Induction of Fas-dependent apoptosis in synovial infiltrating cells in rheumatoid arthritis

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Abstract

Apoptosis is a feature of the synovium of rheumatoid arthritis (RA). We have recently shown that RA synoviocytes were susceptible to anti-Fas mAb and undergo apoptosis in vitro. To investigate whether infiltrating mononuclear cells also undergo Fas-dependent apoptosis, double-labeling techniques combined with immunohistochemical examination with anti-CD3 mAb and the TdT-mediated dUTP-biotin nick end labeling (TUNEL) method to detect apoptotic cells, or in situ RT assay to detect Fas mRNA, were performed using frozen tissue sections. We also examined the in vitro induction of Fas-dependent apoptosis in freshly isolated synovium infiltrating mononuclear cells (SIM), synovial stromal cells (SSC) and peripheral blood lymphocytes (PBL) using tissues from nine patients with RA and three with osteoarthritis (OA). The results showed expression of Fas antigen and apoptotic cells in a number of CD3-bearing cells in RA synovial tissues. In vitro treatment with anti-Fas mAb produced a significant apoptosis of RA SIM and SSC, while none of PBL, and neither SIM nor SSC from OA exhibited apoptosis. Moreover, ~50% of CD4⁺, CD3⁺ and CD45RO⁺ cells, and >90% of Fas-expressing cells of RA SIM underwent apoptosis in response to anti-Fas mAb, as detected by flow cytometry. Our results suggest that RA synovial infiltrating lymphocytes acquire high susceptibility to anti-Fas mAb and undergo apoptosis. Such a phenomenon of infiltrating T cells in RA synovium may play an important pathophysiological role and suggest a possible therapeutic effect for anti-Fas mAb in RA.

Introduction

Current studies have focused on two main cellular pathways involved in the pathogenic process of rheumatoid arthritis (RA). The first involves activation of synovial stromal cells leading to a progressive proliferative synovitis following influx of various inflammatory cells. The other mechanism involves activated lymphocytes, composed mainly of CD4⁺ T cells, producing cytokines associated with synovial cell hyperplasia. Necrosis with fibrotic change is a typical pathological feature of cell death in the rheumatoid synovium (1). However, we have recently demonstrated the presence of apoptosis in synovial stromal cells (SSC) in the rheumatoid synovial tissue (2). We have also found that RA synoviocytes express functional Fas molecules (CD95) and undergo apoptosis characteristically by anti-Fas IgM mAb (2).

The Fas molecule is well known as an apoptosis-associated cell surface protein of 45 kDa that encodes a transmembrane signaling domain (3,4). This molecule is expressed in the thymus, liver, ovary and other tissues (5). The apoptotic process is now recognized as mainstream in embryonic development and tissue homeostasis as well as in the regulation of the immune system and regression of malignant tumors (6).

In order to examine whether infiltrating mononuclear cells undergo apoptosis in the rheumatoid synovial tissues, we...
employed immunohistochemical techniques to detect Fas mRNA expression and apoptotic state in CD3-bearing cells. Moreover, the susceptibility to anti-Fas mAb was also examined in several cell populations, including freshly isolated synovial infiltration mononuclear cells (SIM), SSC and peripheral blood lymphocytes (PBL) by in vitro treatment with anti-Fas mAb. The cells were collected from nine RA patients and three patients with osteoarthritis (OA) serving as a control. Our results demonstrated that the rheumatoid synovial infiltration T cells also undergo apoptosis in situ and can be induced to undergo apoptosis in vitro by anti-Fas mAb. Such an apoptotic process may contribute to the pathophysiology of RA synovitis.

Methods

Cells

Synovial tissue samples were obtained during synovectomy of the knee joint or total knee replacement from nine female RA patients (average age: 62.3 years) and three female OA patients (all female, average age: 70.3 years). The diagnosis of RA was based on the 1987 ACR criteria for RA (7). The experimental protocol was approved by the Hospital Human Ethics Review Committee and a signed consent was obtained from each patient at the time of operation. Heparinized peripheral blood was also obtained from each patient at the time of operation. Heparinized peripheral blood was also obtained from each patient at the time of operation. The synovial tissue was minced into small pieces and digested with 1.0 mg/ml collagenase (Sigma, St Louis, MO). The cells were placed into culture dishes and incubated for 5-10 h in 5% CO$_2$ at 37°C. Cells were divided into adherent (SSC) and non-adherent cells. Non-adherent cells were assessed to a standard gradient centrifugation method to separate mononuclear cells (SIM). PBL were also separated from heparinized peripheral blood using a standard gradient centrifugation method.

