Decoy receptor 3 suppresses B cell functions and has a negative correlation with disease activity in rheumatoid arthritis

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Abstract

Objectives

The decoy receptor 3 (DcR3) is a member of the tumour necrosis factor (TNF) receptor superfamily and may regulate inflammation. The aim of this study was to investigate the role of DcR3 in B cell functions and its correlation to disease activity in patients with rheumatoid arthritis (RA).

Methods

The concentrations of DcR3 and TNF-α were measured by ELISA. B cell proliferation was assessed by quantification of ^{3}H-thymidine uptake. Staphylococcus aureus Cowan (SAC) strain were used to stimulate B cell proliferation and TNF-α production.

Results

Compared to the osteoarthritis (OA) patients, the RA group had higher synovial DcR3 levels (3273.6±1623.2 vs. 1594.8±1190.0 pg/ml, p=0.003), which were negatively correlated with the serum erythrocyte sedimentation rate and Disease Activity Score using 28 joint counts (DAS28) scores (r=-0.560, p=0.002; r=-0.579, p<0.001, respectively). Although the RA B cells have more active characteristics, B cell proliferation induced by SAC was successfully suppressed by recombinant DcR3.Fc fusion protein with an average inhibition of 44.8%. Moreover, DcR3.Fc fusion protein was found to suppress SAC-induced TNF-α production by B cells in 8 RA patients (average inhibition 47.0%).

Conclusion

The results of our study indicated that the inhibition of B cell functions by DcR3 may partially explain the negative correlation between DcR3 level and disease activity in RA patients. Our findings imply that DcR3 may be used as a biomarker for disease activity and a potential therapeutic agent in the treatment of RA.

Key words

decoy receptor 3, rheumatoid arthritis, B cell activation, tumour necrosis factor-α

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Introduction

Rheumatoid arthritis (RA) is the most common form of inflammatory arthritis. This chronic, symmetrical polyarthritis affects females more than males, and has an age of onset of about 50. The pathogenesis of RA is complex and not entirely understood. Multiple cytokines and many cells, including fibroblast-like synoviocytes (FLS), macrophages, T cells, and dendritic cells, are involved in the process of RA. A growing body of evidence suggests that B cells also play a crucial role in RA. B cells contribute to inflammation by producing autoantibodies, secreting cytokines and chemokines, and presenting antigens to autoreactive T cells, and have been treated as a potential therapeutic target (1-5).

Decoy receptor 3 (DcR3), a member in the tumour-necrosis factor (TNF) receptor superfamily, is a secreted protein which is overexpressed in a variety of tumour cells (6-10). DcR3 can neutralise the biological effects of FasL, LIGHT, and the TNF-like molecule 1A (TL1A) (6, 9, 11). DcR3 helps tumour cells escape from immune surveillance during tumourigenesis by blocking FasL- and LIGHT-induced apoptosis (10-12). DcR3 may lead to an increase of tumour angiogenesis and tumour growth by neutralisation of TL1A and modulating macrophages and dendritic cell functions (13-15). Evidence from *in vitro* and animal experiments suggest an anti-inflammation function of DcR3. DcR3 favours the differentiation of Th2 cells and suppresses the differentiation of inflammation-associated Th1 and Th17 cells (7). More recently, we found that that DcR3 has a negative regulatory role on B cells by suppressing the activation of NF-κB (16). It is worth noting that B cells from RA patients show important alterations in the expression of several key surface molecules, including CD86, FcγRIIB, CD95, and complement receptors (17-19). Because CD86 is a costimulatory molecule and FcγRIIB is an inhibitory receptor, up-regulation of CD86 and reduced expression of FcγRIIB observed in the RA B cells imply that these cells are in a more active stage. Furthermore, the RA B cells display a significant increased proliferative response to IL-2 (19). Therefore, whether DcR3 can down-regulate B cell function successfully in RA patients remains largely unknown.

