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Histological Change of Synovium and Clinical Efficacy of Arthroscopic Synovectomy for Effect Attenuation by Etanercept in Rheumatoid Arthritis

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In order to investigate whether arthroscopic synovectomy is effective for rheumatoid arthritis (RA) patients who exhibit an incomplete response to etanercept treatment, we assessed 7 subjects who underwent 8 arthroscopic synovectomies in the knee joint, shoulder joint and elbow joints. We compared c-reactive protein (CRP) and disease activity scores (DAS) 28 before and at 4, 24 and 48 weeks after surgery. Immunohistochemical examination was performed whether tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and receptor activator of nuclear kappa B ligand (RANKL) expressed in synovial cells besides lymphocytes invasion and vascular proliferation occurred in hematoxylin and eosin (HE) staining. After arthroscopic synovectomy we continued etanercept treatment with or without methotrexate (MTX) in a routine manner. We detected synovium proliferation with a vascular increase in the patella femoral (PF) joint, around the meniscus and also on the femoral and tibial side of the anterior cruciate ligament (ACL) in the knee joints. We also found synovium proliferation in the rotator interval (RI) in the glenohumeral joint and fatty change in the subacromial bursa (SAB) in the shoulder. In the elbow joint we found synovium proliferation with white fibrous tissue around the radioulner joint which had developed into bone erosion. The average of CRP at preoperation, 3.8 ± 0.5 mg/dl, was improved to 1.3 ± 0.3 mg/dl at 6 weeks, 0.6 ± 0.2 mg/dl at 24 weeks and 0.7±0.4 mg/dl at 48 weeks after surgery. There were no side effects, not even post surgical infection from arthroscopic synovectomy, during etanercept treatment. DAS28 was improved from 6.3±0.6 to 3.5 ± 1.2 at 6 weeks, 2.8±0.7 at 24 weeks and 2.7±0.9 at 48 weeks after surgery. Histological examination revealed that lymphocyte proliferation, multi-nuclear cells and hyper vascularity emerged in etanercept toleration cases. TNF-α and IL-6 were expressed in the synovium, however RANKL was not expressed. Therefore, it is possible that arthroscopic synovectomy, in order to remove the synovium which produces TNF-α and IL-6, can be an effective method for the continuation of etanercept treatment when its efficacy is decreased in or attenuated for RA patients.

Key words: etanercept, rheumatoid arthritis (RA), histology, arthroscopic synovectomy, attenuation

Introduction

Even in the treatment of rheumatoid arthritis (RA) with anti-tumor necrosis factor (TNF) therapy such as etanercept, there are some cases which fail to control disease activity, prevent structural damage, and maintain the quality of life. Etanercept is a fusion protein consisting of the extracellular ligand-binding domain of the 75-kD receptor for TNF-α and β and the constant portion of human IgG1 which can block the cell signaling to produce inflammatory cytokines. In patients with RA who have an incomplete response to this anti-TNF treatment, methods such as an increase of methotrexate (MTX), steroids or etanercept, or a decrease in the interval period of injection with etanercept can be considered. In our institute we have treated 66 cases of RA by etanercept with or without MTX. However, there is no clear evidence to control the

Table Arthroscopic synovectomy (ASS) in etanercept treatment for RA

cases	age/gender	D.D. (y)	stage	class	surgery	MTX (mg)	steroid (mg)
1	48/F	14	II	II	blt. knee ASS	6	5
2	62/F	12	IV	III	rt. shoulder ASS	(−)	5
3	69/F	19	III	III	lt. knee ASS	(−)	7.5
4	49/M	2	II	II	rt. elbow ASS	6	(−)
5	70/F	21	III	III	rt. elbow ASS	6	5
6	55/F	33	III	III	rt. elbow ASS	8	5
7	75/M	25	III	III	rt. elbow ASS	6	5

D.D. (y): disease duration (year), RA: rheumatoid arthritis, MTX: methotrexate.

