Involvement of TWEAK/Fn14 interaction in the synovial inflammation of RA

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Objective. TWEAK, TNF-like weak inducer of apoptosis, induces not only apoptosis of some tumour cells, but also proliferation of endothelial cells, and angiogenesis. It is known that TWEAK induces production of cytokines that are involved in the pathogenesis of RA. However, it is not clear how TWEAK takes part in the synovitis of RA. In this study, we investigated the role of TWEAK/fibroblast growth factor-inducible 14 (Fn14) interaction in the synovitis of RA.

Methods. TWEAK and Fn14 expression on RA and OA synovial cells (SCs) were analysed by FACS. Synovial fibroblasts (SFs) or freshly isolated SCs were cultured in the presence or absence of recombinant TWEAK (rTWEAK) and anti-TWEAK or anti-Fn14 mAbs. Cell proliferation, cytokine/chemokine production and intercellular adhesion molecule (ICAM-1) expression were measured by WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium monosodium salt], ELISA and FACS, respectively.

Results. TWEAK expression was detected on CD45-positive population in RA synovium, whereas Fn14 was detected on both CD45-positive and CD45-negative populations. Cultured RA and OA SFs showed higher proliferation and produced IL-6, IL-8 and MCP-1 in response to rTWEAK. Cell proliferation and cytokine production of freshly isolated SCs from RA patients were suppressed by anti-TWEAK and anti-Fn14 mAbs. ICAM-1 expression on RA, but not OA, SFs was up-regulated by rTWEAK.

Conclusions. These data suggest that TWEAK/Fn14 interaction plays a substantial role in the synovitis of RA, by directly inducing the proliferation of SFs, and by up-regulating the production of inflammatory cytokines/chemokines as well as the expression of ICAM-1.

KEY WORDS: Rheumatoid arthritis, Cytokines, Chemokines, Adhesion molecules.

Introduction

RA is an autoimmune disease characterized by inflammation of synovial tissue and subsequent joint destruction with unknown aetiology. The major clinical features of RA are inflammation and gradual damage to synovial joints [1, 2]. A massive leucocytic infiltrate mainly consisting of T cells, plasma cells and macrophages is commonly observed in the RA synovium [3–6]. The site of major joint destruction is where the synovium meets the cartilage and bone (pannus) [7]. Development of the destructive synovial pannus in RA is associated with changes in vascular permeability, synovial cell (SC) hyperplasia and angiogenesis [5, 8].

In studies on local immune and inflammatory reaction, RA synovium has been extensively analysed in a variety of ways, such as immunohistological analysis [9], in situ hybridization [10], examination of the synovial fluid contents [11, 12] or short-term culture of SCs [13]. These studies have shown the involvement of various cytokines, both pro-inflammatory and anti-inflammatory ones, and other mediators in the pathogenesis of RA synovitis. To name a few, IL-1, IL-6, IL-10, TGF-β and chemokines such as IL-8 and MCP-1 are the examples of these mediators [3, 4, 14]. Among these, TNF-α is thought to be playing a central role in regulating these mediators. TNF-α and its two receptors (p55 and p75 TNFR) are expressed at many sites within the synovial membrane, including the cartilage/pannus junction [9, 15]. In studies using RA SC culture system and neutralizing anti-TNF-α mAb, blockade of TNF-α abrogated spontaneous production of IL-1, IL-6, IL-8 and GM-CSF [16–18]. Recent clinical evidences using TWEAK blocking mAbs have confirmed the importance of TNF-α in the inflammation and joint destruction in RA. However, non-responders of TNF blockers clearly exist, indicating that some other molecules such as new TNF family members also play a crucial role in the synovitis of RA.

TWEAK, TNF-like weak inducer of apoptosis, is a new member of the TNF family, was originally identified as a weak inducer of apoptosis in some tumour cell lines [19]. To date, it has been reported that TWEAK induces not only apoptosis of some tumour cells but also proliferation of human umbilical vein endothelial cells (HUVECs) in vitro [20–22] and angiogenesis in vivo [20, 22]. It has also been reported that TWEAK could induce up-regulation of adhesion molecules [intercellular adhesion molecule (ICAM-1) and E-selectin] and secretion of chemokines (IL-8 and MCP-1) from HUVEC [23]. Recently, Wiley et al. [22] identified a TWEAK receptor, TWEAKR, by expression cloning using recombinant soluble TWEAK. The TWEAKR turned out to be identical with a fibroblast growth factor-inducible 14-kDa protein (Fn14), which was originally identified as a growth factor-inducible molecule in fibroblasts [24, 25]. More recently, by using newly generated anti-human Fn14 mAbs, we have reported that TWEAK-induced proliferation, migration and cell death is solely mediated by Fn14 [23, 26].

