Overexpression of decoy receptor 3 in synovial tissues of inflammatory arthritis

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Abstract

Objectives

Decoy receptor 3 (DCR3) was a newly identified soluble receptor which was reported to modulate the function of T cells, dendritic cells and macrophages. The aim of this study was to investigate DCR3 expression on the synovial tissue in different types of arthritis.

Methods

We obtained synovial tissues from 17 rheumatoid arthritis (RA), 17 ankylosing spondylitis (AS) and 17 osteoarthritis (OA) patients. Synovial specimens were stained with hematoxylin and eosin. The amount of lymphocytes and mononuclear cell infiltration and vascularity during light microscopic examination was scored from 0–4. The expression of CD3, CD4, CD8, CD68 and DCR3 in lining layer (LL) and sublining layer (SL) cells was stained using the immunohistochemical method and analysed by microscopic examination (score from 0–4, 0=absent, 1=slight, 2=moderate, 3=large, 4=extreme).

Results

OA patients were older than the RA and AS patients (65.9±10.3 years for OA, 58.4±17.7 for RA, and 43.2±16.4 for AS). Synovial tissues in RA patients had significantly increased mononuclear cell infiltration when compared to AS and OA patients (2.3±0.6, 1.9±0.5, 1.6±0.5, respectively, $p<0.05$). There was no striking difference in DCR3 expression in the synovial LL between RA, AS, and OA patients. CD4+ T cells and CD68+ monocytes/macrophages in the SL were more prominent in RA and AS than in OA ($p<0.05$). Similarly, DCR3 in the SL was more overexpressed in RA and AS than in OA (1.83±0.21, 1.71±0.36, 1.39±0.31, respectively, $p<0.01$).

Conclusion

The increased synovial inflammatory cell infiltration in RA and AS was associated with the elevated DCR3 expression.

Key words

DCR3, rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, synovial tissue

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Introduction

Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) are 2 common rheumatic diseases. RA affects more females and the age at onset is about 50 years. In contrast, AS affects young males, and the age at onset is approximately 20 years. Both diseases are genetically determined, particularly the strong relationship between human leukocyte antigen (HLA)-DR4 or -DR1 and RA, and HLA-B27 and AS (1-4). However, the pathogenesis of chronic inflammatory synovitis in either RA or AS is not entirely understood (5). Many studies have demonstrated that different molecules, including tumour-necrosis factor (TNF)-α, matrix metalloproteinase (MMP)-3, Fas, P53, and chemokines, are overexpressed in RA or AS synovial tissues, compared with osteoarthritis (OA) synovial tissues (6-16). The presence of pannus formation, synovial lining cells hyperplasia and large amounts of mononuclear cells in the synovial tissues of RA may be partially due to the defect of either Fas-Fas ligand (FasL) or TRAIL-TRAILR-induced apoptosis (6, 7, 17-19).

Decoy receptor 3 (DCR3) is a new identified protein belonging to the TNF receptor super-family; DCR3 is a secreted protein, since it lacks trans-membrane and cytoplasmic domains (20, 21). DCR3 can neutralise the biologic effects of FasL, LIGHT, and TNF-like molecule 1A (TL1A) (20, 22-24). Initially, elevated DCR3 expression was found in tumour cells (20, 23, 24). The overexpression of DCR3 can help tumour cells escape death by blocking FasL-, LIGHT-, and TL1A induced apoptosis (25-29). DCR3 also has been proven to suppress host immune system by impairment phagocytic activity of macrophage and regulation of dendritic cells, which drive T helper 2 polarisation and lead to tumour growth (30, 31).

Several serologic studies of DCR3 in rheumatic and immune diseases have been conducted. The increase of DCR3 in the serum of SLE may be helpful as a diagnostic parameter, and has indicated high disease activity (32, 33). Similarly, a severe RA stage was associated with highly elevated DCR3 and TL1A serum levels (34). Our pilot study has found that, in comparison with healthy controls, the serum DCR3 level was significantly higher in patients with RA, but not in AS. In addition, the DCR3 level in synovial fluid showed a significant difference between patients with RA and OA. Measurement of DCR3 in RA synovial fibroblasts was done by Hayashi *et al*., who found that increased DCR3 in the fibroblast-like synoviocytes may protect the cells against apoptosis (35). However, the role of DCR3 in AS has not been identified. The aim of this study was to investigate DCR3 expression on synovial membrane cells of RA, AS, and OA. This is the first study to measure DCR3 levels in synovial tissue cells of inflammatory arthritis.

