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ORIGINAL RESEARCH

Increase in Tryptase and Its Role in the Synovial Membrane of Overweight and Obese Patients with Osteoarthritis of the Knee

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Purpose: The mechanisms governing evidence that obesity is a risk factor for osteoarthritis (OA) are not well understood. We previously reported an increase in mast cell (MC) marker expression in the osteoarthritic synovial membrane (SM) of patients with obesity. We hypothesized that an enzyme produced by MC, β-tryptase, may be increased in the SM of obese patients with knee OA and contribute to synovial inflammation. This study investigated the expression of the β-tryptase encoding gene, *TPSB2*, in the SM of obese patients with knee OA and β-tryptase-mediated regulation of IL-1β in synovial cells.

Patients and Methods: A total of 216 patients radiographically diagnosed with knee OA were grouped according to the World Health Organization's body mass index classifications: normal weight (NW; <25 kg/m^2), overweight (OW; 25–29.99 kg/m^2) and obese (OB; ≥30 kg/m^2). Quantitative polymerase chain reaction was conducted to examine *TPSB2* expression in the SM among the three groups. We also examined *TPSB2* and *IL1B* expression in MC-rich (CD3-CD14-CD19-CD90-) and MC-poor (CD3+, CD14+, CD19+, or CD90+) fractions freshly isolated from synovial tissue. Further, the effect of β-tryptase on *IL1B* expression was investigated in cultured CD14-positive (macrophage-rich fraction) and CD14-negative (fibroblast-rich fraction) cells.

Results: There was significantly elevated *TPSB2* expression in the OW and OB groups compared to the NW group. The MC-rich fraction had significantly higher levels of *TPSB2, CD117* and *CD203c* than the MC-poor fraction. Recombinant human β-tryptase stimulated *IL1B* expression in both the synovial fibroblast and macrophage fractions.

Conclusion: Obese patients with knee OA showed elevated *TPSB2* expression in the SM. Tryptase may play a role in synovial inflammation in obese patients with OA.

Keywords: obese, mast cell, osteoarthritis, tryptase, synovial membrane

Introduction

Evidence from a number of studies has implicated obesity as a risk factor for osteoarthritis (OA).[1–5] However, because OA presents in both weight-bearing and non-weight-bearing joints of obese patients,[6,7] mechanical loading may not be the only contributing factor governing the link between obesity and OA. It is likely that other factors also contribute to OA pathology; however, these mechanisms remain to be established.

Mast cells (MC) produce various inflammatory cytokines and growth factors that have been suggested to play a role in acute and chronic inflammatory processes.[8] An increase in MC has been reported in the arthritic synovial membrane (SM) of patients with rheumatoid arthritis (RA),[9–11] OA,[12–14] and spondyloarthritis.[15] MC number is

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associated with the radiographic severity of OA.[13] We recently reported an increase in MC marker expression in the SM of obese patients,[16] suggesting that MC may be involved in OA pathology in obese patients. However, the mechanisms by which MC are involved in OA pathology are not well understood.

Tryptases (α- and β-tryptase) are one of the most abundant proteases found in MC. β-tryptase is the main type of tryptase released during MC degranulation and is involved in inflammatory cytokine production in inflammatory and allergic diseases.[17] Higher β-tryptase levels have been observed in the synovial fluid of OA patients compared to healthy subjects.[18] However, it is unclear whether β-tryptase levels are increased in obese patients with OA.

Studies have reported the involvement of β-tryptase in the production of inflammatory cytokines, including interleukin (IL)-1β and IL-8.[19,20] For example, β-tryptase stimulates IL-1β expression in endothelial cells.[19] Additionally, a number of studies have shown that synovial IL-1β contributes to OA pathology through the actions of matrix metalloprotease and pain-related molecules.[21–25] IL-1β levels in synovial fluid correlate with radiographic OA severity.[26] We hypothesized that β-tryptase levels may increase in the SM of obese patients with OA and contribute to the regulation of IL-1β in the synovium.

Here, we examined the expression of the gene encoding β-tryptase, *TPSB2*, in the SM of obese patients with knee OA and β-tryptase-mediated regulation of IL-1β in synovial cells.