mAb

We used CH11 (IgM; purchased from MBL, Nagoya, Japan) as an anti-Fas mAb and mouse IgM (Dako, Glostrup, Denmark) served as a control. FITC-labeled Nu-T3 (anti-CD3, Nichirei, Tokyo, Japan) was used for immunohistochemical analysis. Phycoerythrin (PE)-labeled Nu-T3, Nu-Th (anti-CD4, Nichirei), Nu-Ts/c (anti-CD8, Nichirei), My4 (anti-CD14, Coulter Immunology, Hialeah, FL), Nu-B20 (anti-CD20, Nichirei), anti-human CD45 (PharMingen, San Diego, CA), UCHL1 (anti-CD45RO, Nichirei), anti-human CD69 (PharMingen), anti-human HLA-DR (PharMingen), UB2 (anti-Fas IgG mAb, does not cross-react with CH11, MBL) and MsIgG (mouse IgG, Coulter Immunology) were used for flow cytometric analysis.

In situ RT assay

Synovial tissue samples were frozen with the OCT compound (Tissue Tek; Miles, Elkhart, IN) and kept at −80°C until the experiment was performed. The synovial tissue specimen was cut into 5–7 µm sections that were mounted onto glass slides. In the next step, in situ RT assay was performed as previously described (8). Briefly, the slides were fixed in 10% phosphate-buffered formalin (Wako, Osaka, Japan) for 4 h at room temperature and digested with 0.1% trypsin (Sigma) in diethylpyrocarbonate (Sigma) treated double-distilled water. After washing with 100% ethanol (Wako), the slides were incubated with a reaction solution containing a primer, digoxigenin-dUTP (Boehringer Mannheim, Indianapolis, IN), dNTP, RNase inhibitor and reverse transcriptase (Gibco, Grand Island, NY). The primer complementary to Fas mRNA segments was 5′-GGCTTTGTCTGTACTCCT-3′ (2). The slides were visualized by anti-digoxigenin-rhodamine (Boehringer Mannheim).

Fig. 1. Fas-expressing lymphocytes and apoptotic lymphocytes in the RA synovium in situ detected by the double-labeling technique of immunohistochemistry and in situ RT assay or the TUNEL method (a) Detection of Fas mRNA in CD3$^+$ cells. Fas mRNA in frozen tissue sections of RA synovium was reverse transcribed under the presence of Fas primer and digoxigenin-dUTP and visualized with anti-digoxigenin-rhodamine (red). The tissue section was assessed immunohistochemically with FITC-labeled anti-CD3 mAb (green). Note the double-positive cells in the sublining area of the RA synovium (yellow) (b) Detection of apoptotic cells among CD3$^+$ cells. DNA nick was reacted with TdT and digoxigenin-dUTP, then visualized with anti-digoxigenin-rhodamine. The tissue section was then reacted with FITC-labeled anti-CD3 mAb. Note the CD3$^+$ cells (green) are TUNEL-positive (with red nuclei) in the lymphatic follicle.
Fig. 2. (a) Effect of anti-Fas mAb on the viability of cells. The percent viability of SSC, SIM or PBL obtained from six patients with RA (triangles) and three OA patients (circles) during the incubation period (h) was examined. We used $5 \times 10^5$ of each cell type. The cells were incubated with 0.1 μg/ml of anti-Fas IgM mAb (CH11) or 1.0 μg/ml of mouse IgM suspended in 500 μl of medium. The number of viable cells was counted at 0, 6, 12 and 24 h, and percent viability was expressed relative to baseline cell number. The relative viability of anti-Fas mAb-treated cells against mouse IgM-treated cells was then calculated at each incubation time. Treatment with anti-Fas mAb caused a significant decrease in the number of live SSC and SIM in RA patients, compared with those of OA patients. (b) Effect of anti-Fas mAb on the viability of cells during extended incubation time. Similar samples from three other RA patients were examined using the same assay described in (a) and the number of live cells was counted at 0, 12, 24 and 48 h, and expressed as percent viability. Treatment with anti-Fas mAb caused a significant decrease in the number of live SSC and SIM in RA patients. However, extended incubation beyond 24 h did not cause a further reduction in viability of these cells.