disease if etanercept fails to control RA activity. For people who exhibit an incomplete response to etanercept treatment, we have also performed arthroscopic synovectomy two weeks after an injection of etanercept, assessed the results, and then continued the injections of etanercept two weeks after surgery. Arthroscopic synovectomy is reported as an effective method for the early stage of RA[1]. However the destructive change of RA could not be completely improved only by arthroscopic synovectomy from the viewpoint of long-term results[2]. We reported recently that the combination of anti-TNF therapy and arthroscopic synovectomy for patients who did not respond after around 3-5 times of infliximab infusion with MTX treatment resulted in significantly improved clinical evaluation[3]. Arthroscopic synovectomy is safe and less painful compared with open synovectomy. Therefore, the hospitalization of patients is for a relatively short period after surgery. Etanercept causes several side effects such as a rash reaction to injections, an increase in blood pressure, headaches due to a slight allergy reaction and interstitial pneumonia as a severe case. If we use those drugs (MTX, steroids and etanercept) too much for RA patients or over a long period, it is possible to induce those side effects, sometimes irreversibly, such as interstitial pneumonia, with or without MTX. To avoid side effects, surgical treatment, such as arthroscopic synovectomy, is one of the safe choices in order to treat RA with etanercept simultaneously. The efficacy of etanercept has already been reported as a long-term therapy and it also prevents joint destruction by using a sharp score[4]. There are no reports, however, about the synovium findings made by arthroscopic/histological examinations during etanercept therapy when there has been a weak response to etanercept. This is the first report about synovium proliferation conditions in knee, shoulder and elbow joints and what the histological findings were in cases of etanercept toleration. We investigated the combination of etanercept and arthroscopic synovectomy, and also disease activity scores (DAS) 28 change, to assess the efficacy of arthroscopic synovectomy for RA patients who had not responded well to etanercept.

Materials and Methods

We performed arthroscopic synovectomy in 7 patients out of 66 etanercept treatment cases for RA (Table). All patients consented to this study before surgery. Twenty-five milligram of etanercept (Enbrel®) were used twice a week. The patients included 2 males and 5 females from 48 to 75 years old with an average age of 62 years old. All patients initially responded to etanercept but gradually the effect decreased and the average of c-reactive protein (CRP) was 3.8±0.5 (2.5-8.6) mg/dl at the time of surgery. The duration of etanercept treatment before surgery was an avarage of 42 (24-56) weeks. The etanercept treatment included the diagnosis of RA based on American College of Rheumatology (ACR) (formerly, the American Rheumatism Association) criteria[5]and categorization according to the criteria of Steinbrocker et al[6]. Two patients were stage II, 4 were stage III and 1 was stage IV. There were 2 patients in Class II, 5 in Class III. Increasing stage means more joint destruction and increasing class means more dysfunction of daily life. They

were medicated during etanercept treatment with 6 mg of MTX (4 patients), 8 mg of MTX (1 patient) and 2 patients had no MTX. Five milligram per day of predonine was used in 5 patients, and 7.5 mg/day of prednisolone was used in 1 patient. Prednisolone sodium succinate (20 mg) was used for all patients as a steroid cover after surgery. They did not take any other disease-modifying antirheumatic drugs (DMARD) during etanercept treatment. The indication of arthroscopic synovectomy is the case of increasing high CRP (2.0 mg/dl<) with limited the numbers of swelling joints including knee, shoulder, ankle, wrist and elbow even using etanercept. Moreover, it is indicated the cases with joint swelling considered to be induced synovium proliferation which cause systemic CRP increasing. At the time of arthroscopic synovectomy, a general anesthetic was administered for shoulder and elbow arthroscopic synovectomy, and lumbar anesthetic was administered in the knee joints. We used a 4.0 mm arthroscope (Smith & Nephew, USA) for the knee and shoulder joints and a 2.7 mm arthroscope (Smith & Nephew) for the elbow joints. We also used a shaver apparatus (Smith & Nephew) and a radiofrequency instrument (VAPR®; Mitek, Norwood, MA, USA) for the operation to remove the synovium.