The increase of DcR3 in the sera from patients with RA has been previously observed but there was no correlation between serum DcR3 level and the level of reported disease activity in humans (20, 21). Using the immunohistochemical staining method, we reported that the level of DcR3 in the synovial sublining layer was higher in RA than in OA, implying that the concentration of DcR3 that existed in SF was higher than that in serum (22). Therefore, we compared the synovial level of DcR3 from RA and OA patients in this study. The correlation between DcR3 level and clinical profiles, laboratory results, and disease activity were analysed. In addition, the influence of DcR3.Fc fusion protein on B cell proliferation was evaluated. The effect of DcR3.Fc fusion protein on the secretion of TNF-α from B cells in RA patients was also studied.

Methods

Patients

Patients and healthy controls (HCs) were enrolled in Taipei Veterans General Hospital. Thirty-two RA and 12 OA patients offered SF samples, with all of them obtained from the knees. Seventeen RA and 17 HCs offered peripheral blood for B cells analysis. The diagnosis of RA was based on the 1987 ACR criteria (23). This study was approved by the institutional ethics committee of Taipei Veterans General Hospital. Informed consent forms were obtained for all patients participating in this study.

Laboratory and clinical assay

Clinical and laboratory assays of the RA patients were recorded. Rheumatoid factors (RF) and C-reactive protein (CRP) were measured with nephelometry, and the erythrocyte sedimentation rate (ESR) was measured with the Westergren method. Since ESR reflects not only inflammation but also anaemia, haemoglobin and haematocrit values were also recorded. Enzyme linked immunosorbent assay (ELISA) was used to detect DcR3 levels in SF

with a detection limit of 93.75 pg/ml (Human DcR3/TNFRSF6B DuoSet ELISA kit, purchased from R&D Systems, Minneapolis, MN, USA). RA disease activity was measured using the Disease Activity Score 28-joint assessment 4 (DAS28-ESR), which was calculated as follows: DAS28 = 0.56 × √ (TJC28) + 0.28 × √ (SJC28) + 0.014 × GH + 0.70 × ln(ESR). Where TJC = tender joint count; SJC = swollen joint count; and GH = general health (patient assessment of disease activity using a 100 mm visual analogue scale) (24). The visual analog scale (VAS) for pain, Lequesne pain and functional index (0-24) (25), and Kellgren-Lawrence score for knees were used to assess the severity of OA (0-4) (26).

B cell proliferation analysis

The recombinant fusion protein DcR3.Fc was expressed with the FreeStyle 293 expression system as described previously (14, 16, 27). Human $CD19^+$ B cells from peripheral blood were purified using antibody conjugated microbeads (Miltenyi Biotec, Auburn, CA). Cell purity was checked with anti-human CD20 antibody staining, and B cell purity was over 90% in all experiments. Purified human IgG1 was used as the negative control. *Staphylococcus aureus* Cowan strain (SAC) was used to stimulate B cell division and TNF-α production in a T cell-independent manner. Purified B cells were incubated in 96-well plates (2×10^4 / well in 200 μl RPMI supplemented with 10% fetal calf serum) for 72 hours with fixed SAC (Sigma) (1:20000 dilution) in the presence or absence of human IgG1 (Calbiochem, La Jolla, CA) (10 μg/ml) or DcR3.Fc (10 μg/ml). Cells pulsed for an additional 24 hours with ^{3}H-thymidine (1 μCi/well, PerkinElmer) were harvested and ^{3}H-thymidine incorporation was assessed with a liquid scintillation counter.

Analysis of TNF-α production

The purified human B cells were incubated in 96-well plates as described above in the medium, or in the medium containing fixed SAC (1: 20000 dilution) in the presence of human IgG1 (10 μg/ml) or DcR3.Fc (10 μg/ml). The supernatant was collected at 12 hours and human TNF-α was measured by ELISA (BD Biosciences) according to the manufacturer's instructions.

Statistical analysis

Comparisons of DcR3 levels between the different groups were analysed with the Mann-Whitney U-test. Spearman's correlation was used to assess the association between DcR3 levels and clinical profiles, laboratory results, and DAS28 scores. The *p*-value was 2-tailed and interpreted as significant when the value was less than 0.05. All statistical analyses were conducted using SPSS software (SPSS 15.0 for Windows, SPSS, Chicago, Illinois, USA).

Table I. Demographic, laboratory, and clinical profiles of patients with rheumatoid arthritis and osteoarthritis who offered synovial fluid.

