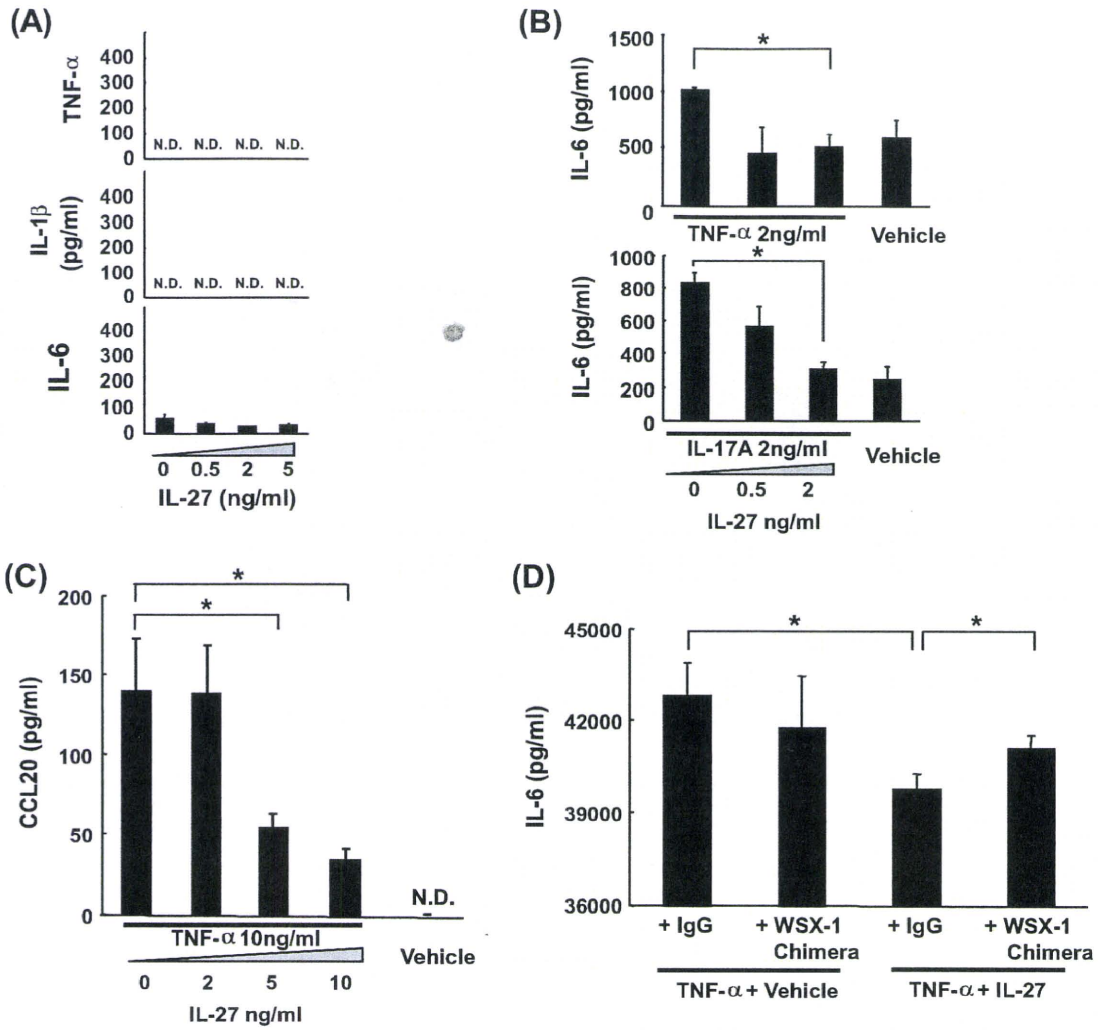

Fig. 5. IL-27 suppresses the production of IL-6 and CCL20 from RA FLSs via WSX-1. FLSs of RA were incubated for 48 h with IL-27 at the indicated concentration and the concentrations of TNF-α, IL-1β or IL-6 in the triplicate supernatants were measured by ELISA. (B) FLSs of RA were incubated for 48 h with 2 ng/ml TNF-α or IL-17A in combination with IL-27 at the indicated concentration, and the IL-6 level in the triplicate supernatants were measured by ELISA. (C) FLSs of RA were incubated for 48 h with 10 ng/ml TNF-α in combination with IL-27 at the indicated concentration, and the CCL20 level in the quadruplicate supernatants was measured by ELISA. (D) FLSs of RA were incubated for 48 h with 2 ng/ml TNF-α in the presence or absence of 2 ng/ml IL-27 in combination with 10 μg/ml IgG control or 10 μg/ml WSX-1 Fc chimera and the IL-6 level in the quadruplicate supernatants was measured by ELISA. Vertical bars represent the means ± SD. Significant differences compared with samples without the addition of IL-27 are indicated by $^*P < 0.05$. N.D. not detectable.

