The Role Of The Tetraspanin CD9 In A Mouse Model Of Antigen-induced Arthritis

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Introduction: Tetraspanins are members of the transmembrane protein family that have four membrane-spanning domains and are present throughout the human body. They reportedly have important roles in functions such as cell adhesion, migration, fusion, and signal transduction. CD9 is one of the tetraspanin protein families and CD9 knockout (CD9 KO) mice have been shown to develop normally without any external or histological abnormalities and with no characteristic features other than infertility in female mice. Previous study reported that CD9 expression is up-regulated in the synovium in cases of osteoarthritis (OA). However, the roles of CD9 in intra-articular inflammation remain unknown. In this study, we tested the hypothesis that CD9 plays important roles in the pathogenesis of arthritis.

The purpose of this study was to elucidate a new mechanism of the pathogenesis of intra-articular inflammation by analyzing the role of the tetraspanin CD9 in transient intra-articular inflammation using a mouse model of antigen-induced arthritis (AIA) and making comparisons between wild-type (WT) and CD9 KO mice.

Methods: To reveal whether CD9 KO mice exhibit onset of arthritis with aging, we examined the knee joints of 1-, 3-, and 6-month-old mice histologically. We also examined the expression of CD9 in synovial fibroblasts (SFBs) and articular chondrocytes of WT mice with and without interleukin (IL)-1β stimulation. Next, we generated an AIA model in the knee joints of 10-week-old WT and CD9 KO mice by inducing transient inflammation via intra-articular injection of methylated bovine serum albumin (mBSA) in mice preimmunized with mBSA and an adjuvant. Seven days after the intra-articular injection, the knee joints were harvested and processed. Sagittal sections of the knee joints of WT and CD9 KO mice were stained with Safranin O and Fast Green for analysis of histopathological differences. The severity of inflammation was evaluated on the basis of an inflammatory score consisting of five parameters: synovitis, joint space exudate, soft tissue inflammation, cartilage degradation, and bone damage. For each parameter, a score of 0 indicated none, 1 indicated mild, 2 indicated moderate, and 3 indicated severe change. We also analyzed the expression of inflammatory cytokines in the serum of AIA model mice using a Bio-plex assay and the expression of genes related to inflammation and chondrocytes in SFBs and chondrocytes stimulated with IL-1β using real-time polymerase chain reaction analysis.

Results: The knee joints of 1-, 3-, and 6-month-old CD9 KO mice did not show early onset of degenerative changes compared with those of WT mice, and development of these joints appeared normal (Fig. 1). In WT mice, CD9 expression was up-regulated in SFBs, but down-regulated in chondrocytes. In the AIA model (Fig. 2), inflammation in CD9 KO mice was suppressed, and the associated inflammation scores were significantly lower for all five parameters and thus the total score...
The respective inflammatory scores in WT and CD9KO mice were 2.8 ± 0.4 (2~3) and 2.2 ± 0.6 (1~3) points (P = 0.02) for synovitis, 2.6 ± 0.7 (1~3) and 1.8 ± 0.7 (1~3) points (P < 0.01) for joint space exudate, 2.9 ± 0.3 (2~3) and 2.3 ± 0.6 (1~3) points (P < 0.01) for soft tissue inflammation, 2.6 ± 0.6 (1~3) and 1.7 ± 0.7 (1~3) points (P < 0.01) for cartilage degradation, and 2.5 ± 1.0 (0~3) and 1.5 ± 1.1 (0~3) points (P = 0.04) for bone damage, with total scores of 13.3 ± 1.7 (10.5~15) and 9.7 ± 3.0 (5~14) points (P < 0.01).

In the serum of both WT and CD9 KO mice, tumor necrosis factor (TNF)α was up-regulated after the induction of AIA, and TNFα was lower in serum of CD9 KO mice than in WT mice. On the other hand, the up-regulation of IL-10 was greater in CD9 KO mice. The expression of the inflammatory genes IL-6, matrix metalloproteinase (MMP)-13, and Adams5 was suppressed in SFBs and chondrocytes of CD9 KO mice after IL-1β stimulation compared with their expression in these cells from WT mice.

Discussion: Mice with double knockout of CD9 and CD81, which has homology with CD9, reportedly exhibit osteopenia. However, another study reported the abundant expression of CD9 in activated osteoclasts in ovariectomy-induced osteoporosis model mice. Thus, no consensus exists regarding the roles of CD9 in bone and joint disease. In the lung, lipopolysaccharide stimulation accelerates the proliferation of inflammatory cells in CD9 KO mice. However, in this study, transient intra-articular inflammation was suppressed in CD9 KO mice. CD9 KO female mice have been reported to have infertility caused by deficiencies in exosome secretions, including microRNAs. Exosomes are microvesicles with diameters are 30-100nm. And tetraspanin (CD9, CD81 etc) is one of the construction proteins of exosome. And exosomes transfer from the cell of origin to the recipient cells, various bioactive molecules such as miRNAs. In previous report, exosome release in CD9 KO mice reported to be reduced. Previously we also showed that exosomes derived from IL-1β-stimulated SFBs and OA SFBs induce OA-like changes in normal chondrocytes. These exosomes also induced a significant increase in proteoglycan release from cartilage explants as well as migration and tube formation in endothelial cells. In this study, transient intra-articular inflammation in CD9 KO mice was suppressed, and we investigated deficiencies in exosome secretions in CD9 KO mice. We propose that in CD9 KO mice, arthritis is suppressed by a reduction in the amount of exosomes released, which reduces the secretion of factors that increase the intensity of arthritis.

Significance: In this study, although CD9 KO itself did not lead to joint degeneration during development, intra-articular inflammation was suppressed in CD9 KO mice compared with WT mice. Our results indicate that CD9 accelerates the development of arthritis.
Fig. 1 The histological section of the knee joints of 1-, 3-, and 6-month-old WT and CD9 KO mice

Fig. 2 Sagittal sections of the knee joints of WT and CD9 KO mice induced AIA and stained with Safranin O and Fast Green
Fig. 3 The inflammatory scores of WT and CD9 KO mice

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