	RA n=32	OA n=12
Age, years	50.7 ± 2.9	62.0 ± 3.5
Female (%)	27 (84.3)	8 (66.7)
RF (IU/ml)	122.9 ± 24.6	-
ESR (mm/hour)	39.6 ± 4.4	-
CRP (ng/ml)	2.6 ± 0.5	-
Haemoglobin (g/dL)	12.3 ± 1.4	-
Haematocrit (%)	36.4 ± 3.5	-
DAS28 score	5.0 ± 0.3	-

RF: rheumatoid factor; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: Disease Activity Score using 28 joint counts; values shown as mean ± SE.

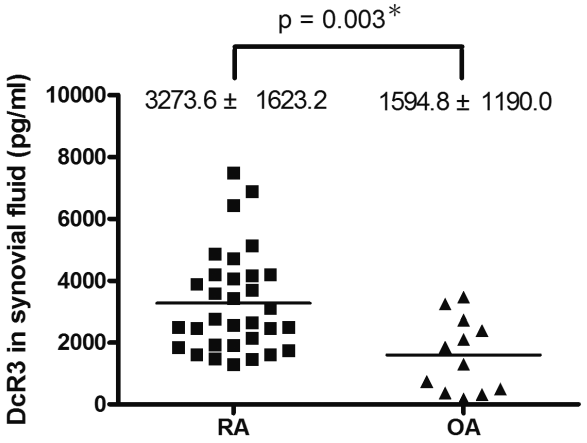

Fig. 1. The DcR3 levels in the synovial fluid from RA and OA patients.
The synovial fluid was collected and aliquot frozen at -70°C. The level of DcR3 was detected by ELISA according to the manufacturer's instructions.
*Significant *p*-value <0.05.

Results

Clinical characteristics of RA and OA patients

Table I shows the demographic, laboratory, and clinical characteristics of all the studied RA and OA subjects. The average age of the RA patients who offered SF samples was 50.7 years, and 62.0 years for the OA patients. Twenty-seven (84.3%) RA patients and 8 (66.7%) OA patients were female. The gender ratio was not significantly different between the RA and OA patients. ESR, CRP, haemoglobin, and haematocrit values (mean ± SD) in RA patients were 39.6±4.4 mm/hour, 2.6±0.5 ng/ml, 12.3±1.4 g/dL, and 36.4±3.5%, respectively. Disease-modifying anti-rheumatic drugs, including methotrexate (MTX), sulfasalazine, hydroxychloroquine, or leflunomide, were the main treatment for the RA patients. Non-steroidal anti-inflammatory drugs (NSAIDs) and/or oral glucocorticosteroids were used according to the patient's condition. In treatment-resistant cases, biologics were used. None of the patients received B cell depletion therapy. In the case of the OA patients, we administered analgesics or NSAIDs. None of the patients received intra-articular injections of corticosteroid or hyaluronic acid before SF samples were collected.