Our recent study has shown that administration of neutralizing anti-murine TWEAK mAb ameliorated joint swelling of collagen-induced arthritis (CIA), a well-established model for RA, indicating that TWEAK/Fn14 interaction is involved in the pathogenesis of CIA via induction of chemokine production and angiogenesis [27]. Perper et al. [28] also reported the function of TWEAK as a novel arthritogenic mediator using murine CIA model. However, contribution of TWEAK/Fn14 interaction to the synovitis of RA remains to be elucidated.

In this study, we have investigated how TWEAK/Fn14 interaction contributes to the pathogenesis of synovitis in RA. TWEAK and Fn14 were expressed on the freshly isolated RA SCs. Production of inflammatory cytokines/chemokines and up-regulation of ICAM-1 on RA synoviocytes were observed in response to recombinant TWEAK (rTWEAK), and these effects were abrogated by anti-TWEAK and anti-Fn14 mAbs. Cell proliferation and cytokine production of RA SCs were suppressed
by anti-TWEAK and anti-Fn14 mAbs. ICAM-1 expression on RA, but not OA, synovial fibroblasts (SFs) was also up-regulated by rTWEAK. Our present results suggest that TWEAK/Fn14 interaction plays a substantial role in the pathogenesis of synovitis in RA.

Materials and methods

Patients

Patients with RA were diagnosed according to the revised criteria of the ACR [23]. RA was in a chronic phase (disease duration >2 yrs) and active (number of swollen joints > 4, CRP > 2.0 mg/dl, ESR > 40 mm/h) in all of the RA patients studied. Synovial tissues from RA or OA patients were obtained at the time of synovectomy or total knee arthroplasty. Prior to the study, written informed consent was obtained from all the patients, and the study was approved by the Ethics Committee of Juntendo University School of Medicine.

Cells

To isolate SCs, surgical specimens from RA or OA patients were subjected to collagenase and DNase I treatment. In brief, synovial specimens were incubated in Minimum Essential Medium alpha (MEM; Gibco, Auckland, New Zealand) supplemented with collagenase (1 mg/ml) and DNase I (166 μg/ml) at 37°C for 3 h. Resulting cell suspension (defined as ‘SCs’) was filtered and subjected to further in vitro analyses. In this study, ‘SCs’ are defined as SCs cultured from the third until ninth passages, and 99.9% CD45-negative as estimated by FACS analysis. The cells were cultured in α-MEM supplemented with 10% fetal calf serum (FCS; Gibco, Auckland, New Zealand), 0.2 mg/ml streptomycin/penicillin at 37°C in 5% CO2.

Reagents

The human CD8-human TWEAK fusion protein (CD8-TWEAK) and the neutralizing anti-human TWEAK mAb (CARL-1, mouse IgG3) and the neutralizing anti-human Fn14 mAb (ITEM-2, mouse IgA) were prepared as described previously [23]. The biotinylated anti-human Fn14 mAb (ITEM-4, mouse IgG2b) was prepared as described previously [26]. Phycoerythrin (PE)-labelled anti-human ICAM-1 mAb (MEM111, mouse IgG1) was purchased from BD-PharMingen (San Diego, CA, USA). FITC-labelled anti-human CD45 mAb (HI30, mouse IgG1) was purchased from BD-PharMingen (San Diego, CA, USA). ELISA kits for IL-6, IL-8 and MCP-1 were purchased from BD-PharMingen. 3,3,5,5-Tetramethylbenzidine dihydrochloride (TMB) was obtained from Sigma (St Louis, MO, USA).