Patients and methods

Patients

During a 2-year period, synovial tissues were obtained from 17 RA, 17 AS and 17 OA patients who underwent surgery or arthroscopic examination. The diagnosis of RA was based on the 1987 ACR criteria (36), while the diagnosis of AS was based on the modified New York criteria (37). Seventeen OA patients were used as controls for a comparison with RA and AS synovial pathology (38). The study was approved by the institutional ethics committee of Taipei Veterans General Hospital. All patients were required to sign a consent form to participate in this study.

In RA, synovial tissues were obtained from 10 patients during total knee arthroplasty, from 1 patient who received knee arthroscopy for diagnostic biopsy, from 4 patients during total hip replacement surgery, and from 2 patients who received wrist arthroscopy for diagnostic biopsy. In AS, synovial tissues were obtained from 15 patients during total hip replacement surgery, from 1 patient who received knee arthroscopy for diagnostic biopsy, and from 1 patient who received knee arthroscopic synovectomy. In OA, synovial tissues were obtained from 14 patients during total knee arthroplasty and from 3 patients during total hip replacement surgery. All synovial samples from the surgical procedures were immediately fixed in 10% formalin and processed

Table I. Pathologic findings in different rheumatic diseases.

	RA (n=17)	AS (n=17)	OA (n=17)	*p*-value RA *vs.* OA	AS *vs.* OA	RA *vs.* AS
Synovial lining cells (layer)	4.2 ± 1.7	4.0 ± 1.4	3.7 ± 1.1	0.586	0.586	0.973
Mononuclear cell infiltration[a]	2.3 ± 0.6	1.9 ± 0.5	1.6 ± 0.5	0.001*	0.131	0.018*
Vascularity[a]	2.8 ± 0.6	2.7 ± 0.7	2.3 ± 0.7	0.062	0.193	0.586

[a] score from 0 to 4: 0=absent; 1=slight; 2=moderate; 3=large; 4=extreme.
*Statistical analysis: data were analysed using SPSS 15.0. The Kruskal-Wallis tests were used, as appropriate, to assess the statistical significance of differences between groups.

through paraffin embedding. Five micrometer sections were cut from each synovial tissue and then mounted on a glass slide.

Immunohistochemical staining

Serial sections were stained with the following monoclonal antibodies: anti-CD3 (clone SP7, Lab Vision), anti-CD4 (clone 4B12, Lab Vision), anti-CD8 (clone SP16, Lab Vision), anti-CD68 (clone PG-M1, Dako), and anti-DCR3 (clone 3H5/DCR3, Santa Cruz). Staining procedures were performed according to the previously described 3-step method (39). Each 5μm synovial tissue was incubated with primary antibody for 60 minutes. After washing with phosphate-buffered saline (PBS), the sections were incubated with a biotinylated anti-mouse secondary antibody for 15 minutes, using a streptavidin-labelled peroxidose complex kit (ABC, Signet). The colour reaction was developed using diaminobenzidine (DAB) as a chromogen, and lastly the slides were stained with haematoxylin. Negative controls were performed using irrelevant isotype-matched monoclonal antibodies. For the positive control, we used tumour cells from one woman with breast cancer. The endogenous peroxidase was blocked with 1% hydrogen peroxide.

Microscopic analysis

The expression of CD3, CD4, CD8, CD68, and DCR3 by each positive cell was randomly analysed and the total number of cells was estimated on the same sections counterstained with haematoxylin. Two regions, including the lining layer (LL) and the sublining layer (SL), were selected for evaluation. At least 4 consecutive high-power fields in either the LL or SL were observed and counted. The percentage

