OB groups

To determine whether CD5L expression is elevated in obese KOA patients, we analyzed expression levels in the SM of KOA patients. CD5L expression was significantly higher in the OB group than in the NW and OW groups

Table 2. Patient demographic profiles in the < 65 and ≥ 65 years groups

Variable	< 65 years (n = 15)	≥ 65 years (n = 75)	P value
Age	58.4 ±5.4	75.3 ±6.0*	< 0.001
Gender (F/M)	9/6	55/20	0.298
BMI	27.3 ±4.6	29.4 ±4.5	0.111
K/L grade (2/3/4)	0/8/7	1/20/54	0.120

Asterisk (*) indicates a significant difference of $p < 0.05$ between the < 65 and ≥ 65 groups.
BMI – body mass index, K/L – Kellgren/Lawrence grade

($p < 0.001$ and $p < 0.001$, respectively), but comparable between the NW and OW groups ($p = 0.999$) (Fig. 1).

Patient demographic profiles in the < 65 and ≥ 65 years groups

Kellgren/Lawrence (K/L) grade 2/3/4 ratio and male/female ratio and were both similar between the groups (Table 2).

CD5L expression in SM of < 65 and ≥ 65 years groups

Age significantly differed between the OB and other groups. To investigate the effect of aging, we compared CD5L expression between the < 65 and ≥ 65 years groups. There was no difference between the two groups ($p = 0.629$) (Fig. 2).

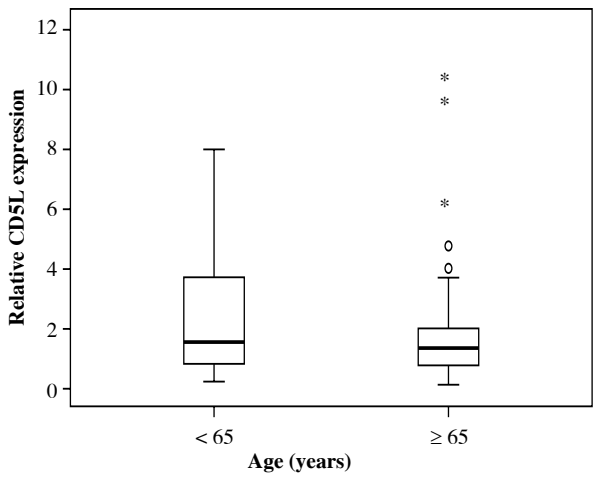

Fig. 2. Effect of aging on synovial CD5L expression. Small circles indicate mild outliers (greater than 1.5 interquartile ranges from an edge of the box). Stars indicate extreme outliers (greater than 3.0 interquartile ranges from an edge of the box)

Expression of CD5L in synovial Mϕ

qRT-PCR analysis also revealed that CD14 mRNA levels in $CD14^+$ fractions from the SM of KOA patients were higher than in $CD14^-$ fractions ($p = 0.037$, Fig. 3A). CD5L expression was observed in $CD14^+$ cells in all patients. However, there was no detection in $CD14^-$ fractions in any patients (Fig. 3B).

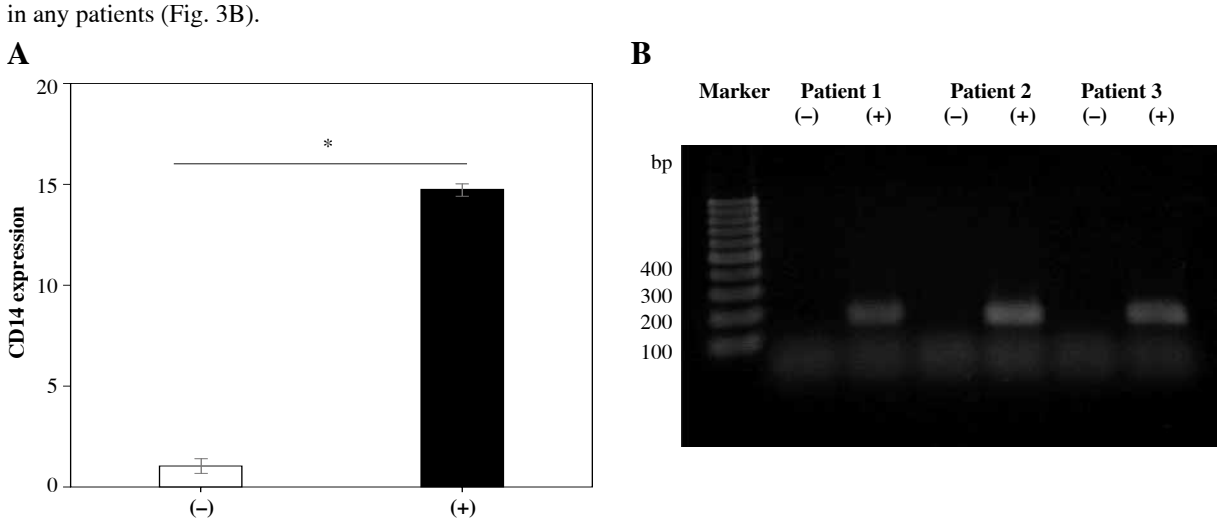

Fig. 3. Expression of CD5L in $CD14^+$ and $CD14^-$ fractions. **A**) Expression of CD14 and CD5L in $CD14^+$ and $CD14^-$ fractions. *$p < 0.05$. **B**) Agarose gel electrophoresis of the qRT-PCR product of CD5L

Discussion

Epidemic research has suggested a link between obesity and KOA progression [1, 25, 26]. KOA patients with obesity are approximately 7 times more likely to experience KOA progression than those of normal weight [1]. A meta-analysis revealed that the odds ratio for obese subjects with KOA development was 2.6 times that of normal weight subjects [25]. In the present study, KOA patients with obesity who underwent total knee arthroplasty (TKA) were significantly younger than OW and NW subjects. Together with previous reports, our observations suggest that obesity is associated with the pathology of KOA.

Inflammation plays a role in the progression and pain of KOA. Among findings to date, obesity was associated with synovial inflammation in human and animal studies [27, 28]; a high-fat diet led to synovial inflammation and promoted OA in rats [28]; and interleukin 6 (IL-6) levels were higher in the SF of obese hip OA patients than in hip OA patients of normal weight [27]. Regarding CD5L, levels were elevated in the course of metabolic and cardiovascular pathologies, such as in adipose tissue in obese mice [19] and in regions of atherosclerosis in mice [29]. CD5L contributes to Mϕ infiltration and inflammation in mice [19]. In the present study, CD5L expression in KOA patients with obesity was significantly higher than in normal and overweight patients. Obese groups contained a younger population than the normal weight group; however, CD5L expression did not differ between them. We speculate that CD5L contributes to synovial inflammation in OA patients with obesity, and that the elevation of CD5L in SM may partly explain the relationship between KOA pathology and obesity.

Synovial Mϕ contributes to synovial inflammation via the production of inflammatory cytokines and thereby contributes to OA pathology [15, 16, 30]. CD5L supports the survival of infiltrating macrophages, and overexpression of CD5L in CD5L transgenic mice remarkably increased the number of Mϕ in tissues [31]. CD5L deficiency also results in a reduction in macrophage infiltration in adipose tissue in obese mice [19]. In the present study, CD5L was specifically expressed in synovial Mϕ. CD5L may play an important role in synovial inflammation through the Mϕ in KOA.

In this study, we found that CD5L expression was elevated in SM of KOA patients with obesity. Further investigation of the precise role of CD5L in the relationship between KOA pathology and obesity is required.

The authors declare no conflict of interest.

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