**TdT-mediated dUTP-biotin nick end labeling (TUNEL) method in RA synovium**

The modified TUNEL method was performed as described previously (2). Briefly, the same tissue sections as in situ RT assay, described above, were used. The proteins were stripped from the nuclei by incubation with 20 μg/ml proteinase K at room temperature. The sections were immersed in TdT buffer containing 30 mM Trizma base, pH 7.2, 140 mM sodium cacodylate, 1.0 mM cobalt chloride (Boehringer Mannheim). Then, TdT (0.3 U/μl) and digoxigenin-11-dUTP in TdT buffer were added. The reaction was terminated by placing the slides in the TB buffer containing 300 mM sodium chloride and 30 mM sodium citrate, and the samples were covered with 2% aqueous solution of human serum albumin or bovine serum albumin, followed by rhodamine-labeled anti-digoxigenin antibody (Boehringer Mannheim) reaction.

**Immunohistochemical analysis**

Tissue samples were prepared as described above. Sections treated with in situ RT assay and TUNEL method were assessed immunohistochemically. For this purpose, the specimen was blocked with 4% skimmed milk for 20 min at room temperature and then incubated with FITC-labeled anti-CD3 mAb (Nu-T3).

**Induction of apoptosis in various cells obtained from RA and OA patients**

Aliquots of 500 μl of $1 \times 10^6$ cells/ml SSC, SIM and PBL from six RA and three OA patients were suspended in 0.1 μg/ml of anti-Fas mAb or 1.0 μg/ml of mouse IgM in 10% FCS/RPMI and cultured for up to 24 h. The number of viable cells was counted using the Trypan blue exclusion test at 0, 6, 12 and 24 h, and the percent viability was calculated relative to the
cell number at 0 h of treatment with each Ig, and then the relative viability of anti-Fas mAb-treated cells against mouse IgM-treated cells was calculated at each incubation time. SSC, SIM and PBL from three other patients with RA were analyzed using the same assay with extended incubation time. Live cell numbers were counted at 0, 12, 24 and 48 h in each cell population.

To confirm that the reduction in live cell number was dependent on apoptotic cell death, SSC and SIM with or without anti-Fas treatment from three RA patients were stained with propidium iodide (PI; Sigma), after fixation with 1% formalin (Wako) and 70% ethanol (Wako). The samples were analyzed by flow cytometry (FACS Calibur, Becton Dickinson, San Jose, CA) and apoptotic population was calculated using ModFit software.

Detection of DNA fragmentation

SSC, SIM and PBL cells (2×10^6) obtained from RA patients were incubated for 8 h with and without 1.0 μg/ml of anti-Fas mAb, and harvested for DNA extraction and electrophoresis to detect apoptotic ladder formation, as described previously (9, 10). Briefly, the cells were incubated in 0.5 mg/ml proteinase K (Sigma)/TNE (10 mM Tris, pH 7.5, 0.1 M NaCl, 1 mM EDTA)/SDS solution at 50°C for 1 h, then treated with 0.1 mg/ml RNase A (Nippongene, Tokyo, Japan) for an additional hour. Then, 10 μl of DNA was labeled with adenosine 5'-triphosphate, tetra(tributylammonium) salt, [γ-32P] (DuPont Company, Wilmington, DE), applied to 1% agarose gel, and exposed to an X-ray film (Fuji Photo Film, Tokyo, Japan).

Time-lapse series of morphological change in SSC and SIM by anti-Fas mAb treatment

The technique of time-lapse cinemicroscopy (Arriflex 351IB) was used at a rate of 30 frames/min to examine serial changes occurring during apoptosis of synovial infiltrating T cells by adding 1.0 μg/ml of anti-Fas mAb. A representative cell was observed for 30 min. A slower speed of one frame every 50 s was used for synovial adherent cells and observed for 21 h.

Flow cytometric analysis of RA SIM

RA SIM were harvested after 24 h of incubation with or without 0.1 μg/ml anti-Fas mAb and stained with various PE-labeled mAb, including anti-CD3, CD4, CD8, CD14, CD20, CD45, CD45RO, CD56, CD69, HLA-DR and Fas mAb. Briefly, 2×10^5 cells were incubated with various mAb at the optimal concentration in 1×PBS containing NaN3 and BSA for 60 min on ice. After three washing with the same PBS, the cells were immediately assessed by flow cytometric analysis (FACS Calibur) using appropriate software (Cell Quest).