We observed the synovium in the articular joints directly by arthroscopy of the knee, shoulder and elbow to analyze what condition the synovium was in and where it had proliferated in the joints during etanercept treatment. Furthermore, we investigated the change in CRP (mg/dl), DAS28 (CRP), and ACR20, ACR50, and ACR70 before and after arthroscopic synovectomy[7]. In 1 histological examination, we analyzed the synovium from an arthroscopic synovectomy 3 years before and 1 year after treatment with etanercept for 1 case (case numer 1, Table). We checked the synovium by histology with hematoxylin and eosin (HE) in all cases as to whether lymphocyte proliferation, hyper vascularity, or macrophage proliferation had appeared. For immunohistochemistry, the tissue sections were blocked for 10 min in phosphate buffer saline (PBS) containing 20% rabbit serum and then incubated overnight at 4℃ with the following antibodies: anti-TNF-α mouse monoclonal antibody (1: 1,000; Biogenesis, Pool, UK), anti-human interleukin (IL)-6 rabbit polyclonal antibody (Rockland Inc., Gilbertsville, PA, USA), and the anti-human receptor activator of nuclear kappa B ligand (RANKL) (FL-317) rabbit polyclonal antibody (1: 200; Santa Cruz Biotechnology, CA, USA). After treating with a second antibody at room temperature for 10 min, sections were then incubated for 10 min with appropriate Vectastain ABC reagent (Vector), using 3,3'-diaminobenzidine-4HCL (DAB) (Sigma) for the color reaction for 5 min, which resulted in brown staining of antigen-expressing cells. Then we analyzed the expression patterns of TNF-α, IL-6 and RANKL in synovium of all cases of etanercept toleration. For statistical analysis, we used the Wilcoxon signed rank test to compare the CRP, DAS28, ACR20, ACR 50, and ACR70 before plus 24 and 48 weeks after surgery. P values of less than 0.05 were considered significant.

Results

In our knee arthroscopic findings, we detected high vascular synovium between the disc and tibial cartilage and also at the notch of the femoral chondyle attached to the anterior cruciate ligament (Fig. 1A and C). We shaved and removed the synovium and the joint cavity was made clear and clean after arthroscopic synovectomy (Fig. 1B and D). In shoulder arthroscopy, we found synovium proliferation with white fibrous tissue at the rotator interval (Fig. 2A) and around the anteroinferior glenohumeral ligament (AIGHL) (Fig. 2B). The anterior limb was floating with the synovium over the long head of the biceps tendon (LHB). In regard to the biggest difference between the knee and the shoulder synovium, the latter had white fibrous tissue which induced a joint impingement in the glenohumeral joint. In the subacromial bursa, the fatty tissue increased in the bursal synovium (Fig. 2C) and we removed those tissues with the shaver and VAPR® without bony resection for arthroscopic debridement (Fig. 2D). In the elbow arthroscopic findings, we detected synovium proliferation between the tibiofibular joints which had in-