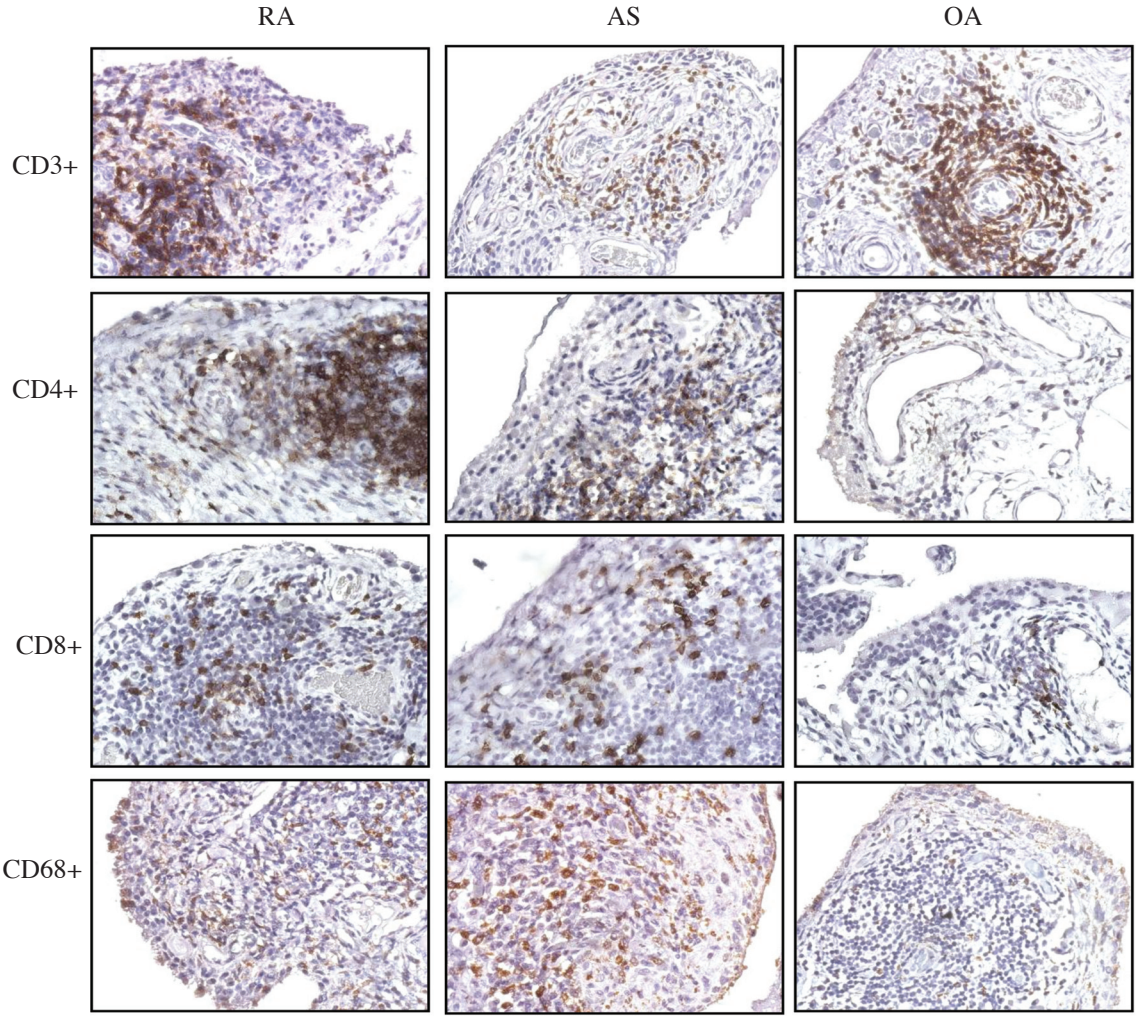

Fig. 1. The distribution of positive CD3, CD4, CD8, and CD68 cells in synovial and subsynovial lining layers in patients with rheumatoid arthritis (RA), ankylosing spondylitis (AS) and osteoarthritis (OA). The synovial tissues were obtained from knees of RA patients, hips of AS patients, and knees of OA patients.

of positive cells relative to total cells was calculated for each sample, counting at least 80 and up to 200 cells. The amount of positive cells relative to total cells was scored for each sample, from 0 to 4 (0: 0%; 1: <25%; 2: 25–49%; 3: 50–74%; 4: ≥75%) (6, 40).

Histologic evaluation by haematoxylin and eosin (HE) stain

Synovial specimens were fixed in 10% formalin and processed through paraffin embedding and sectioning, and then they were stained with HE. Histologic findings, including synovial lining cell hyperplasia, degree of mononuclear cell infiltration, and degree of vascularity, was evaluated according to a semiquantitative scoring system (0–4 scores, where 0=absent, 1=slight increase, 2=moderate, 3=large, 4=extreme) (8, 41).