Results

Detection of Fas expression and apoptosis in CD3+ cells

To detect Fas antigen expression in CD3-bearing cells, RA tissue sections were assessed by the double-labeling technique combined with in situ RT assay and immunohistochemistry. mRNA of Fas antigen was detected with rhodamine-labeled anti-digoxigenin antibody, while CD3 molecule was detected with FITC-labeled anti-CD3 mAb. As shown in Fig. 1(a), some of the cells with Fas expression (red) also expressed the CD3 molecule (green), mainly at the sublining area of the synovium, detected as a yellow color. The RA tissue section was also examined by double labeling with the TUNEL method and anti-CD3 mAb. Apoptotic cells were detected with rhodamine-labeled anti-digoxigenin antibody, while CD3-bearing cells were detected with FITC-labeled anti-CD3 mAb.

Fig. 2(a) shows a lymphatic follicle of RA synovium and in the center of follicle, some apoptotic cells (red) were detected in aggregation with CD3-bearing cells (green).

Induction of apoptosis by anti-Fas IgM mAb in vitro

To examine whether SIM undergo apoptosis by anti-Fas mAb, SSC, SIM and PBL obtained from six RA and three OA patients were treated with 0.1 μg/ml of anti-Fas mAb and 1.0 μg/ml of mouse IgM for up to 24 h. The number of viable cells was counted after 0, 6, 12 and 24 h of treatment with each Ig, and the percent viability of anti-Fas-treated cells relative to mouse IgM-treated cells was calculated. The mean percentage of each cell population in RA and OA patients was calculated, and the results were compared using Student's t-test. As shown in Fig. 2(a), treatment with anti-Fas mAb at a concentration of 0.1 μg/ml reduced the mean percent viability of SSC and SIM in RA patients within 24 h. In contrast, PBL did not exhibit apoptosis in significant numbers during the first 24 h. Similarly, treatment had no effect on all cell types in OA patients. After longer incubation of RA samples with anti-Fas mAb (up to 48 h), the viability at 24 h was not significantly different from that at 48 h, as shown in Fig. 2(b) (PBL: 100.3 ± 7.8 to 96.1 ± 6.5%, SIM: 74.6 ± 15.3 to 73.5 ± 16.9%, SSC: 61.6 ± 7.9 to 58.2 ± 10.2%). Figure 2(a and b) shows the mean viability with the SD of each cell population for RA and OA patients.

To confirm that the apoptotic cells contributed to the
Fas-dependent apoptosis in RA synovial cells

Fig. 4. Time-lapse series of photographs showing changes in cellular morphology of synovial adherent cells and non-adherent cells after treatment with anti-Fas mAb. (A) A non-adherent synovial cell (×400) observed for 30 min after treatment with 1.0 μg/ml of anti-Fas mAb. Note the typical apoptotic change shown in one mononuclear cell. Changes in cell morphology and condensation of chromatin in the periphery of the nucleus were observed and followed by gradual destruction into apoptotic bodies. (B) Larger view of synovial adherent cells (×100) observed for 21 h after treatment with anti-Fas mAb. An increased number of shrunken cells was observed in a time-dependent manner.

reduction in the number of live cells, SSC and SIM from the latter three RA patients were also examined by flow cytometry after treatment with or without anti-Fas for 24 h. The results showed that treatment increased the percentage of apoptotic cell from 36.5 ± 17.9% to 79.8 ± 12.8% in SSC and 34.0 ± 1.0% to 56.0 ± 2.6% in SIM. These figures were compatible to those of the Trypan blue exclusion test.

Detection of DNA fragmentation in anti-Fas-treated SSC and SIM
To determine whether the decrease in percent viability was due to apoptosis, DNA was obtained from SSC, SIM and PBL from RA patients after 8 h of treatment with or without anti-Fas mAb, and ladder formation was examined by electrophoresis. The results are shown in Fig. 3. A slight ladder formation
was observed in SIM without treatment of anti-Fas mAb. With anti-Fas treatment, a marked ladder formation appeared in SSC and augmentation of ladder formation was observed in SIM. In contrast, PBL did not exhibit ladder formation even when treated with anti-Fas mAb.