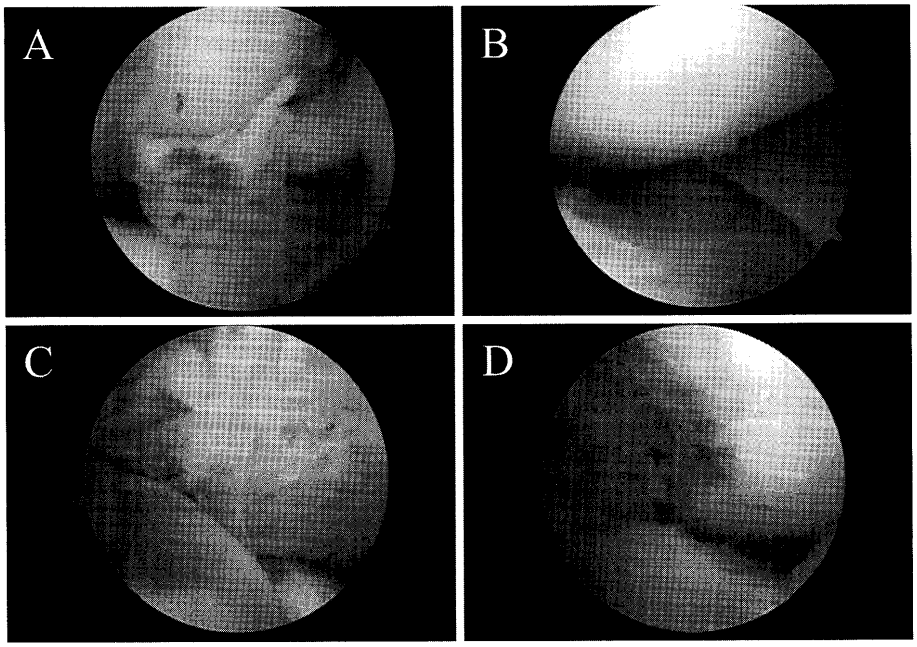

Fig. 1 Arthroscopic finding and synovectomy during etanercept treatment for RA
A: high vascular synovium between the disc and tibial cartilage.
B: after arthroscopic synovectomy.
C: synovium at the notch of femoral chondyle attached the anterior cruciate ligament.
D: the view cleaned up after arthroscopic synovectomy.

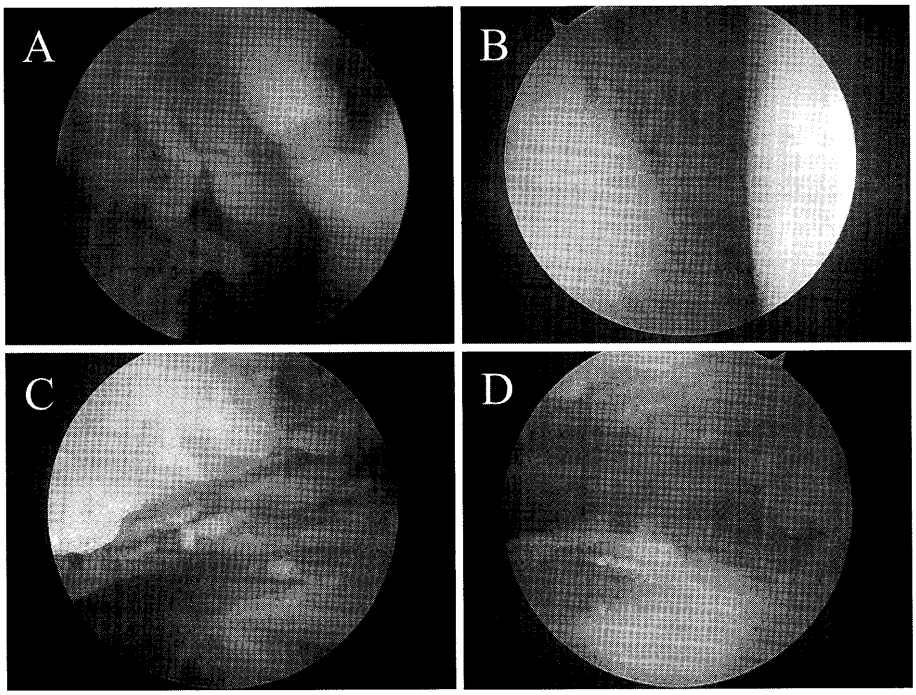

Fig. 2 Arthroscopic finding in shoulder arthroscopy in etanercept treatment
A: synovial proliferation with white fibrous tissue at rotator interval.
B: after synovectomy.
C: subacromial bursa before surgery.
D: subacromial bursa after surgery.