Statistical analysis

All the clinical and laboratory data were analysed using SPSS 15.0 for Windows (SPSS, Chicago, Illinois, USA). Group differences were analysed by Mann-Whitney U-test. Correlations between variables were determined using the Spearman's rank correlation test. A *p*-value of <0.05 was regarded as being significant.

Results

Clinical analysis

We enrolled 17 RA patients, 17 AS patients and 17 OA patients in this study. The mean age was 58 (58.4±17.7) years for RA, 43 (43.2±16.4) years for AS and 66 (65.9±10.3) years for OA patients, and there was a significant difference among the 3 groups (p= 0.001). The gender ratio also disclosed differences, with males more predominant than females, particularly in AS. Treatment for AS was mainly non-steroid anti-inflammatory drugs (NSAID). Sulfasalazine was used only for AS patients with peripheral arthritis. Methotrexate and other disease-modifying antirheumatic drugs (DMARDs) and NSAIDs were used for RA patients. No biologic agent was administered to AS or RA patients. For the OA patients, we administered analgesics or NSAIDs. No patient received an intra-articular injection of corticosteroid or hyaluronic acid before synovial samples were collected.

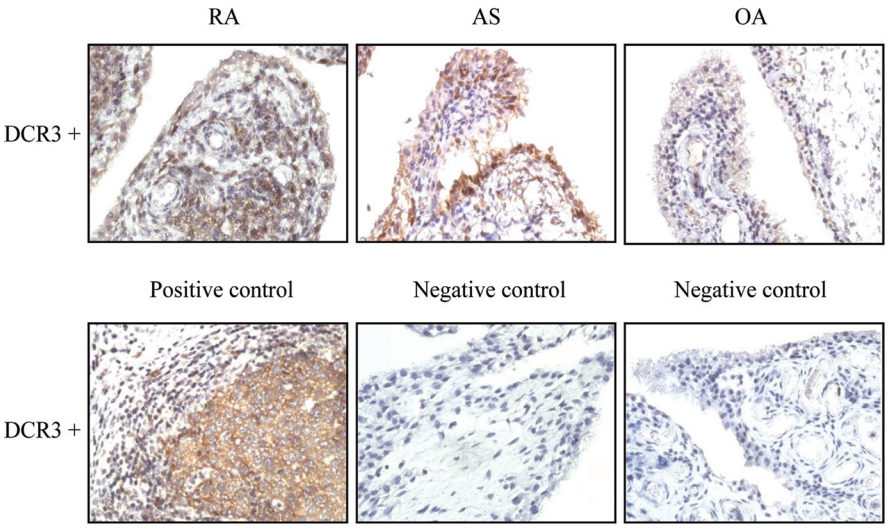

Fig. 2. The distribution of DCR3 positive cells in synovial and subsynovial lining layers in rheumatoid arthritis (RA), ankylosing spondylitis (AS) and osteoarthritis (OA). The synovial tissues were obtained from knees of RA, AS, and OA patients. We used tumour cells from one woman with breast cancer as a positive control. Negative controls were obtained from hip parts of 2 AS patients and performed using irrelevant isotype-matched monoclonal antibodies.

Light microscopic study

The results of light microscopic examination of the synovial biopsy specimens of these 3 rheumatic diseases showed that the synovial LL cell hyperplasia ranged from 1 to 13 layers and mean ± SD in RA was 4.2±1.7; in AS, 4.0±1.4; and in OA, 3.7±1.1 (Table I). There was no significant difference between the rheumatic diseases (all p>0.05). Similar to the LL cell hyperplasia, there was no significant difference in vascularity among the 3 different groups (2.8±0.6 in RA, 2.7±0.7 in AS, 2.3±0.7 in OA, all p>0.05). However, the mononuclear cell infiltration in the SL of RA patients was comparatively higher than that of AS and OA patients (RA *vs*. AS, 2.3±0.6 *vs*. 1.9±0.5, p=0.018; RA *vs*. OA, 2.3±0.6 *vs*. 1.6±0.5, p=0.001).