3.5. IL-27 suppresses the production of IL-6 or CCL20 induced by stimulation of RA FLSs with proinflammatory cytokines

To investigate the influence of IL-27 on RA FLSs, we measured the production of proinflammatory cytokines by RA FLSs in the presence or absence of IL-27. Because the concentration of IL-27 in plasma or synovial fluid in RA was at most 1 ng/ml (Fig. 1), we used IL-27 at a maximum concentration of 10 ng/ml. IL-27 alone failed to induce TNF-α, IL-1β, or IL-6 (Fig. 5A). However, IL-27 significantly suppressed the IL-6 production induced by TNF-α or IL-17A from FLSs (Fig. 5B). IL-27 also significantly suppressed the CCL20 production induced by TNF-α (Fig. 5C). These results suggest that IL-27 plays anti-inflammatory roles in two ways: both directly via the suppression of IL-6 production and indirectly by regulating recruitment of $CCR6^+$ cells including Th17 cells, via the suppression of CCL20 production. Furthermore, the addition of decoy WSX-1 to these experiments significantly abrogated the suppression of IL-6 production induced by TNF-α from FLSs (Fig. 5D). These results indicate that stimulation of IL-27R with IL-27 has a suppressive effect on the production of proinflammatory cytokines.

3.6. IL-27 has positive correlation with IFN-γ but weak negative correlation with IL-17A in the synovial fluid of RA

IL-27 is known to induce Th1 development and suppress Th17 development [9,12–15] and reduced the production of CCL20 (Fig. 5C). To evaluate the possible regulation in the development and the migration of Th cells by IL-27 in inflamed joints of RA, we measured the concentration of IFN-γ and IL-17A in the synovial fluid of RA analyzed in Fig. 1. The concentrations of IFN-γ and IL-17A were 0.026 ng/ml on the average ranging from 0.004 to 0.057 ng/ml, and 0.052 ng/ml ranging from 0 to 0.21 ng/ml, respectively. The IL-27 level had significant positive correlation with the IFN-γ level ($r = 0.56$, $p = 0.03$), but weak negative correlation with the IL-17A level ($r = -0.30$, $p = 0.27$) (Fig. 6). These results indicate that IL-27 in the inflamed joints of RA has effect in the increase of

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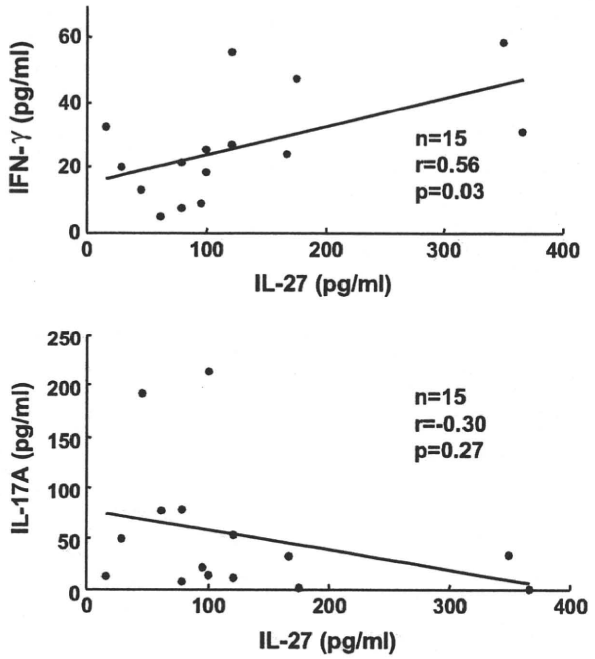

Fig. 6. The correlation of IL-27 with IFN-γ or IL-17A in the synovial fluid of RA. The concentrations of IFN-γ or IL-17A in the synovial fluid of RA patients analyzed in Fig. 1 were measured by ELISA ($n = 15$). Correlations of the IL-27 level with the IFN-γ level or the IL-17A level are shown. Pearson product-moment correlation coefficients (r) and P values are shown.

IFN-γ producing cells and reduction of IL-17A producing cells, implying induction of Th1 differentiation and suppression in the differentiation or the migration of Th17 cells by IL-27.