Patients and Methods

All participants received total knee arthroplasty (TKA) at our hospital. Power analysis was performed with an alpha of 0.05, power of 0.80, and N2 (number of patients with normal weight)/N1 (number of overweight and obese patients) ratio of 0.64 using G*POWER3 to determine the optimal sample size. The analysis indicated the need for 84 patients with normal weight and 132 overweight/obese patients for a detectable difference in *TPSB2* between normal weight and overweight/obese groups. A total of 216 SM samples were extracted during TKA from the subjects, who were diagnosed with radiographic knee OA. A fraction of each SM sample was snap frozen in liquid nitrogen and stored at −80°C until the RNA extraction procedure. SM samples randomly obtained from 10 patients were used to evaluate *TPSB2* expression in MC, while those from 5 patients were used to evaluate the effect of β-tryptase on *IL1B* expression. The study protocol was approved by the Institutional Review Board for Clinical Research and Treatment of Kitasato University (approval number: KMEO B19–259). Written informed consent was obtained from all participants one day before surgery to participate in this study and to remove and use their SM. This study complied with the principles of the Declaration of Helsinki.

The subjects were grouped according to the World Health Organization's body mass index (BMI) classifications: normal weight (NW; <25 kg/m^2), overweight (OW; 25–29.99 kg/m^2) and obese (OB; ≥30 kg/m^2). Table 1 shows the patients' clinical characteristics according to group. Quantitative PCR (qPCR) was used to examine *TPSB2* expression in the SM among the three groups.

Quantification of mRNA Expression by qPCR

RNA extraction, cDNA synthesis and qPCR using the SYBR green method were performed using previously reported methods.[11,15] Primer sequences are listed in Table 2. *GAPDH* was used to normalize gene expression. Relative expression was determined from mean values from all control samples (SM from the NW group or vehicle-treated cells in vitro).

Expression of *TPSB2* and *IL1B* in MC

The expression of *TPSB2* and *IL1B* was examined in MC-rich and MC-poor fractions of cells obtained from synovial tissue. Magnetic isolation using a CD117 or CD203c antibody failed to enrich the MC fraction. We therefore attempted to enrich the MC fraction using negative selection. Synovial tissue was immediately digested with 2 mg/mL type I collagenase solution at 37°C for 2 h. Following collagenase digestion, the extracted cells were incubated with cell staining buffer (BioLegend, San Diego, CA) containing biotin-conjugated CD3 (Clone, OKT3; T cell marker), CD19

Table 1 Patients' Clinical Characteristics

	Normal (n=84)	Overweight (n=91)	Obese (n=41)	P
Age (years)	76.1±0.8	73.1±0.8[a]	69.0±1.3[a,b]	<0.001
Male/Female, n	14/70	24/67	8/33	0.298
KL(3/4), n	26/58	28/63	11/30	0.907
BMI (kg/m^2)	22.3±0.2	27.3±0.1[a]	32.8±0.3[a,b]	<0.001

Notes: Values indicate mean±standard deviation unless otherwise indicated. [a]P<0.05 compared with the normal group. [b]P<0.05 compared with the overweight group.
Abbreviations: KL, Kellgren and Lawrence grade; BMI, body mass index.

Table 2 Primer Sequences

Gene	Direction	Primer Sequence (5′–3′)	Product Size (bp)
TPSB2	F R	CGCAAAATACCACCTTGGCG GTGCCATTCACCTTGCACAC	138
IL1B	F R	GTACCTGTCCTGCGTGTTGA GGGAACTGGGCAGACTCAAA	153
CD14	F R	TCCCTCAATCTGTCGTTCGC ATTCCCGTCCAGTGTCAGGT	150
CD90	F R	GACCCGTGAGACAAAGAAGC CCCTCGTCCTTGCTAGTGAA	138
CD117	F R	TGACTTACGACAGGCTCGTG CCACTGGCAGTACAGAAGCA	126
CD203c	F R	CGACTGCACTATGCCAAGAA GGTCCATGTGCCAGAAAGAT	164
GAPDH	F R	TGCCACTCAGAAGACTGTGG TTCAGCTCTGGGATGACCTT	129