Flow cytometric analysis

For the detection of TWEAK/Fn14 expression in RA or OA synovium, freshly isolated SCs were subjected to the FACS analysis immediately after the collagenase and DNase I treatment. These SCs were incubated with FITC-labelled anti-human CD45 mAb, biotinylated CARL-1 or biotinylated ITEM-4 for 20 min at 4°C, followed by PE-labelled avidin (BD-PharMingen). To examine ICAM-1 up-regulation, SFs were cultured in the presence or absence of CD8-TWEAK and CARL-1 or ITEM-2 for 24 h. These cells were trypsinized and collected, and then were incubated with PE-labelled anti-human ICAM-1 mAb for 20 min at 4°C. After washing with PBS, the stained cells (live-gated on the basis of forward and side scatter profiles and propidium iodide exclusion) were analysed on a FACS Caliber (Becton-Dickinson, San Jose, CA, USA) and data were processed using the CellQuest program (Becton-Dickinson).

Proliferation assay

SFs fed 1 day before use were trypsinized and resuspended at 5 × 10^5 cells/ml in α-MEM. An equivalent number of cells (2.5 × 10^3/well) in 50 μl were plated onto each well of a flat-bottomed 96-well microtitre plate and allowed to adhere for 24 h at 37°C. CD8-TWEAK and mAbs or control Ig were prepared in this medium at 2× concentration, and 50 μl each was added to the wells, resulting in 100 μl of culture. The plate was incubated for 72 h at 37°C. 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulphonyl)-2H-tetrazolium monosodium salt (WST-8, Dojindo, Kumamoto, Japan) was added (10 μl/well) to each well. The plate was further incubated at 37°C for 4–7 h until colour development. Absorbance was read at 450 nm on a Microplate reader with Microplate manager (Bio-Rad, Richmond, CA, USA). For the proliferation assay of freshly isolated RA or OA SCs, cells were resuspended at 4 × 10^5 cells/ml. An equivalent number of cells (2 × 10^3/well) in 50 μl were plated onto each well of a flat-bottomed 96-well microtitre plate and allowed to adhere for 24 h at 37°C. Anti-TWEAK and anti-Fn14 mAbs or control Ig were prepared in this medium at 2× concentration, and 50 μl each was added to the wells, resulting in 100 μl of culture.

ELISA of cytokines and chemokines

SFs fed 1 day before use were trypsinized and resuspended at 5 × 10^5 cells/ml in α-MEM. An equivalent number of cells (2.5 × 10^3/well) in 50 μl were plated onto each well of a 24-well microtitre plate and allowed to adhere for 24 h at 37°C. CD8-TWEAK and mAbs or control Ig were prepared in this medium at 2× concentration, and 500 μl each was added to the wells, resulting in 1 ml of culture. The plate was incubated for 24 h at 37°C, and then the culture supernatant was collected and stored at −20°C until further experiments. For cytokine quantification of freshly isolated RA or OA SCs, cells were resuspended at 4 × 10^6 cells/ml. An equivalent number of cells (2 × 10^5/well) in 500 μl were plated onto each well of a 24-well microtitre plate and allowed to adhere for 24 h at 37°C. Anti-TWEAK and anti-Fn14 mAb or control Ig were prepared in this medium at 2× concentration, and 500 μl each was added to the wells, resulting in 1 ml of culture. Cytokine content was measured using specific ELISA kits (PharMingen) according to the manufacturer’s instruction.

Statistical analysis

All comparisons between two groups were analysed using Student’s unpaired t-test. P-values <0.05 were considered statistically significant.

Results

Expression of TWEAK and Fn14 on freshly isolated RA SCs and RA SFs

To detect TWEAK/Fn14 expression at the site of synovial inflammation, we stained freshly isolated RA and OA SCs with anti-human TWEAK and anti-human Fn14 mAbs [29]. As represented in Fig. 1A, both TWEAK and Fn14 were expressed on RA SC. However, the distribution was different between TWEAK and Fn14. Fn14 was expressed on both CD45-negative and CD45-positive populations, whereas TWEAK expression was limited to CD45-positive population. These data indicate that TWEAK is expressed on synovial leucocytes whereas Fn14 is expressed on leucocyte and non-leucocyte population, most likely represents SFs, in RA synovium. On which sub-populations of the CD45-positive leucocytes are TWEAK and Fn14 expressed is yet to be elucidated.