4. Discussion

In this study, we showed that the concentration of IL-27 in plasma of RA and OA patients and HV was at most 1 ng/ml, while that in synovial fluid of RA and OA was at most 400 and 40 pg/ml, respectively (Fig. 1). Recently, IL-27 at 10–100 ng/ml was reported to activate RA FLSs as an inflammatory cytokine [23]. In our laboratory, a high concentration (100 ng/ml) of IL-27 tended to induce IL-6 production from RA FLSs. However, based on the detected concentration of IL-27 in plasma or synovial fluid, we considered that a concentration of less than 10 ng/ml better reflects physiological conditions than a concentration of 10–100 ng/ml.

Experimental autoimmune animal models or *in vitro* human studies suggest that Th17 cells play a crucial role in RA [1–4]. Indeed, clinical treatment for RA targeting IL-17A has shown significant effects [25]. Th17 cells specifically express the chemokine receptor CCR6, and its ligand CCL20 recruits Th17 cells into sites of inflammation [1,5]. IL-27 has been reported to suppress the development of Th17 cells and the production of cytokines including IL-17A from $CD4^+$ T cells by blocking RORC expression in humans and in several experimental animal models, whereas it induces Th1 differentiation [9,12–15]. In our experiment of RA synovial fluid, the IL-27 level had significant correlation with the IFN-γ level but weak negative correlation with the IL-17A level (Fig. 6). Although IFN-γ and IL-17A can be produced by other cells than Th cells, these results imply that IL-27 in the inflamed joints of RA also induced Th1 development and suppress the development or the migration of Th17 cells.

IL-27 also suppresses the production of CCL20 induced by TNF-α from human keratinocytes [10]. In our experiment with FLSs, IL-27 suppressed the production of CCL20 induced by TNF-α and the production of IL-6 induced by TNF-α or IL-17A. These data collectively indicate that in RA, IL-27 suppresses not only the proinflammatory cytokine network but also the recruitment of $CCR6^+$ cells including Th17 cells.

IL-27R is a heterodimeric receptor consisting of WSX-1 and gp130. gp130 is known to be expressed on RA synovial cells and although IL-27 binds to WSX-1, signal transduction in response to IL-27 is never seen without the combination of both components [7,17,24]. Therefore, the addition of decoy WSX-1 successfully neutralized the suppressive effect of IL-27 in our experiments (Fig. 5D). WSX-1 is expressed on FLSs, and is also ubiquitously distributed on RA synovium (Fig. 4A and C). These results indicate that IL-27 might have a widespread suppressive effect on the synovium in RA.

We have shown that $CD14^+$ cells infiltrate RA synovium and produce IL-27 (Fig. 3B). DCs as well as monocytes/macrophages might be included among these $CD14^+IL\text{-}27^+$ cells, because human circulating $CD14^+$ monocytes have the capacity to differentiate into macrophages or DCs after immigration into extravascular tissues [26]. In particular, it was reported that human monocyte-derived DCs transdifferentiate into osteoclasts in the RA microenvironment, and that in the intermediate stage of the transdifferentiation they express CD14 and function as APCs [27].

From these results, we suggest that IL-27-producing $CD14^+$ cells might be circulating in peripheral vessels irrespective of RA or OA, but that these cells rarely migrate to normal synovium or synovial fluid. This may be why the concentration of IL-27 in joints was lower. IL-27-producing $CD14^+$ cells might preferentially infiltrate the synovium in RA joints and contribute to modification of joint inflammation. Notably, the sequence of anti-inflammatory mechanisms of IL-27 produced by $CD14^+IL\text{-}27^+$ cells infiltrating into RA synovium is one of the negative feedback systems acting against synovial inflammation in RA. Because IL-27 also suppresses the development of Th17 cells [9,12–15], IL-27 is considered to protect joints from inflammation induced by Th17 cells. However, the IL-27 level in RA synovial fluid was lower than in RA peripheral blood (Fig. 1). This lower IL-27 level in RA joints might result in incomplete regulation of Th17 cells in RA joints and contribute to the severity and perpetuation of joint inflammation. This may be partly because the poor regional blood flow in joints causes less accumulation of $CD14^+IL\text{-}27^+$ cells compared with the peripheral circulation. Alternatively, the cytokine environment of the joint might affect the production of IL-27 by $CD14^+$ cells. In this respect, reinforcement of the suppressive function of IL-27 might be a novel therapeutic agent targeting RA, although the role of IL-27 in human immunity is so complex that further research is required.

In conclusion, $CD14^+IL\text{-}27^+$ cells specifically infiltrate the synovium after the onset of RA, and IL-27 plays anti-inflammatory roles in RA. IL-27 inhibited not only the production of IL-6 from RA FLSs but also the recruitment of $CCR6^+$ cells including Th17 cells into RA joints by suppressing the production of CCL20 from RA FLS. This sequence is regarded as one of the negative feedback systems against inflammation in RA.

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