Time-course of morphological change in SSC and SIM after treatment with anti-Fas mAb

The apoptotic process was confirmed by the time-lapse series of pictures obtained by cinemicroscopy, shown in Fig. 4. Figure 4(a) is a high power view of a representative SIM undergoing apoptosis with 1.0 µg/ml of anti-Fas mAb. Changes in cell morphology and condensation of chromatin in the periphery of the nucleus were observed at baseline, and the following pictures show the gradual destruction of a particular cell until the formation of apoptotic bodies at 30 min. Figure 4(b) shows a longer observation period of SSC after treatment with anti-Fas mAb for up to 21 h. The number of cells undergoing apoptosis increased in a time-dependent manner. Shrinking of cells undergoing apoptosis was evident and destruction of these cells finally occurred by converting into several pieces or ballooning into several blebs. We also noticed through cinemicroscopy that the apoptotic cells and bodies were quickly removed by migrating macrophages.

**CD3⁺, CD4⁺ and CD45RO⁺ cells were the main populations undergoing apoptosis by anti-Fas mAb**

To determine the subpopulations of SIM undergoing apoptosis in RA patients, flow cytometric analysis was performed using SIM from three RA patients by staining with PE-labeled anti-CD3, CD4, CD8, CD14, CD20, CD45, CD45RO, CD56, CD69, HLA-DR and Fas mAb. SIM with or without anti-Fas mAb treatment for 24 h were analyzed and the percentage of positivity with each mAb was determined. As shown in Fig. 5, treatment caused a significant reduction in the percentage of CD3⁺, CD4⁺ and CD45RO⁺ cells (66.7 ± 4.0 to 35.0 ± 5.4%, 45.2 ± 7.7 to 21.4 ± 3.8% and 62.3 ± 4.9 to 28.1 ± 13.5% respectively). Since the percent decrease in CD45-expressing cells (74.4 ± 3.7 to 39.0 ± 4.3%) and CD45RO-expressing cells was almost the same (~35.4 and 34.2%), the major component of the reduction of CD45-expressing cells was considered to be CD45RO-expressing cells. Moreover, Fas-expressing cells also decreased markedly from 18.5 to 1.1%. CD69 is known to be expressed on activated T and B cells. According to the present data, these activated cells were also susceptible to anti-Fas treatment and underwent apoptosis (23.4 ± 3.5 to 5.1 ± 2.8%). CD3⁺, CD4⁺ and CD45RO⁺ cells are the most predominant cells present in the rheumatoid synovium, and are considered potentially capable of altering the immunological processes in the RA synovium (1).

**Discussion**

The major finding of the present study was that the infiltrating T cells (CD3⁺ cells) express Fas mRNA and undergo apoptosis in RA synovium, as evident by immunohistochemical analysis and *in situ* RT assay or TUNEL method. Using *in vitro* experiments, freshly isolated SSC and SIM from nine RA and three OA patients showed that RA SIM and SSC, but not PBL, were highly susceptible to anti-Fas mAb and underwent apoptosis. In contrast, these cells did not undergo apoptosis in OA patients. Apoptosis accounted for the reduced number of viable cells, as confirmed by flow cytometry, DNA electrophoresis and cinemicroscopic analysis. Flow cytometric analysis revealed that the major subset of SIM were CD3⁺, CD4⁺ and CD45RO⁺ cells, and that these cells were highly susceptible to anti-Fas mAb *in vitro*.

The rheumatoid synovium is characterized by proliferation of synoviocytes, followed by influx of inflammatory cells, consisting mainly of CD4⁺ and CD45RO⁺ T cells. Even though the pathogenesis of synovial proliferation remains obscure,
two major distinct cellular pathways have been considered recently. The first is based on altered T cell function, while the second focuses on hyperplasia of synovitis as the primary pathological event (11,12). Several animal models of arthritis induced by type II collagen or adjuvant suggest that the development of an immune reaction is a major cause of arthritis. Moreover, a certain type of the MHC is a key factor for the progression of synovitis (13–16). On the other hand, c-fos transgenic mice demonstrate that proliferative synovitis is followed by destruction of the bone and cartilage tissue (11). This process occurs without immunological cell infiltration, thus supporting the latter pathway, i.e., hyperplasia of synovocytes as a primary phenomenon in RA.

We have recently demonstrated both in vivo and in vitro that the presence of apoptosis is a characteristic feature of the RA synovium (2). Such a process is highly accelerated in vitro by anti-Fas mAb. Even though Fas molecules are expressed on OA synovial cells, they did not undergo apoptosis by anti-Fas mAb (2). The present study extended these early findings and demonstrated that in the RA synovium a proportion of the synovial infiltrating cells bearing CD3 molecules was in an apoptotic state, and that was enhanced in the presence of anti-Fas mAb in vitro. Moreover, the majority of T cells susceptible to anti-Fas mAb in SIM were CD3+, CD4+ and CD45RO+ T cells. In the rheumatoid synovium, the distribution of the mononuclear cell subset is quite different from that of peripheral cells and the majority of infiltrated T cells are CD45RO+ (17) that are considered to play a pathogenic role in RA synovitis.

