Silencing the Expression of Connexin 43 Decreases Inflammation in Rat Synovium

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INTRODUCTION:
Rheumatoid arthritis (RA) is a chronic inflammatory disease that leads to destruction of multiple joints. Previous studies have indicated the importance of proinflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin (IL)-1β, and IL-6 in synovium of RA patients. [1] Remission of RA has been achieved since the appearance of biological treatments that target these cytokines. However, there are cases in which complete control of arthritis is not possible even with suppression of proinflammatory cytokines at the top of the cytokine cascade. This suggests that RA has pathophysiological features that involve factors other than the cytokine cascade.

Connexin (Cx) is a family of structurally-related transmembrane proteins that assemble to form gap junction. More than 20 isoforms of Cx have been found in humans. Of these, Cx43 is the most widespread, and also expressed in human synovium. [2] Recent studies show that Cx43 is activated by the inflammatory response, and that inhibition of Cx43 expression reduces inflammation. [3] It is thus likely that Cx43 is involved in synovitis of RA. However, the role of Cx43 in RA is largely unknown. To clarify the role of Cx43 in the pathophysiological features of synovitis, we analyzed in vitro the effects of small interfering RNA targeting Cx43 on proinflammatory cytokines, and investigated in vivo the therapeutic effects of siCx43 in rat models of arthritis.

METHODS:
The study was conducted according to the regulations regarding animal research of Kyoto Prefectural University of Medicine. In vitro, to investigate whether interference of Cx43 gene expression affect proinflammatory cytokine, fibroblast-like synoviocytes (FLSs) of rat were transfected with 50nM of siRNA 24hr before stimulation with lipopolysaccharide (LPS / 0.1 μg/ml). After 6hr stimulation with LPS, total RNAs were extracted from the cells. The expression levels of Cx43, TNF-α, IL-1β, and IL-6 mRNA were analyzed by quantitative real time RT-PCR. In vivo, to transfer siCx43 into the left knee joint of collagen-induced arthritis (CIA) rat, electroporation-assisted siRNA transduction were used. siRNA was transfected every 3 days (i.e., 7, 10, 13, and 16 days after immunization). After electro-transduction of siRNA, foot volume was measured as paw swelling. For histological analysis, the left ankle joints of CIA rat were excised 28days after immunization. Statistical significance was defined as a p-value of less than 0.05 by Tukey’s test.

RESULTS:
In vitro, after stimulation with LPS to FLSs, the expression of Cx43 was stimulated compared to the cells without LPS. (Figure 1) When transfection with siCx43 was performed, TNF-α, IL-1β, and IL-6 expression were markedly reduced to 56.1±9.3, 40.8±8.7, and 18.5±8.2 (%), respectively, compared to the cells which were transfected of non-specific siRNA. In vivo, paw swelling was significantly suppressed 21–28 days after immunization. (Figure 2A) Histological examination revealed that rats treated with siCx43 showed mild outgrowths of synovial membrane and infiltration of the inflammatory cells. (Figure 2B)

DISCUSSION:
The most highly expressed Cx in normal human synovial membranes is Cx43. [2] Previously, we also reported that Cx43 is more highly expressed in human RA synovial membranes than in synovial membranes of osteoarthritis patients. In the present study, we investigated the response of Cx43 to an inflammatory stimulus in rat FLSs. There was a significant rise in Cx43 gene expression when FLSs were stimulated with LPS. The inhibition of Cx43 expression simultaneously suppressed the gene expression of the proinflammatory cytokines which were stimulated by LPS. These results suggest the possibility that increased expression of Cx43 in synoviocytes induces proinflammatory cytokines, thus exacerbating synovitis in RA.

CIA rats have been reported as RA models. In the present study, Ankle swelling resulting from CIA were significantly suppressed in the group of siCx43 transduction in comparison to the group of non-specific siRNA. Histological examination also revealed clear suppression of synovitis. These results suggest that Cx43 inhibition may suppress synovitis. Although future studies are needed to compare the therapeutic effects of siRNA specific to other cytokines such as TNF-α, IL-1β, IL-6, and RANKL, the development of drugs to suppress Cx43 could be an important key in the novel treatment of synovitis in RA.

SIGNIFICANCE:
Cx43 has many potential roles in the pathophysiology of RA synovitis and may be a valuable target for therapeutic intervention.

REFERENCES:

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