Immunohistochemical analysis

CD3+ lymphocytes, CD4+ T cells, CD8+ T cells, CD68+ monocytes/macrophages in the synovial LL and SL were stained and analysed using immunohistochemistry (Figs. 1, 2). No T cells were found in the synovial LL of RA, AS or OA. There was no significant difference for CD68 cells in the LL between RA, AS, and OA (all p>0.05) (Table II). The expression of DCR3 in the LL showed a result similar to CD68.

Compared with OA, a significant increased mononuclear cell infiltration was observed in the SL of RA, including CD3+ lymphocytes (2.11±0.29 *vs*. 1.76±0.28, p=0.002), CD4+ T cells (1.74±0.32 *vs*. 1.29±0.20, p<0.001), CD8+ T cells (1.59±0.36 *vs*. 1.25±0.28, p=0.009), CD68+ monocytes/macrophages (1.27±0.17 *vs*. 0.97±0.22, p<0.001) (Table II). In AS group, CD4+ T cells and CD68+ monocytes/macrophages in SL were more predominant than in OA (1.62±0.17 *vs*. 1.29±0.20, p<0.001; 1.22±0.22 *vs*. 0.97±0.22, p=0.002, respectively). The expression of DCR3 in the SL cells (Figs. 1, 2) was also significantly increased in RA and AS, compared with OA (RA *vs*. OA, 1.83±0.21 *vs*. 1.39±0.31, p<0.001; AS *vs*. OA, 1.71±0.36 *vs*. 1.39±0.31, p=0.009). There was no significant difference for mononuclear cells and DCR3 expression in SL between RA and AS.

Next, the differences of various mononuclear cell infiltrations in different part of joints were analysed. In RA group, CD68+ macrophages infiltration in the specimens from knees was higher than from hip joints, both in LL (2.30±0.34 *vs*. 1.87±0.10, p=0.026) and SL (1.31±0.15 *vs*. 1.10±0.14, p=0.010). Similar results were not observed in OA and AS.

Table II. The comparison of different cell populations and DCR3 expression in synovial cells among the patients with RA, AS and OA.

	RA (n=17)	AS (n=17)	OA (n=17)	*p*-value RA *vs*. OA	AS *vs*. OA	RA *vs*. AS
Lining layer						
CD68 + [a]	2.16 ± 0.34	2.04 ± 0.24	1.94 ± 1.48	0.057	0.218	0.394
DCR3 + [a]	2.46 ± 0.32	2.42 ± 0.44	2.25 ± 0.40	0.179	0.231	0.958
Sublining layer						
CD3 + [a]	2.11 ± 0.29	1.98 ± 0.29	1.76 ± 0.28	0.002*	0.053	0.182
CD4 + [a]	1.74 ± 0.32	1.62 ± 0.17	1.29 ± 0.20	<0.001*	<0.001*	0.150
CD8 + [a]	1.59 ± 0.36	1.37 ± 0.20	1.25 ± 0.28	0.009*	0.182	0.053
CD68 + [a]	1.27 ± 0.17	1.22 ± 0.22	0.97 ± 0.22	<0.001*	0.002*	0.357
DCR3 + [a]	1.83 ± 0.21	1.71 ± 0.36	1.39 ± 0.31	<0.001*	0.009*	0.345

[a] score from 0 to 4: 0=absent; 1=slight; 2=moderate; 3=large; 4=extreme.
*Statistical analysis: data were analysed using SPSS 15.0. The Kruskal-Wallis tests were used, as appropriate, to assess the statistical significance of differences between groups.