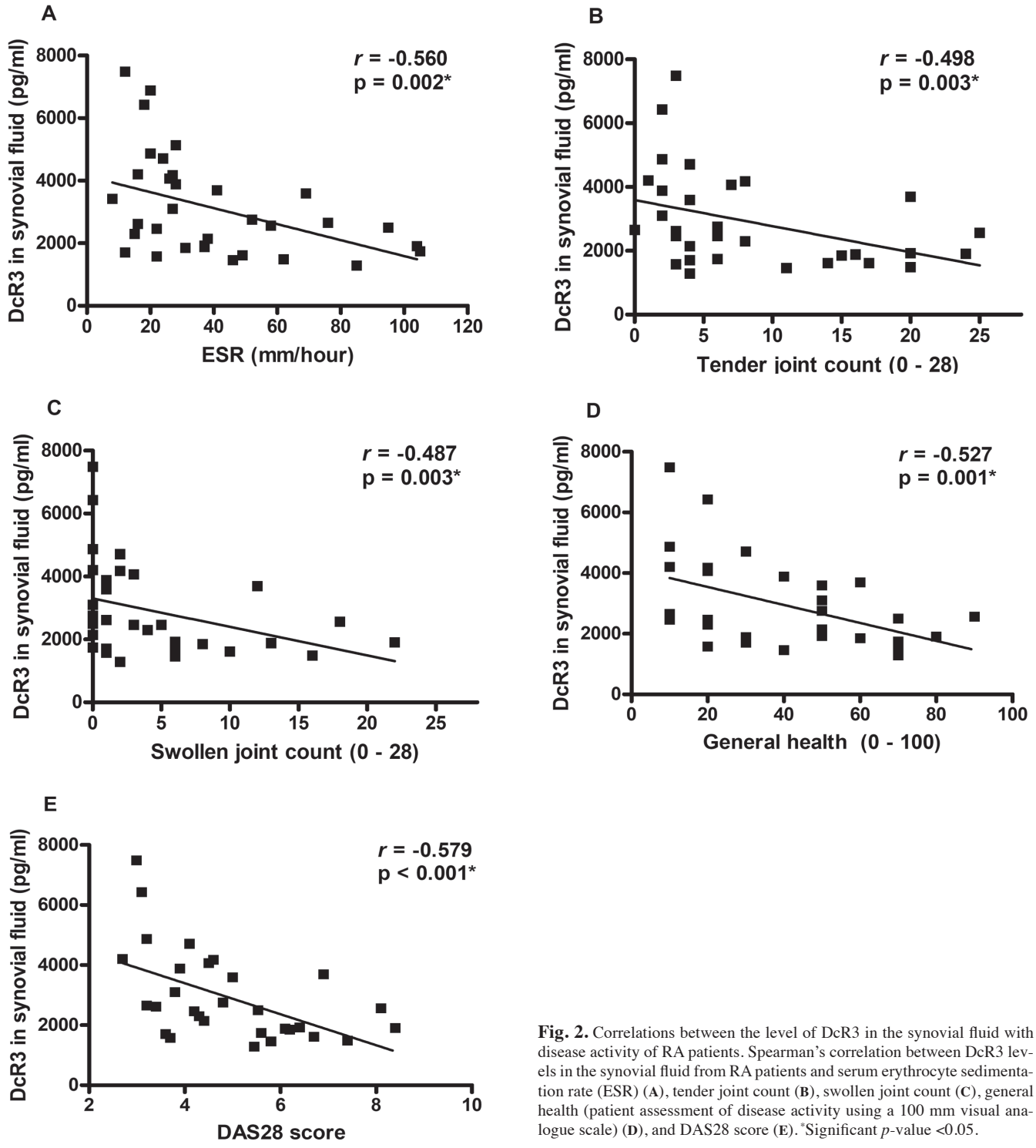

Fig. 2. Correlations between the level of DcR3 in the synovial fluid with disease activity of RA patients. Spearman's correlation between DcR3 levels in the synovial fluid from RA patients and serum erythrocyte sedimentation rate (ESR) (**A**), tender joint count (**B**), swollen joint count (**C**), general health (patient assessment of disease activity using a 100 mm visual analogue scale) (**D**), and DAS28 score (**E**). *Significant *p*-value <0.05.

DcR3 levels in SF from RA and OA patients and the correlation with disease activity

DcR3 was detected in the SF of all RA and OA patients. The median of DcR3 levels in SF was 2630.5, range 1289.5–7480.8 pg/ml in RA patients, whereas it was 1539.5, range 176.2–3464.8 pg/ml in OA patients. As shown in Figure 1, DcR3 levels in SF were higher in RA patients than in the age- and sex-matched OA patients (3273.6±1623.2 vs. 1594.8±1190.0 pg/ml, p=0.003).

To study the significance of this DcR3 elevation, we analysed the association between DcR3 levels and laboratory characteristics and disease activity. We found that DcR3 levels in SF had a negative correlation with DAS28 scores and its components, including serum ESR, TJC, SJC, and GH (r=-0.579, p<0.001; r=-0.560, p=0.002; r=-0.498, p=0.003; r=-0.487, p=0.003; r=-0.527, p=0.001, respectively) (Fig. 2). However, no significant association between the DcR3 levels in SF with RF or CRP was observed (r=0.139, p=0.411; r=-

0.115, p=0.484, respectively). In contrast, there was no correlation between synovial DcR3 levels and the VAS pain score, Lequesne pain and functional index, or Kellgren-Lawrence grade of knee in OA group (both p>0.05).