As discussed later, RA or OA SFs from the third to ninth passage were subjected to several experiments such as proliferation assay or cytokine/chemokine production in this study.
Therefore, we assessed the expression of TWEAK/Fn14 on RA SF prior to further experiments. We also assessed the ratio of CD45-positive and CD45-negative populations by FACS analysis (Fig. 1B). As shown in Fig. 1B, after the third or more passages, 99.9% of the surviving RA SCs were CD45-negative, and Fn14 but not TWEAK expression was detected on RA SF. As for OA, 99.9% of the cultured OA SCs were CD45-negative after the third or more passages, and as is the case with RA, Fn14 but not TWEAK was expressed on OA SFs. The average expression level of Fn14 on OA SFs was slightly lower than that on RA SFs; however, the difference was not statistically significant. These results indicate that Fn14 but not TWEAK is expressed on RA and OA SFs and that there were no leucocytes existing in the culture of RA or OA SFs used in the following experiments.

**TWEAK/Fn14 interaction contributes to proliferation of RA and OA SFs**

Previous studies have shown that TWEAK induces proliferation and migration of endothelial cells [20, 22]. It has also been shown that TWEAK-induced proliferation of endothelial cells is mediated by Fn14 [23]. To examine whether TWEAK/Fn14 interaction affects proliferation of RA SF, we cultured RA or OA SF with various concentrations of rTWEAK in the presence or absence of neutralizing anti-TWEAK or anti-Fn14 mAbs [23]. After a 72-h culture, cell proliferation was determined by the WST-8 assay. Proliferation of RA and OA SF was enhanced by rTWEAK stimulation in a dose-dependent manner. Addition of either anti-TWEAK or anti-Fn14 mAb in this culture abrogated the rTWEAK-induced cell proliferation (Fig. 2). These results show that the TWEAK/Fn14 interaction can enhance the proliferation of RA and OA SFs.

Since TWEAK expression was detected on CD45-positive population of RA SC (Fig. 1A), we next examined whether spontaneous proliferation of freshly isolated RA SC could be inhibited by anti-TWEAK and anti-Fn14 mAbs. As shown in Fig. 3, both anti-TWEAK and anti-Fn14 mAbs significantly inhibited the spontaneous proliferation of freshly isolated RA SC in three out of four patients tested. On the other hand, neither anti-TWEAK nor anti-Fn14 mAbs affect the spontaneous
It has been reported that TWEAK can induce production of several pro-inflammatory cytokines, such as IL-8 and MCP-1, by human endothelial cells [23, 30]. Recently, Perper et al. [28] showed that administration of neutralizing anti-TWEAK mAb can reduce concentration of TNF-α, RANTES (regulated on activation, normal T expressed and secreted), MMP-9, MIP (macrophage inflammatory protein)-1β and MCP-1 in serum of CIA mice. In our recent study using murine CIA model, we have also shown that treatment with neutralizing anti-TWEAK mAb could reduce MCP-1 and MIP-2, a murine homologue for human IL-8, contents in serum and joint washouts [27]. To investigate the effect of TWEAK stimulation upon RA and OA SF, we cultured RA or OA SF with various concentrations of CD8-TWEAK in the presence or absence of neutralizing anti-TWEAK or anti-Fn14 mAbs. After a 24 h culture, cell-free culture supernatants were collected and cytokine/chemokine contents were determined by specific ELISA. Production of IL-6, IL-8 and MCP-1 by RA SF was significantly enhanced by rTWEAK stimulation in a dosage-dependent manner. Both anti-TWEAK and anti-Fn14 mAbs abrogated the rTWEAK-induced cytokine/chemokine production (Fig. 4). RA SFs also responded to the stimulation with rTWEAK and cytokine/chemokine production was enhanced in a dosage-dependent manner except for IL-8 production. These results show that the TWEAK/Fn14 interaction can mediate production of IL-6, IL-8 and MCP-1 by RA and OA SFs.

We also examined the effect of anti-TWEAK and anti-Fn14 mAbs on the spontaneous production of IL-6, IL-8 and MCP-1 by freshly isolated RA and OA SC. As shown in Fig. 5, both anti-TWEAK and anti-Fn14 mAbs significantly inhibited the production of IL-6, IL-8 and MCP-1 by freshly isolated RA SC, although some individual differences were observed. Neither anti-TWEAK nor anti-Fn14 mAbs showed such inhibitory effect upon OA SCs; however, there was an exception observed (IL-6 in OA Patient 2). We also compared the inhibitory effect of anti-IL-6 and anti-TNF-α mAbs with those of anti-TWEAK and anti-Fn14 upon cytokine/chemokine production of freshly isolated RA/OA SCs. Although anti-TWEAK and anti-Fn14 had suppressive effect comparable with those of anti-IL-6 and anti-TNF-α upon cytokine/chemokine production of freshly isolated RA SCs, blockade of TNF-α seemed to be more efficient in attenuating proliferation and IL-6 production of freshly isolated RA SCs. In addition, only anti-TNF-α could reduce IL-6 and IL-8 secretion from freshly isolated OA SCs (data not shown). These results suggest that the TWEAK/Fn14 interaction is involved in the spontaneous production of pro-inflammatory cytokines/chemokines in RA, and in some cases in OA, synovium.