(Clone, HIB19; B cell marker), CD14 (Clone, M5E2; macrophage marker), and CD90 (Clone, 5E10; fibroblast marker) antibodies for 30 minutes at 4°C. All antibodies were purchased from BioLegend and used at a dilution of 1:100. After washing twice with PBS, the cells were added to streptavidin-conjugated magnetic particles (BD Biosciences, CA, USA) and separated in a magnetic separation system (BD IMagTM cell separation system, BD Biosciences) into negative (MC-rich; CD3-CD14-CD19-CD90-) and positive (MC-poor; CD3+, CD14+, CD19+, or CD90+) fractions. Expression of *TPSB2* and *IL1B* in the negative (MC-rich) and positive fractions was evaluated using qPCR analysis without cell culture. To confirm successful enrichment of MC, expression of MC markers (CD117, CD203c) was also evaluated using qPCR analysis.

β-Tryptase Stimulation of Synovial Fibroblasts and Macrophages

To examine the role of β-tryptase in the synovium, synovial fibroblasts and macrophages were extracted from the SM of 5 knee OA patients. CD14-positive (macrophage-rich) and CD14-negative (fibroblast-rich) fractions were isolated using a magnetic separation system, as described elsewhere.[24] CD14-negative and CD14-positive cells were cultured for 7 days in six-well plates containing α-MEM. To confirm the cell population in each fraction after 7 days of culture, the expression of synovial fibroblast (CD90) and macrophage markers (CD14) was evaluated using qPCR analysis. The cells were subsequently stimulated with vehicle (serum-free media) or 15 mU/mL of recombinant human β-tryptase (rh-β-tryptase; Promega, Madison, WI, USA) for 8 and 24 hours. β-tryptase concentrations were determined using methods described in a previous study.[20] After stimulation, *IL1B* expression was evaluated using qPCR analysis.

Statistics

Statistical analyses were conducted using SPSS 25.0. Continuous variables were analyzed using the Wilcoxon signed-rank test (two groups) and Kruskal–Wallis test (three groups), and categorical variables using the Fisher exact test. $P<0.05$ indicated statistical significance.

Results

Patients' Clinical Characteristics Among BMI Groups

Patients were significantly younger in the OW and OB groups compared to the NW group (Table 1). The ratio of patients by sex (male/female) and Kellgren and Lawrence grade (3/4) was comparable among the groups ($P=0.298$ and $P=0.907$, respectively; Table 1).

Synovial *TPSB2* Expression Among Obesity Groups

TPSB2 expression in the OW and OB groups was significantly higher than in the NW group (Figure 1; $P=0.039$

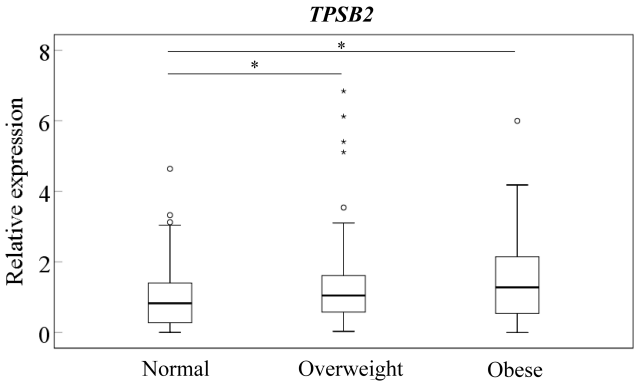

Figure 1 Expression of *TPSB2* in the synovium of normal, overweight, and obese groups. *P<0.05 compared to the normal groups.

and P=0.021, respectively). No differences were observed between OW and OB groups (P=0.515).