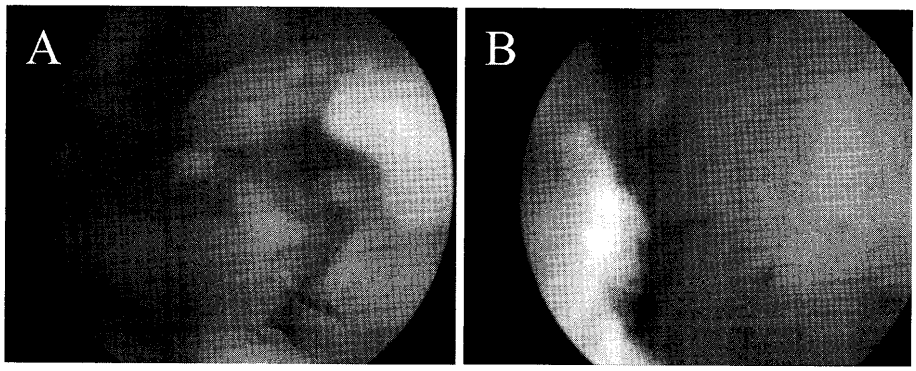

Fig. 3 Arthroscopic finding in elbow arthroscopy
A: synoium proliferation around radio-ulnar joint.
B: after arthroscopic synovectomy.

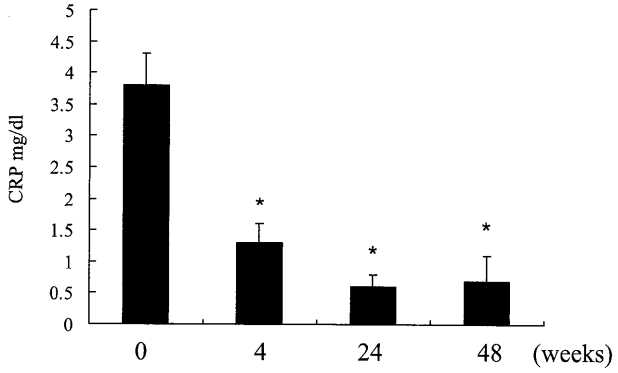

Fig. 4 Serum CRP changes after 0, 4, 24 and 48 weeks by arthroscopic synovectomy
* is significant decrease compared with 0 week ($p<0.05$).

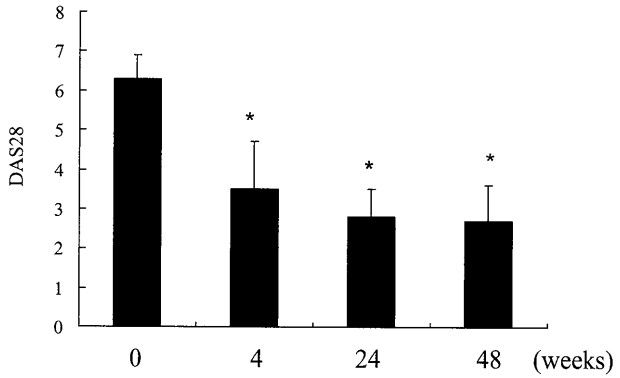

Fig. 5 DAS28 after 0, 4, 24 and 48 weeks by arthroscopic synovectomy
* is significant decrease compared with 0 week ($p<0.05$).

vaded and eroded the tibia (Fig. 3A) and we removed those tissues so as to clearly see the joint cartilage which had not induced impingement (Fig. 3B). The average of CRP at preoperation, 3.8 ± 0.5 (2.5-8.6) mg/dl, was significantly improved to 1.3 ± 0.3 (0.6-1.7) mg/dl 4 weeks later ($p<0.05$), and it then changed to 0.6 ± 0.2 (0.2-1.2) mg/dl 24 weeks later ($p<0.05$) and 0.7 ± 0.4 (0.1-0.9) mg/dl 48 weeks later ($p<0.05$) (Fig. 4). Therefore, CRP was significantly improved after 4 weeks and continued for 48 weeks after arthroscopic synovectomy during etanercept treatment. DAS28 was calculated to be 6.3 ± 0.6 at 0 week, 3.5 ± 1.2 at 4 weeks($p<0.05$), 2.8 ± 0.7 at 24 weeks ($p<0.05$) and 2.7 ± 0.9 at 48 weeks ($p<0.05$) after surgery (Fig. 5). DAS28 was significantly improved after 4 weeks and continued for 48 weeks after arthroscopic synovectomy. Therefore, arthroscopic synovectomy was clinically effective for the patients who tolerated the effect of etanercept. ACR20 was 82%, ACR50 was 54% and ACR70 was 35% at 24 weeks, and ACR20 was 76%, ACR50 was 47% and ACR70 was 32% at 48 weeks. Therefore, the efficacy of etanercept was clinically enhanced by arthroscopic synovectomy. In histological examination, 3 years before etanercept treatment, arthroscopic synovectomy was performed in the case of number 1 of Table, then after 1 year of etanercept treatment, the effect of attenuation was recognized and arthroscopic synovectomy was performed again. In the case of attenuation of the etanercept effect, the synovium contained lymphocyte proliferation with hyper vascularity and in the surface layer multi-nuclear cells were found (Figs. 6A-D, and Fig. 7A). In immunohistochemical examination,