Over recent years, evidence has accumulated indicating that apoptosis is not limited to immature thymocytes but can be similarly triggered in mature extrathymic T cells (19–23). Several studies have demonstrated that activated human T cells with αβ and γδ TCR undergo apoptosis by anti-Fas or anti-CD3-TCR mAb (20,21). Furthermore, stimulation of human CD4+ T cell clones by anti-TCR mAb induces cell shrinkage within 3 h and DNA fragmentation is usually detectable after 5 h. The mechanism of apoptosis of mature T cell is not very clear. Wesselborg et al (21) suggested that these cells must be activated by antigens before they become susceptible to anti-CD3 TCR mAb-mediated apoptosis. These findings suggest that the sensitivity of Fas-dependent apoptosis in peripheral T cells depends on the level of antigen stimulation and the susceptibility of SIM to anti-Fas mAb may be induced by a yet unknown antigen expressed on cells present in the synovium that in turn activates SIM in situ.

In the present study, as most of the Fas expressing cells diminished in the same assay on anti-Fas treatment, the resistant SIM population was considered to be Fas-negative cells. Based on these findings, it seems that the susceptibility of mononuclear cells present in the synovium to anti-Fas mAb depends on Fas expression. However, in SSC, susceptibility to anti-Fas and Fas expression seemed to be independent of such expression, according to our previous data demonstrating that the expression of these cells did not differ between RA and OA synovial cells.

Anti-Fas mAb treatment did not cause an increase in a subpopulation of SIM. It is possible that apoptotic cells change their surface antigenic status rapidly so that the cells undergoing apoptosis do not express a surface antigen. Thus, it is likely that all the subsets that were examined in the present study had some susceptibility to anti-Fas mAb and undergo apoptosis in rheumatoid synovium. However, these cells were mainly CD3+, CD4+ and CD45RO+ cells, which are known to affect the susceptibility to anti-Fas mAb in RA SIM.

The present results are different from data reported previously by our laboratory (2) that showed apoptotic DNA ladder formation in freshly isolated SIM without anti-Fas mAb treatment. However, in the present experiment, DNA was extracted 8 h after separation from RA synovium, while it was extracted from freshly prepared tissue in our previous study (2). SIM is considered to be activated in situ so that these cells may undergo apoptosis by placing them into the culture medium through either Fas-dependent or independent pathways and 8 h of incubation can be considered long enough to induce apoptosis.

Clinical improvement of RA has been reported recently following the administration of mAbs, such as anti-CD4 and anti-tumor necrosis factor (TNF)-α (24). In particular, the efficiency of anti-TNF-α as a treatment for RA is well established. Anti-Fas mAb clearly induced cell death in the present study through apoptosis of both SSC and SIM, which are considered as the two main pathogenic cellular components of RA. In this regard, various Fas-expressing cells, such as hepatocytes (5), and immune responsible stem cells, may undergo apoptosis following the systemic administration of anti-Fas mAb (25). Although such treatment with anti-mouse Fas mAb in several strains of adult mice induces apoptosis in the thymus or spleen cells, we have never detected abnormalities in laboratory blood tests indicative of lethal adverse reaction (26). Based on these data, systemic adverse reactions may be minimized by local administration of this antibody into the tissue lesion, e.g., injection into the joint cavity. Moreover, as confirmed in the present study by cine-microscopy, cells undergoing apoptosis are rapidly cleared by the phagocytic process. Therefore, the induction of apoptosis of rheumatoid synovial cells would appear to protect other tissues from the harmful consequences of exposure to the toxic construct from dying cells.

Based on these findings, it is clear that some of the CD3-bearing cells express Fas antigen and undergo apoptosis in the RA synovium, and these synovial infiltrating T cells, particularly CD45RO+ T cells, are susceptible to anti-Fas mAb and undergo apoptosis. Our study suggests that the anti-Fas mAb may serve as an effective tool for the treatment of RA.

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Abbreviations

OA osteoarthritis
PBL peripheral blood lymphocyte
PE phycoerythrin

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