Expression of *TPSB2* and *IL1B* in MC

Expression levels of *TPSB2* and *IL1B* in MC were examined in MC-rich and MC-poor fractions of cells obtained from 10 osteoarthritic SM (3 and 7 SM from normal and overweight subjects, respectively). Expression levels of *CD117* and *CD203* were significantly higher in the MC-rich fraction than the MC-poor fraction (Figure 2A and B; *CD117*, P=0.005; *CD203c*, P=0.005; *TPSB2*, P=0.005). Expression levels of *TPSB2* were also significantly higher in the MC-rich fraction than the MC-poor fraction (Figure 2C; P=0.005). In contrast, *IL1B* expression was significantly higher in the MC-poor fraction than the MC-rich fraction (Figure 2D; P=0.005).

Effect of β-Tryptase on *IL1B* Expression in Synovial Fibroblasts and Macrophages

As mentioned above, *IL1B* expression was higher in the MC-poor fraction than the MC-rich fraction. Next, we examined the effect of rh-β-tryptase on *IL1B* expression in synovial fibroblast- and macrophage-rich fractions obtained from 5 osteoarthritic SM (2, 2, and 1 SM from normal, overweight, and obese subjects, respectively). CD14 expression in the CD14-positive fraction was significantly higher than that in

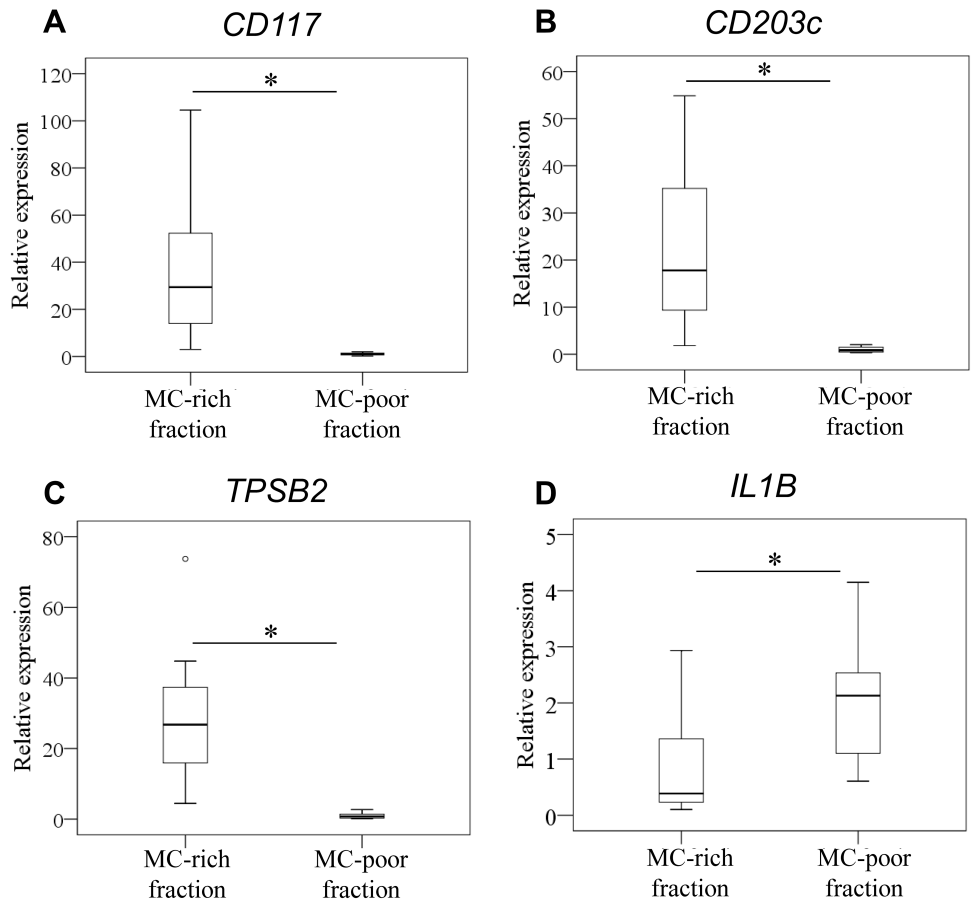

Figure 2 Expression of *TPSB2* and *IL1B* in mast cells. Expression of *CD117* (**A**), *CD203c* (**B**), *TPSB2* (**C**), and *IL1B* (**D**) in MC-rich (CD3-CD14-CD19-CD90-) and MC-poor (CD3+, CD14+, CD19+, or CD90+) fractions. *P<0.05 compared to the MC-poor fraction.