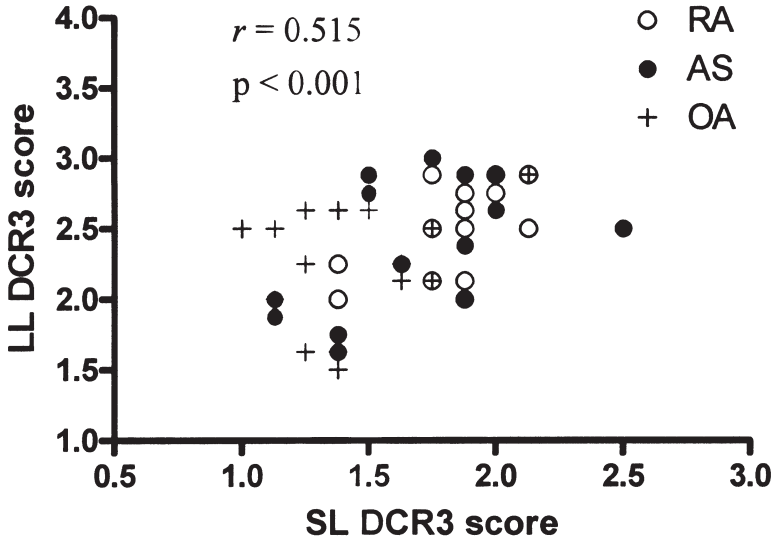

Fig. 3. A positive correlation was shown between DCR3 expression score in synovial lining cells (LL) and sub-lining layer cells (SL).
*Statistical analysis: the results were analysed using SPSS 15.0. The non-parametric correlations of Spearman's tests were used as appropriate, to assess the statistical significance of differences between groups. Open circles, rheumatoid arthritis (RA); closed circles, ankylosing spondylitis (AS); plus sign, osteoarthritis (OA).

Correlation of SL DCR3 and LL DCR3

Using Spearman's correlation coefficient analysis, positive correlations were observed between SL DCR3 and LL DCR3 scores in RA and AS, but not in OA patients (r=0.525, p=0.037; r=0.540, p=0.025; r=0.143, p=0.585, respectively). We analysed all 51 patients (RA, AS, and OA) and found SL DCR3 score was significantly associated with LL DCR3 score (r=0.515, p<0.001) (Fig. 3).

Discussion

The findings of increased synovial mononuclear cells in RA or AS (*vs*. OA) in this study reconfirmed previous results (6, 42-44). Significant overexpression of DCR3 in RA and AS (*vs*. OA) was also demonstrated and may be associated with the increased inflammation. The importance of macrophage in RA has been well established; increased numbers of macrophages were noted in RA synovium, they can contribute proinflammatory cytokines, such as TNF-α and interleukin (IL)-1β, etc, and were correlated with disease activity (45, 46). Studies to investigate the role of macrophages in the pathogenesis of AS were found to be limited. Smith *et al*. reported that deficient interferon-gamma production was found in macrophages derived from AS (47). It has been reported that DCR3 can drive macrophages towards an M2 phenotype, which has an anti-inflammatory property characterised by secreting the anti-inflammatory cytokine IL-10 and down-regulates the production of proinflammatory cytokines (30). Vandooren *et al*. reported that spondylarthritis synovitis displayed strongly reduced synovial fluid levels of M1-derived mediators (48). Taking together, in RA and AS, elevated DCR3 in synovial SL may drive macrophages to M2 phenotype and attenuate the inflammatory status. Indeed, DcR3 was suggested to be able to attenuate joint inflammation by neutralising TL1A, LIGHT, and FasL (21).

In this study, monocytes/macrophages infiltration and DCR3 expression were compared between LL cells and SL cells in RA, AS, and OA patients. Both were equally present in the LL of all arthritis, but were more prominent in the SL of RA and AS, while compared with OA. Since LL had less cell population when compared to the SL, the significant difference of monocytes/macrophages or DCR3 expression between inflammatory arthritis in SL may be different from in LL.

RA is characterised by symmetrical pain and swelling of small joints, including the proximal interphalangeal, metacarpal, and wrist joints of the hands and the metatarsal joints of the feet (4). Arthritis in large joints, including shoulders, knees, and hips, was also observed in RA. In this study, the most frequent synovial tissues we obtained in RA were knees, followed by hips and wrists. In contrast, the most frequent synovial tissues in AS were hips. In AS patients, peripheral arthritis mainly occurs in lower limbs with an asymmetrical pattern. Hip involvement in AS patients can lead to destruction and patients might receive total hip replacement surgery at a relatively young age (49). Interestingly, we found more CD68+ macrophages infiltration in the specimens from knees than hip joints in RA group, suggesting synovitis of knees in RA was more severe than hips.

This is the first study to use immunohistochemical staining to measure the

DCR3 in synovial LL and SL cells. However, there are some limitations in this study. A limitation is that the mean age differed among the RA, AS, and OA patients. Another limitation is that we did not compare DCR3 levels in the serum or synovial fluid with in synovial tissues.

In the present study, the increased DCR3 expression on the synovial tissues is associated with the increased inflammatory cell infiltration in RA and AS. Whether DCR3 can be used as a disease activity biomarker or a diagnostic parameter in autoimmune or inflammatory joint diseases is unknown and requires further study.

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