Suppression of cell proliferation by DcR3 in B cells from RA patients

DcR3 has been reported to regulate the functions of macrophages, dendritic cells, and T cells, but its role in B cell activities is largely unknown. Because B cells from the patients with autoimmune diseases usually have alterations in the surface and/or intracellular proteins, whether DcR3 can regulate the functions of B cells from RA patients is still not clear. To test this, peripheral blood CD19$^+$ B cells from HCs and patients with RA were purified and treated with fixed SAC. At the same time, DcR3.Fc fusion protein or human IgG1 (serving as a control protein) were added to test for their effects on B cell proliferation. B cell proliferation in RA patients was similar to that in HCs (data not shown). DcR3.Fc reduced SAC-induced B cell proliferation of all 7 RA patients we tested, with an average inhibition percentage of 44.8% (Table II). The inhibitory effect of DcR3.Fc on B cell proliferation was also observed in cells from 12 out of 13 HCs, with a similar average inhibition percentage (49.3%) when compared to RA groups.

The influence of DcR3 on TNF-α production from B cell in RA and HC

To further confirm the impact of DcR3 on B cells, we measured the TNF-α production by B cells from 8 HCs and 8 RA patients who did not receive biologic therapy. The TNF-α levels secreted from B cells without stimulation were detected in 8 RA patients (56.6±19.3 pg/ml). Treating with SAC successfully induced TNF-α production by B cells with an average of 998.9±738.7 pg/ml in RA patients and 462.2±345.5 pg/ml in HCs (p=0.105). Moreover, we found that DcR3.Fc reduced SAC-induced TNF-α production in 8 RA patients and 4 out of 8 HCs. The inhibition percentage by DcR3.Fc was calculated and shown in Figure 3. Compared to human IgG1, DcR3.Fc suppressed TNF-α production by B cells stimulated by SAC with an average inhibition of 47.0±20.8% in RA patients (p<0.001) (Fig. 3A). The inhibitory effect of DcR3 on SAC-induced TNF-α production by B cells did not show significant difference in 8 HCs when compared to human IgG1 (average inhibition rate: 8.8±36.0%, p=0.442) (Fig. 3B).

Table II. DcR3.Fc suppresses Staphylococcus aureus-induced B cell proliferation from 7 patients with rheumatoid arthritis.

	Medium	SAC + hIgG1	SAC + DcR3.Fc	Percentage of inhibition#
No 1	272 ± 73*	1180 ± 176	729 ± 168	38%
No 2	105 ± 34	5001 ± 340	2794 ± 332	44%
No 5	128 ± 69	482 ± 40	214 ± 8	56%
No 16	129 ± 32	8097 ± 807	4718 ± 375	42%
No 18	98 ± 21	7863 ± 274	5468 ± 891	30%
No 20	83 ± 22	7685 ± 979	4267 ± 798	44%
No 29	123 ± 23	2120 ± 187	850 ± 53	60%

*Fifty thousand CD19$^+$ B cells purified from peripheral blood were seeded in 96-well plates and incubated with Staphylococcus aureus (SAC, 1:20000 dilution) in the absence or presence of human IgG1 (hIgG1, 10 μg/ml) or DcR3.Fc (10 μg/ml) for 72 hours. Cells pulsed for an additional 24 hours with ^{3}H-thymidine (1 μCi/well) were harvested and ^{3}H-thymidine incorporation was assessed with a liquid scintillation counter. Data shown as mean ± SE.

#Percentage of inhibition was calculated as (1- SAC plus DcR3/SAC plus hIgG1) × 100%.