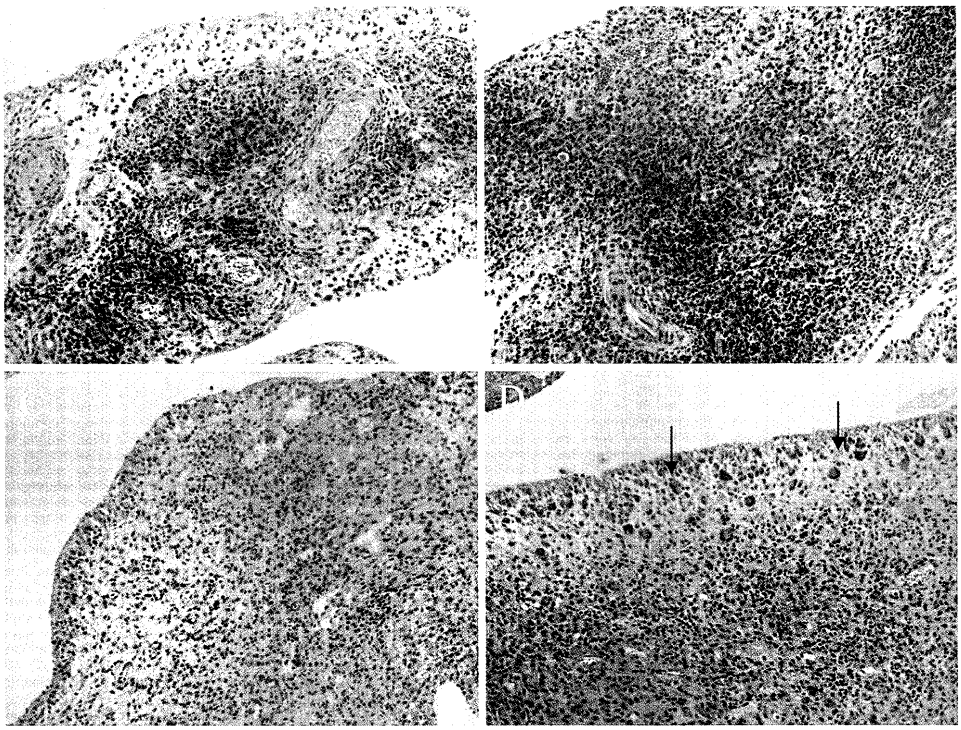

Fig. 6 The histological examination before (A, C) and after (B, D) etanercept treatment in rt. knee joint (×100). Arrow is showing multinuclear macrophages located in the superficial layer of synovium.