the CD14-negative fraction (Figure 3A; P=0.025). In contrast, CD90 expression in CD14-negative cells was significantly higher than that in CD14-positive cells (Figure 3B; P=0.001). We found that rh-β-tryptase stimulated *IL1B* expression in both the synovial fibroblast- and macrophage-rich fractions at 8 h after stimulation (Figure 3C and D; P=0.043 and P=0.043, respectively). In contrast, there was no difference in *IL1B* expression at 24 h after stimulation in either fraction (Figure 3C and D; P=0.893 and P=0.138, respectively).

Discussion

Previous studies have reported a strong link between the development of KOA and obesity.[1–5] Several studies have also reported an association between obesity and the need for TKA in OA patients.[1,27,28] Coggon et al studied subjects older than 45 years who had received TKA and found that the median BMI was 28.1 kg/m^2, or overweight.[1] In our study, 61% of patients were considered overweight or obese. While approximately 70% of patients from all 3 groups had a Kellgren and Lawrence grade of 4 independent of obesity grade, patients in the OW and OB groups received TKA at a younger age than those in the NW group. Together with previous studies, our findings suggest that increasing BMI is associated with KOA progression.

Previous studies have reported elevated levels of tryptase in adipose tissue and serum from obese human subjects.[29–31] BMI is significantly correlated with serum tryptase concentration and the prevalence of allergic respiratory disease symptoms.[30] Circulating β-tryptase levels are correlated with BMI and the presence of carotid plaques.[31] In our