**TWEAK/Fn14 interaction induces cytokine/chemokine production by RA SFs**

**TWEAK induces up-regulation of ICAM-1 expression on RA SFs**

It has been reported that TWEAK induces up-regulation of adhesion molecules such as ICAM-1 and E-selectin on HUVEC [23]. Thus, we examined if TWEAK-induced up-regulation of these molecules could also be observed in RA and OA SFs. RA or OA SFs were stimulated with CD8-TWEAK in the presence or absence of anti-TWEAK or anti-Fn14 mAb, and the expression level of ICAM-1 was determined by flow cytometry after 24 h of culture. As shown in Fig. 6, TWEAK significantly up-regulated the expression level of ICAM-1, and either anti-TWEAK or anti-Fn14 mAbs abrogated the up-regulation of ICAM-1. OA SFs did not respond to the stimulation with CD8-TWEAK in the aspect of ICAM-1 up-regulation. These results indicate that the TWEAK/Fn14 interaction can induce the up-regulation of ICAM-1 on RA SFs.

**Discussion**

In a recent study, we have shown that neutralizing anti-mouse TWEAK mAb can ameliorate joint swelling in murine CIA model [27]. To investigate the spontaneous production of pro-inflammatory cytokines/chemokines in RA, and in some cases in OA, synovium.
model. Pathological analysis revealed that synovial hyperplasia was significantly reduced by the anti-TWEAK mAb treatment. Another recent study reported by Perper et al. [28] has also shown the ameliorating effect of neutralizing anti-TWEAK mAb upon synovial joint inflammation in murine CIA. In light of these findings in murine arthritis model, we examined whether TWEAK could induce the proliferation of RA SFs.

Prior to examine the effect of TWEAK upon proliferation of RA SF, we examined the expression of Fn14 on RA SF. As shown in Fig. 1B, cultured SF from RA patients highly expressed Fn14, but not TWEAK. SFs isolated from OA patients

**Fig. 3.** Effects of anti-TWEAK and anti-Fn14 mAbs on the proliferation of freshly isolated SCs. Freshly isolated SCs from RA or OA patients were cultured in the presence of anti-TWEAK mAb, anti-Fn14 mAb or isotype-matched control Ig (10 μg/ml). After 72 h culture, cell proliferation was measured by the WST-8 assay. Data are represented as mean ± S.D. of triplicate samples. Data with SC from four RA or OA patients are represented. *P < 0.05 as compared with the control Ig.
also expressed Fn14, but not TWEAK. So, we next conducted an experiment to see if exogenous TWEAK could induce proliferation of cultured RA or OA SFs via interaction with Fn14. Figure 2 shows that proliferation of RA and OA SFs is significantly enhanced by rTWEAK in a dose-dependent manner, and that both anti-TWEAK and anti-Fn14 mAbs can abrogate the TWEAK-induced proliferation. Members of the TNF family are characterized as mediators of various cellular responses including proliferation, differentiation and death [31, 32]. As a member of the TNF family, TWEAK has been shown to induce cell death in some tumour cell lines [19, 33] and to induce proliferation of endothelial cells [23]. Our finding shown in Fig. 2 suggests that TWEAK functions as a mediator of cellular proliferation rather than that of cell death in RA and OA SFs. In the present study, we prepared SFs by culturing cell suspension including leucocytes and SFs extracted from synovial tissue. During several passages, leucocytes and other cell populations except for SFs were removed along with the drained culture media. If the TWEAK-induced enhanced proliferation of RA SF proliferation plays a role in the synovial hyperplasia in RA, there must be certain cell population that expresses TWEAK in the synovial tissue of RA patients. To explore the source of TWEAK in the RA synovial tissue, we divided freshly isolated RA SCs into two populations on the basis of CD45 expression, and stained these two different populations with anti-TWEAK and anti-Fn14 mAbs to detect expression of TWEAK and Fn14. This experiment revealed that TWEAK expression in RA synovium was limited to CD45-positive population whereas Fn14 was expressed on both CD45-positive and CD45-negative populations (Fig. 1A). We also measured concentration of soluble TWEAK in the culture supernatant of RA and OA SC/SF by ELISA. Although TWEAK expression on the cell surface of CD45-positive population of RA SC was detected, concentration of soluble TWEAK was below detectable level in the culture supernatant of both RA and OA SC/SF (data not shown). To assess the functional interaction between TWEAK expressed on CD45-positive population of RA SC/SF and anti-TWEAK and anti-Fn14 mAbs into the primary culture of RA or OA SCs, and found that both anti-TWEAK and anti-Fn14 mAbs could inhibit the spontaneous proliferation of RA but not OA SC. This finding strongly suggests that at least some part of RA SC proliferation is mediated by TWEAK/Fn14 interaction and that blockade of TWEAK can be a therapeutic strategy against synovial hyperplasia in RA. The character of CD45-positive leucocytes expressing TWEAK in RA SC is yet to be determined by further study. Macrophages are the primary candidates, since we previously demonstrated that TWEAK could be expressed on IFN-γ-stimulated monocytes but not on activated lymphocytes [29].