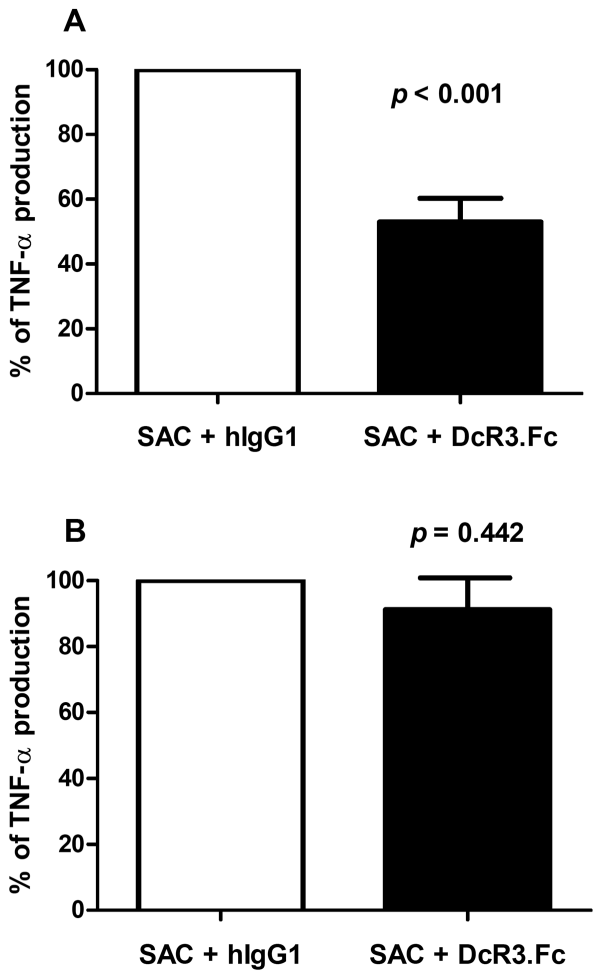

Fig. 3. The influence of DcR3.Fc fusion protein on TNF-α production by B cells in RA patients (A) and healthy controls (B). CD19$^+$ B cells were purified to approximately 90% purity using magnetic beads from peripheral blood obtained from 8 RA patients and 8 healthy controls. 2 × 10^4 cells were seeded in 96-well plates and cultured with indicated reagents. Fixed Staphylococcus aureus Cowan (SAC) strain (1:20000 dilution) were added in the presence of 10 μg/ml human IgG1 (hIgG1) or DcR3.Fc. The supernatant was collected 12 hours later and TNF-α was measured using ELISA. The percentage of TNF-α production was calculated as follows: 100% x (TNF-α level in sample tested)/(TNF-α level in hIgG1 treated control). The summary of all RA and HC donors in three independent experiments is shown.

Discussion

In this study, we found that the level of DcR3 in the SF from RA patients was significantly higher than that from OA

patients and had a negative correlation with serum disease activity. We speculated that DcR3 may have an inhibitory function on the immune response in RA; DcR3.Fc fusion protein suppressed B cell proliferation induced by SAC. In addition, DcR3.Fc fusion protein was proven to reduce TNF-α production by B cells from RA patients after cells were stimulated with SAC. These results indicate that DcR3 may suppress inflammation in RA patients at least partly via regulating B cell proliferation and TNF-α production.

At present, the question of whether therapy induces disease remission as well as when these treatments should be discontinued in RA patients are two major issues (28, 29). Establishing a biomarker is useful in determining the optimal treatment strategy. Previous studies have observed an elevated level of serum DcR3 in RA patients; Bamias *et al*. analysed the serum DcR3 levels of 81 Greek RA patients and 51 HCs and found that the serum DcR3 levels were significantly higher in the RA patients (20); in a Japanese study, DcR3 expression in the serum was statistically higher in 28 RA patients compared to 10 OA patients (21). However, no correlation of serum DcR3 with RA clinical activity was found in these two reports. In this study, we successfully proved that the DcR3 levels in SF negatively correlated with disease activity and inflammation, indicating that DcR3 levels in SF was a biomarker of disease activity in patients with RA. Previous studies have demonstrated that different inflammatory related molecules, including TNF-α, matrix metalloproteinase, Fas, and chemokines, are overexpressed in RA compared to OA synovial tissues, suggesting that RA patients had more severe inflammation than OA patients (30-35). Therefore, OA was recognised as a low-grade inflammatory disease and usually used as control. Recently, we reported that DcR3 on the synovial tissue was more overexpressed in RA than in OA patients and associated with the degree of inflammatory cell infiltration (22), implying that DcR3 may be related to synovial inflammation. Indeed, DcR3 level in SF is significantly higher in our RA patients than that in OA patients, which is similar to previous study (21). Therefore, DcR3 levels in SF may be treated as a biomarker of disease activity and reflect local inflammatory status in RA, offering evidences for anti-rheumatic agents selection and timing of step-down. The expression of DcR3 in autoimmune patients is reported to be stimulated by TNF-α, one of most important pro-inflammatory cytokines, via the NF-κB pathway (36), therefore, the reason why its levels were up-regulated in the SF from RA patients may be due to high inflammation conditions. Generally ESR and CRP are correlated in patients with RA. Indeed, there was a significant positive correlation noted between serum ESR and CRP in the present study ($r=0.352$, $p=0.045$). However, we observed that in our patients the ESR, but not serum CRP, were associated with DcR3 level in SF. The reason why poor correlation between SF DcR3 level and serum CRP may be our patients' characteristics. Previous studies have reported that normal ESR and CRP values were seen in a substantial proportion of RA patients (37, 38). Over 80% (26 out of 32, 81.25%) of our RA patients had an elevation of ESR (≥20 mm/hour) and only about 60.0% (20 out of 32, 62.5%) had abnormal CRP (>1.0 ng/ml). Further studies are needed to confirm these results.