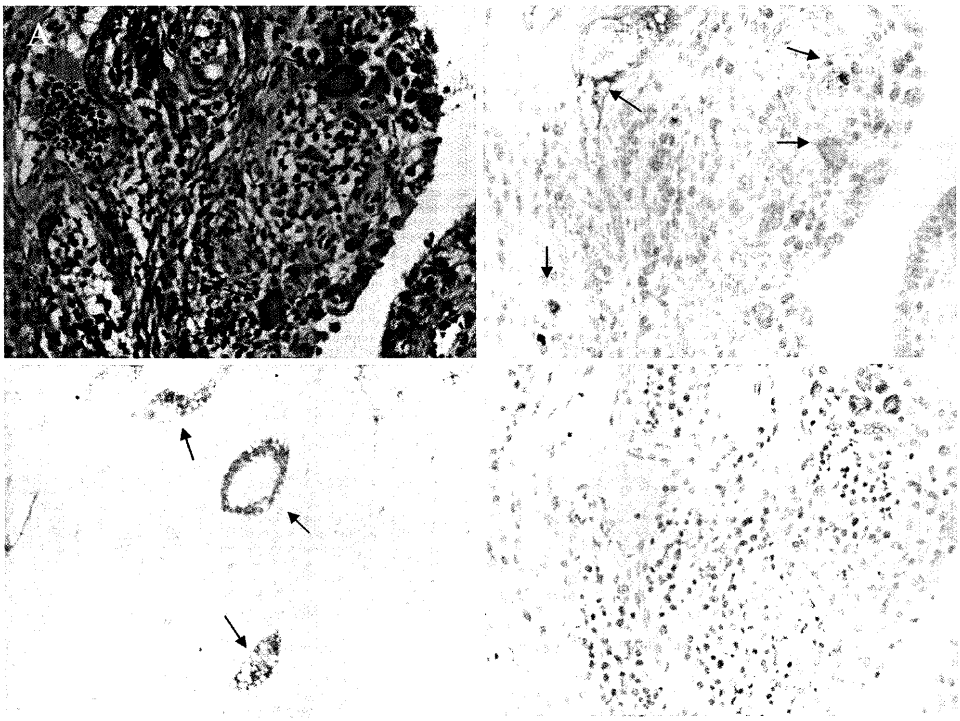

Fig. 7 Immunohistochemical examination of synovium in etanercept-tolerated case (case number 3)
A: HE (×200), arrow is showing multi-nuclear cells.
B: TNF-α (×200), arrow is showing positive TNF-α.
C: IL-6 (×100) arrow is showing positive IL-6 on endothelial cells.
D: RANKL (×200).

TNF-α was expressed in all over the synovial cells even after treatment with etanercept in all cases (Fig. 7B). IL-6 was expressed in only the endothelial cells of the vessels in the synovium (Fig. 7C). In evaluation of TNF-α and IL-6 expressions, interstitial cells of synovium showed TNF-α positive in 7 out of 8 cases (87.5%) and IL-6 positive 0 out of 8 cases (0%), on the other hand endothelial cells showed TNF-α positive in 6 out of 8 cases (75%) and IL-6 positive in 7 out of 8 cases (87.5%). RANKL was not expressed in synovium cells in any case at all (Fig. 7D). Therefore, TNF-α and IL-6 were produced in the synovium in etanercept attenuation cases but RANKL, which differentiates the osteoclasts in synovium, was not expressed.

Discussion

The treatment goals of RA are long-term substantial relief of the signs and symptoms of joint inflammation, including pain, joint damage, and impaired function[8]. Ideally, treatment will induce disease remission and restore normal functionality. Currently accepted treatment options for RA include non-biological DMARD and biological therapies, the latter often combined with MTX therapy[9]. TNF inhibitors have been shown to significantly reduce the progression of joint damage and ensure long-term symptomatic relief of RA, as measured by DAS[10][11]. However, the effect of biological agents such as etanercept attenuates gradually in some cases. There are several ways to salvage those attenuation cases by other biologic agents such as infliximab, an increased dosage of MTX or steroids, or adding other DMARDs. In histological examination, there were numerous multi-nuclear macrophages, like osteoclasts in the synovium in the etanercept attenuation cases in our results. Therefore, if proliferating synovium continues to exist in a joint cavity, it is possible to develop joint destruction even after treatment with etanercept. To solve this problem, arthroscopic synovectomy is useful to remove the synovium with numerous multi-nuclear macrophages for subsequent continuity of etanercept treatment. This surgical procedure resembles cancer treatment, by which a tumor is removed before or after chemotherapy.