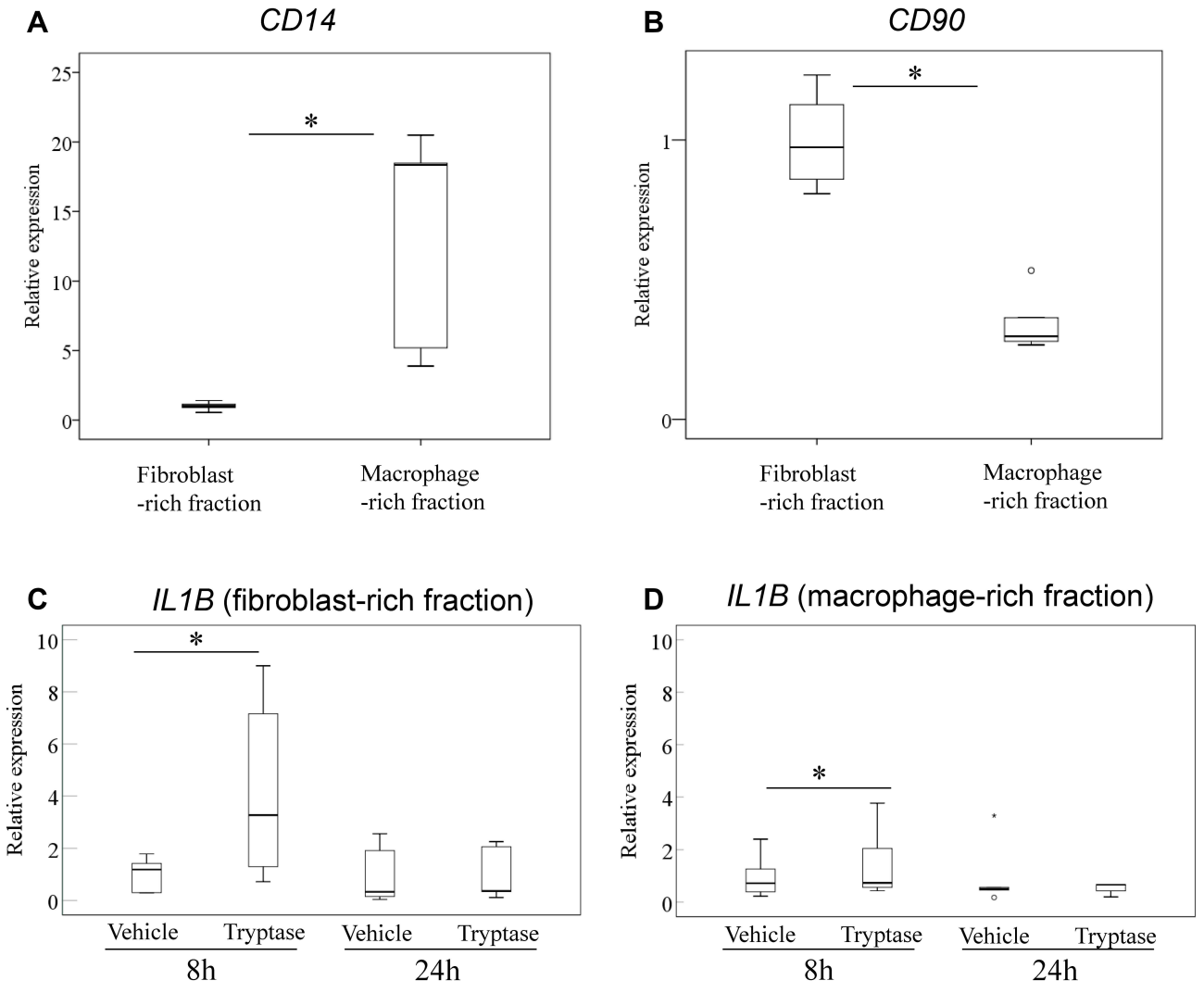

Figure 3 Effect of β-tryptase on *IL1B* expression in synovial fibroblasts and macrophages. Expression of CD14 (**A**) and CD90 (**B**) in CD14-negative (fibroblast-rich) and CD14-positive (macrophage-rich) fractions. *P<0.05 compared to the macrophage-rich fraction. Effect of β-tryptase on *IL1B* expression in CD14-negative (fibroblast-rich) (**C**) and CD14-positive (macrophage-rich) (**D**) fractions. *P<0.05 compared to the vehicle-treated cells.

study, higher *TPSB2* expression levels were observed in overweight and obese subjects with OA. In addition, higher *TPSB2* expression was observed in the MC-rich fraction of cells obtained from synovial tissue. We previously reported an increase in the expression of MC markers, *CD117* and *CD203c*, in the synovium of obese patients with OA.[16] Taken together, our findings suggest that synovial tryptase levels may increase in OA patients with increasing BMI. Further, a previous study reported that the number of tryptase-positive cells is increased in the adipose tissue of obese patients.[29] Synovial tissue contains an adipose tissue. Therefore, the increase in *TPSB2* observed in obese and overweight patients may reflect the increase in MC and tryptase expression in the adipose tissue of these patients.

MC may directly and indirectly contribute to IL-1β production. MC have been shown to produce IL-1β[32] and to interact with several other cell types including macrophages, T cells, and endothelial cells.[33–37] An in vitro study showed that β-tryptase stimulates the production of IL-1β in endothelial cells from human umbilical veins in vitro.[20] Further, MC activation induces *IL1B* expression in CD68-positive synovial macrophages in cultured SM cells derived from RA patients.[36] In our study, *IL1B* expression was significantly lower in the MC-rich fraction than in the MC-poor fraction. However, β-tryptase stimulated *IL1B* expression in synovial macrophages and fibroblasts. β-tryptase may therefore indirectly contribute to synovial inflammation through activation of synovial fibroblasts and macrophages in obese patients with OA. There were several limitations in the present study. First, we did not examine protein levels. Second, we did not determine differences in *TPSB2* and *IL1B* expression in the MC-rich fraction among normal, overweight, and obese groups. Third, we did not determine the localization of MC in synovial membrane. Finally, the mechanism by which an increase in tryptase contributes to OA pathology remains to be determined. Histological analysis and correlation analysis between β-tryptase and inflammatory and catabolic factors are needed.

These limitations notwithstanding, we found that *TPSB2* expression was elevated in the SM of obese patients with knee OA. Tryptase may therefore play a role in synovial inflammation in obese patients with OA.

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Disclosure

The authors report no conflicts of interest in this work.

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