Previous studies have shown that TWEAK can induce secretion of pro-inflammatory cytokines such as IL-6, IL-8 and MCP-1 from human astrocytes [34], human dermal fibroblasts [30] or HUVEC (human umbilical vein endothelial cells) [23]. Also, our recent study using murine CIA model have demonstrated that treatment with anti-TWEAK mAb can ameliorate synovial inflammation by reducing chemokine contents in serum and joint washouts and that rTWEAK can induce pro-inflammatory chemokine production by CIA SF [27]. It has been reported that significant reduction of MCP-1 expression in synovial tissue accompanies the reduction in synovial lining-layer macrophages and sublining inflammatory cell infiltrates [35] and that IL-8 induces transendothelial migration of neutrophils to sites of inflammation [36, 37]. As for IL-6, which is known as a growth factor for activated T and B lymphocytes, there is increasing evidence that IL-6 may inhibit bone formation and induce bone resorption through its stimulatory effects on osteoclasts [38]. Thus, it is interesting that IL-6 gene-knockout mice do not develop erosions of bone [39]. The results shown in Fig. 4 suggest that RA SF secrete these cytokines/chemokines in response to TWEAK via its interaction with Fn14 on RA SF. The involvement of TWEAK/Fn14 interaction in a persistent production of pro-inflammatory cytokines/chemokines in RA synovium was further substantiated by the inhibitory effects of anti-TWEAK and anti-Fn14 mAbs on the spontaneous production of IL-6, IL-8 and MCP-1 by freshly isolated RA SCs (Fig. 5). In addition to their inhibitory effects upon spontaneous cytokine production by RA SC, anti-TWEAK and anti-Fn14 mAbs also inhibited IL-6 secretion by OA SC (Fig. 5B). Although our results shown in Fig. 1A indicate that cell surface expression of neither TWEAK nor Fn14 was observed in SCs...
freshly isolated from two OA patients, the data in Fig. 5B (the inhibition of IL-6 secretion from OA SCs) suggests a possibility that, in some cases, TWEAK/Fn14 interaction might also be involved in the inflammation of OA synovium.

In addition to proliferation and pro-inflammatory cytokine production, TWEAK has been shown to induce cell surface expression of adhesion molecules such as ICAM-1 and E-selectin on human endothelial cells [23]. ICAM-1 (CD 54), which is
Stimulation index was calculated as follows; stimulation index = (mean fluorescence intensity (MFI) of each group)/(MFI of medium alone group). Data are represented as mean ± s.o. of three independent experiments. *P < 0.05 as compared with TWEAK alone.

In conclusion, the present study has shown that TWEAK expressed on CD45-positive population of RA SCs is possibly one of the mediators to up-regulate adhesion molecule in RA synovial tissue, and that the blockade of TWEAK/Fn14 interaction may be another therapeutic target to abrogate inflammatory cell migration to RA synovium.

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