Since the major inflammation occurs in the joints of RA patients, we hypothesised that high concentrations of DcR3 accumulated locally may attenuate the inflammatory process. The primary causes of the initiation of the inflammatory status in RA are complex, and various factors are considered to play a role in the pathogenesis. Helper T cells, FLS, and macrophages are proven to contribute to the immune response in RA, and accumulated evidence supports the theory that DcR3 plays an anti-inflammatory role. First, DcR3 was suggested to be able to attenuate joint inflammation by neutralising TL1A, LIGHT, and FasL (7). Another study found that DcR3 can drive macrophages toward an M2 phenotype, which has an anti-inflammatory property characterised by secreting the anti-inflammatory cytokine IL-10 and down-regulating the production of proinflammatory cytokines (14). The up-regulation of DcR3 attenuated the T helper 1 response (15, 27). DcR3 can block LIGHT-lymphotoxin-β receptor (LT-βR)-mediated signaling ,which activates dendritic cells and induces a cytotoxic T cell response (39). All together, it suggests that DcR3 may be a negative regulator in the immune response. The finding that higher DcR3 levels in SF associated with lower DAS28 scores in this study supports our hypothesis. It is possible that DcR3 can be kept in high levels in the synovial membrane and therefore regulates the functions of infiltrating leukocytes. This helps explain why elevated DcR3 in the SF is associated with lower disease activity in RA patients. In contrast, we failed to demonstrate the correlation between synovial DcR3 levels and disease severity in the OA group. The low-grade inflammatory status of OA may be one of possible reasons.

Researchers have recently come to believe that B cells are responsible for the process of RA. First, B cells produce autoantibodies, including RF, anti-citrullinated protein antibodies, etc., which may contribute to the pathological processes of RA (40-42). Second, B cells are able to secrete cytokines, such as lymphotoxin, IL-10, and transforming growth factor-β, and regulate inflammation in the synovial tissues of RA (43-45). B cells also produce the chemokines which contribute to leukocyte infiltration and the development of ectopic lymphoneogenesis within the inflammatory synovium in RA (2, 46). Third, as antigen presenting cells, B cells can present antigens and provide co-stimulatory signals which induce autoreactive T cell activation (47, 48). In addition, B cells were proven to be able to activate FLS, which contributes to bone erosion by producing inflammatory cytokines and proteases (4, 49). The efficacy of B cell depletion therapy on RA also supports these findings (50-52).

Our previous study found that DcR3 attenuated the activation of normal B cells by suppressing the activation of NF-κB (16). Because accumulated evidence show that B cells from RA patients express activation markers and

