A crucial role for lectin-like oxidized LDL receptor-1 signal on cartilage destruction in inflammatory arthritis

Introduction
Rheumatoid arthritis (RA) is one of the immune-mediated inflammatory diseases. LOX-1 (Lectin-like oxidized low-density lipoprotein receptor 1), one of functional receptors for oxidized LDL (ox-LDL), is expressed in various cells, including endothelial cells and chondrocytes, and its expression is enhanced by oxidative stress, inflammatory cytokines (1). Several studies have reported that the ox-LDL/LOX-1 axis modulates cartilage degradation in RA (2) (3). Although the importance of LOX-1 in RA is apparent, little is known whether LOX-1 is expressed in the human joint synovium, which is a major site of systemic inflammation in RA, or whether an anti-LOX-1 treatment can block articular cartilage degradation in vivo. In this study, we investigate the presence of LOX-1 protein in human synovium, and the functional roles of LOX-1 in the pathogenesis of inflammatory arthritis, such as RA.

Methods
Synovial tissues samples with RA patients were prepared at the total knee replacement. Ethical approval was granted by the institution’s ethics committee. Frozen synovial tissues embedded in OCT compound were cut into 5-µm sections, fixed in acetone for 10 min, and air-dried. C57BL/6 mice (10 weeks old, male) were used for the knee joint inflammation in vivo experiments. The mice in the experimental group were subjected to intraarticular injection (10 ml, once daily) for 7 days of ox-LDL (2 mg/ml), native LDL (2 mg/ml) or phosphate-buffered saline (PBS) into the right knee joint. One hour before ox-LDL injection, a group of mice was subjected to intraarticular injection (20 ml) with TSS8 (10 µg/ml), human recombinant LOX-1 protein (1.0 mg/ml) or control IgG (10 µg/ml). Specimens were processed to paraffin-embedded sections with a thickness of 7 µm. The following antibodies were used for immunohistochemistry: anti-LOX-1, anti-ox-LDL, an anti-MMP-3 antibody or control IgG.

The severity of joint inflammation and proteoglycans depletion were scored on an arbitrary scale of 0–4. Joint inflammation and proteoglycans depletion were scored by 2 blinded, independent observers in each section, and the averages were recorded.

All data were reported as the mean ± SEM. Bonferroni method was used for statistical analyses. P < 0.05 was considered significant.

Results
First, we assessed the expression of the ox-LDL and LOX-1 proteins in human synovial tissue. The ox-LDL and LOX-1 (Figure 1) protein were colocalized in the synovia of RA samples, particularly in the lining layer and around blood vessels.

Next, to assess the role of ox-LDL and LOX-1 in the pathogenesis of arthritis in vivo, we injected ox-LDL, native LDL, or PBS into unilateral knee joints for 7 days and evaluated joint inflammation (Figure 2). Immunohistochemistry study showed prominent LOX-1 expression in the cartilage and synovium in the ox-LDL-treated group, whereas the native LDL and PBS-treated group hardly displayed any positive staining.

Hematoxylin–cosin staining (Figure 3) revealed that treatment with ox-LDL caused massive synovial hyperplasia with significantly increased inflammatory cellular infiltration and pannus formation, compared with the native LDL-treated controls. In contrast, joints pretreated with the anti-LOX-1 antibody or with the recombinant LOX-1 significantly inhibited synovial hyperplasia induced by ox-LDL treatment. Safranin O-staining revealed significantly higher depletion in the ox-LDL-treated joint compared with the joints treated with native LDL. In contrast, pretreatment with the anti-LOX-1 antibody or the recombinant LOX-1 significantly prevented the proteoglycan loss of the articular cartilage.

To clarify the involvement of proteoglycans in the loss of proteoglycans, MMP-3 expression was examined using immunohistochemical analysis. The MMP-3 protein was observed at higher levels in the synovium and articular cartilage after administration of ox-LDL plus control IgG. MMP-3 expression was markedly reduced in joints pretreated with the anti-LOX-1 antibody or recombinant LOX-1.

Discussion
Inflammation is one of hallmarks in RA. Although the evidence has accumulated that ox-LDL–LOX-1 axis is involved in inflammation, to date, the function role for cartilage is not fully understood, especially in the field of RA. We have shown that the ox-LDL and LOX-1 protein are expressed in the lining layer of the human RA synovium. From present in vivo studies, ox-LDL induced not only the joint inflammation but cartilage destruction, which would be mediated by the production of MMP-3. Furthermore, we showed that blockade of ox-LDL–LOX-1 interaction suppressed arthritic changes and MMP-3 expression induced by ox-LDL in the knees of mice.

LOX-1 gene is an immediate early gene that process nuclear factor kappa B response (4) and is dynamically modulated by proinflammatory mediators. Since LOX-1 is easily upregulated by cytokines, LOX-1 would have an important role for amplifying local inflammatory response in the systemic inflammation such as RA. Therefore, blockade of LOX-1 signal would be beneficial for prevention of the inflammation and cartilage destruction in the inflammatory arthritis, such as RA.

References