Surgical treatment of RA is one of the choices if the patient does not respond well to medical treatment. The synovium produces many cytokines and chemokines which develop into joint cartilage destruction[12]. Synovectomy is a surgical method to reduce those cytokines and chemokines, especially in joint fluid[13]. In our results, we detected TNF-α and IL-6 expressed in cases of etanercept toleration. TNF-α was produced by synovium cells besides vessels, but IL-6 was mainly produced by the epithelial cells of vessels. Therefore, the synovium in etanercept toleration produces those cytokines even after etanercept treatment. In histological examination, multi-nuclear cells were detected. We checked as to whether they were osteoclast-like cells producing RANKL but RANKL was not detected in those multi-nuclear cells. These findings might indicate that the synovium had little potentiality to develop osteoclast differentiation for the destruction of bones and joints in cases of etanercept toleration.

Synovectomy of the knee in early inflammatory arthritis appears to be successful in decreasing swelling and pain when the underlying disorders are unresponsive to aggressive medical therapy. The indications for the need for synovectomy may be debated, but generally the criterion of failure of appropriate medical management for a period of 6 to 12 months in the absence of significant radiographic changes may be accepted as an appropriate indication for consideration of synovectomy. Multiple reports on the outcome of arthroscopic synovectomy appear to indicate that loss of motion is less of a problem than is seen following open synovectomy, and that the outcome of arthroscopic synovectomy in terms of palliation of the arthritic syndrome is equally effective[14]. A review by Doets et al, reported that although arthroscopic synovectomy in the setting of RA produced fair or good results in 50% of the cases, one-half of the 83 patients in this series had undergone total replacement at a mean interval of 4 years after synovectomy[15]. However, if we combine anti-TNF therapy such as etanercept and arthroscopic synovectomy, the efficacy of synovectomy may be more continuous than

synovectomy only. In our data, DAS28 had decreased at the latest by 48 weeks after surgery. This clinical improvement may be useful for continuous use of etanercept without severe side effects. In addition, the inhibition or improvement of bone erosion or joint destruction by the combination of etanercept and arthroscopic synovectomy to remove the synovium which produces cytokines should be investigated in the future.

Conclusions

This study indicates that cases of etanercept attenuation responded to the effect of arthroscopic synovectomy, leading to significant improvement of CRP and DAS28 at the latest by 48 weeks after surgery. Histological examination revealed that lymphocyte proliferation, multi-nuclear cells and hyper vascularity emerged in etanercept attenuation cases. TNF-α and IL-6 were expressed in the synovium but RANKL was not expressed. Therefore, the removal of the synovium which produces TNF-α and IL-6 may be effective in continuing treatment with etanercept.

Acknowledgement

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関節リウマチに対するエタネルセプト効果減弱例の関節鏡視下滑膜切除術の臨床的効果と関節滑膜の組織学的検討

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関節リウマチ（RA）の治療において生物学的製剤であるエタネルセプトが近年使用されているが，その効果減弱例に対して関節鏡視下滑膜切除を施行した7例8関節，男性2例，女性5例，平均年齢62（48～75)歳について術前 c-reactive protein（CRP）と disease activity scores（DAS）28を経時的に48週まで調べ，術中採取した滑膜を組織学的に tumor necrosis factor-alpha（TNF-α），interleukin-6（IL-6）および receptor activator of nuclear kappa B ligand（RANKL）の発現について調べた．その結果 CRP は術前 3.8±0.5 mg/dl から術後48週で 0.7±0.4 mg/dl に有意に低下し，DAS28 は術前 6.3±0.6 から術後48週で 2.7±0.9 に有意に低下した．エタネルセプト効果減弱例の滑膜はリンパ球浸潤，血管増生を示し，滑膜表層に多核の組織球の発現を認めた．免疫組織化学的検討では TNF-α の発現は滑膜全体の細胞に見られ，IL-6 は滑膜細胞は抑制され血管のみ発現していた．破骨細胞の分化を促進する RANKL は滑膜に発現していなかった．以上よりエタネルセプト効果減弱例の RA では組織学的に TNF-α の発現が見られる滑膜を切除することにより，局所からのサイトカイン産生を抑制する関節鏡視下滑膜切除は有効であり，エタネルセプトの効果を持続できる一つの手段として考えられる．