may have a tendency to have a stronger proliferative response, we were uncertain if DcR3 affects RA B cells as well (19). Given the fact that B cells play a critical role in the pathogenesis of RA, we decided to study whether DcR3 reduced the disease activity of RA by modulating B cell functions. We noticed that DcR3.Fc had an inhibitory effect on B cell proliferation in RA subjects. In a mouse collagen-induced arthritis model, the injection of DcR3 plasmid attenuates disease severity, and the activation of T cell and B cell (53), indicating that DcR3 may be used as a drug to treat RA in humans as well. Although the cause of RA is unknown, the importance of T cells, B cells and abnormal T-B cell interaction in the pathogenesis of RA has been recognised. T cells not only play a very critical role in the proper activation and proliferation of B cells, but also in the differentiation of plasma cells, which produce large amounts of autoantibodies in the RA patients. TL1A, one of the ligands of DcR3, may also involve the function of B cells. Sun *et al*. found that TL1A levels of serum and SF in RA were significantly higher than that in OA, and serum TL1A concentrations have a positive correlation with autoantibody production in RA (54). Moreover, TL1A has been proven to augment TNF-α production by T cells and contribute to the pathogenesis of RA (55). An elevated level of DcR3 in SF may neutralise TL1A in SF and then inhibit autoantibody and proinflammatory cytokine production. Therefore, our observation further supports the potential of DcR3.Fc as a therapeutic agent in the treatment of RA.

In the present study, we discovered that DcR3 attenuated proliferation and TNF-α production in human B cells. Although DcR3 is believed to neutralise the biological effects mediated by FasL, LIGHT, and TL1A, it also serves as a ligand to induce osteoclast formation (13), monocyte adhesion (14, 56), or inhibit synovial fibroblast proliferation (57). In an attempt to identify ligands to DcR3 on B cells, our results showed that human peripheral blood B cells do not express FasL, LIGHT, or TL1A, and that the addition of receptor fusion proteins, which bind FasL, LIGHT, and TL1A, did not have a suppression effect on human B cells (16). Therefore, these three molecules may not be DcR3 receptors on B cells. In myeloid cells, DcR3 is reported to modulate cell functions via heparan sulfate proteoglycans (56, 58). However, we demonstrated that heparin did not neutralise the inhibitory activity of DcR3.Fc on B cells (16), implying that DcR3 does not suppress B cell proliferation by binding to heparan sulfate proteoglycans. Based on these observations, we postulate that a novel receptor mediates the biological functions of DcR3 on human B cells.

TNF-α is one of the most important cytokines involved in RA and is secreted by activated T cells, macrophages, NK cells, and neutrophils and primarily produced by synovial macrophages in RA. However, relatively little research has been conducted on the role of TNF-α produced by B cells in the pathogenesis of RA. We accidentally observed that human B cells secrete significant amounts of TNF-α after SAC stimulation, suggesting that B cells may also be TNF-α producer in RA. Next, we investigated whether DcR3 reduced TNF-α production by B cells from RA patients. Our data show that although B cells from RA patients seemed to produce higher TNF-α levels when compared to those from HCs, DcR3.Fc successfully reduced SAC-induced TNF-α production in RA patients with a higher inhibition percentage when compared to that in HCs. All together, these results support the hypothesis that B cells have different characteristics in RA and DcR3 may contribute a critical negative regulatory role on them.

The elevation of serum DcR3 has been reported in various inflammatory diseases, including RA and SLE (20, 21, 59). Because TNF-α has been proven to stimulate DcR3 expression in in vitro studies (36, 60, 61), the increase of DcR3 expression in inflammation conditions may be caused by TNF-α production. However, whether DcR3 suppresses or promotes inflammation depends on different disease conditions. On one hand, DcR3 is thought to be a negative regulator of inflammation by its neutralising effects on FasL, LIGHT, and TL1A (7). The discovery that DcR3 favours the differentiation of Th2 cells and suppresses the differentiation of inflammation-associated Th1 and Th17 cells (15, 27, 62)—along with the fact that DcR3 induces DC apoptosis (58)—is supporting evidence that DcR3 possesses an anti-inflammatory function. On the other hand, DcR3 enhances T cell proliferation (20) and overexpression of DcR3 in mice causes a lupus-like syndrome (63), suggesting that DcR3 plays a role in the pathogenesis of SLE. The reason why DcR3 plays different roles in different autoimmune diseases warrants further investigation. However, it is highly likely that the increase of DcR3 in various inflammatory conditions is due to the up-regulation of inflammatory cytokines, especially TNF-α.

Conclusions

This is the first study to reveal that RA patients with higher DcR3 levels in the SF had less disease activity, indicating that DcR3 in SF may be used as a biomarker of disease activity in RA. DcR3.Fc may be considered as a potential therapeutic agent in the treatment of RA for its ability to reduce B cell numbers and attenuate TNF-α production.

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