Gene Therapy (2000) 7, 527–533

www.nature.com/gt

ACQUIRED DISEASES ***RESEARCH ARTICLE***

Novel gene therapy for rheumatoid arthritis by FADD gene transfer: induction of apoptosis of rheumatoid synoviocytes but not chondrocytes

T Kobayashi[1], K Okamoto[1], T Kobata[1,2], T Hasunuma[1], T Kato[1], H Hamada[3] and K Nishioka[1]

[1]*Rheumatology, Immunology, and Genetics Program, Institute of Medical Science, St Marianna University School of Medicine, Kawasaki;* [2]*Division of Immunology, Institute for Medical Science, Dokkyo University School of Medicine, Tochigi; and* [3]*Department of Molecular Biotherapy Research, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan*

Synovial cells in the rheumatoid synovium show abnormal proliferation, leading to joint destruction. Rheumatoid synovial cells express functional Fas antigen and are susceptible to Fas-mediated apoptosis. We have proposed the induction of apoptosis by Fas/Fas ligand system of proliferative rheumatoid synovium as a novel therapy for rheumatoid arthritis (RA). We have recently reported that Fas-associated death domain protein (FADD) plays a key role in Fas-mediated apoptosis of synovial cells in patients with RA. In this study, we determined whether FADD gene transfer could induce apoptosis of RA synoviocytes in vitro and in vivo. Transfection of FADD gene by adenoviral vector into cultured RA synoviocytes induced up-regulation of FADD expression and apoptosis. In addition, local injection of FADD adenovirus (Ad-FADD) eliminated synoviocytes in vivo by induction of apoptosis of proliferating human rheumatoid synovium engrafted in severe combined immunodeficiency mouse, which is the most suitable animal model of RA for the evaluation of treatment strategy in vivo. In addition, Ad-FADD-induced apoptosis was limited to cells of the synovium tissue and did not affect chondrocytes. Our results strongly suggest that FADD gene transfer can induce apoptosis of RA synoviocytes both in vitro and in vivo, suggesting that FADD gene transfer might be effective in the treatment of RA. Gene Therapy (2000) **7,** 527–533.

Keywords: *gene therapy; rheumatoid arthritis; Fas-associated death domain protein; adenovirus; synoviocytes*

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by abnormal synovial hyperplasia associated with local infiltration of various inflammatory cells, which leads to cartilage and bone destruction.[1] Because the pathogenesis of RA is still unknown, at present, treatment of RA is directed against various components of the chronic inflammatory process.[2] However, there are few curable therapies for RA.

Apoptosis, particularly mediated by Fas (APO-1/CD95)/Fas ligand (FasL) system, is closely associated with the pathophysiology of RA.[3] Synovial hyperplasia, a unique feature of RA, is not limitless, and spontaneous regression has been reported.[4,5] In addition, it has been demonstrated that proliferative synoviocytes as well as infiltrating mononuclear cells in rheumatoid synovium express Fas antigen and these cells undergo Fas-mediated apoptosis both *in vivo* and *in vitro*.[6–8] However, intractable synovial hyperplasia is often observed during the course of the disease, suggesting that Fas/FasL system may be incapable of fully eliminating cells in the proliferative rheumatoid synovium. In this regard, elimination of proliferating synoviocytes in the rheumatoid synovium by regulating the apoptotic processes seems to be a potentially effective treatment modality for RA.[9,10]

Gene therapy has proved to be an effective therapeutic modality for certain diseases, such as hereditary diseases and cancer.[11] Recent studies have shown that FADD gene transfer by adenoviral or retroviral vectors potently induced apoptosis of human malignant glioma cells.[12,13] In these studies, Fas-associated death domain protein (FADD), binds to intracellular death domain of Fas and promotes signaling pathways of Fas-mediated apoptosis. In this regard, we have recently reported that FADD is a key signaling molecule for Fas-mediated apoptosis of synoviocytes in patients with RA,[14] suggesting that FADD gene transfer could also induce apoptosis of RA synoviocytes.

Based on these findings, we investigated here the effects of FADD gene transfer into cells of the rheumatoid synovium *in vitro* and *in vivo* using adenoviral vector, which is reported to be the most efficient vector for gene transfer into synovial cells both *in vitro* and *in vivo*.[15,16] Our results demonstrated that transfection of FADD gene into RA synoviocytes enhanced apoptosis of these cells both *in vitro* and *in vivo*. These results suggest that FADD gene transfer might be an effective novel therapy for RA. This is the first report that proposes the application of FADD gene transfer for the treatment of RA, one of the most representative autoimmune diseases.

Correspondence: K Nishioka, Rheumatology, Immunology, and Genetics Program, Institute of Medical Science, St Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki 216-8512, Japan
Received 1 April 1999; accepted 31 October 1999

Results

Induction of apoptotic cell death by FADD gene transfer into cultured RA synoviocytes

We first investigated cell viability and FADD expression in cultured RA synoviocytes infected with replication-defective recombinant adenovirus containing human FADD gene (Ad-FADD). Infection of cultured RA synoviocytes with Ad-FADD resulted in the death of these cells in time- and dose-dependent manners (Figure 1). Infection with Ad-FADD at multiplicities of infection (MOIs) of 100 and 200 resulted in the death of almost all cultured RA synoviocytes after 48 h culture (Figures 1 and 2c). In contrast, untreated cultured RA synoviocytes and LacZ adenovirus (Ad-LacZ)-infected synoviocytes did not show cell death (Figure 1a and Figure 2a and b). As shown in Figure 2d, almost all cultured RA synoviocytes infected with Ad-LacZ at a MOI of 100 expressed β-galactosidase (β-gal), indicating that these cells were highly infected with the adenoviral vector. Furthermore, the expression of FADD was augmented in cultured RA synoviocytes infected with Ad-FADD but not Ad-LacZ, corresponding with the induction of cell death (Figure 2e). In addition, after staining with Hoechst 33258, DNA fragmentation and nuclear condensation were observed in cultured RA synoviocytes infected with Ad-FADD but not Ad-LacZ (Figure 3). Combined together, these results confirmed that overexpression of FADD through its gene transfer induces apoptotic cell death of cultured RA synoviocytes.

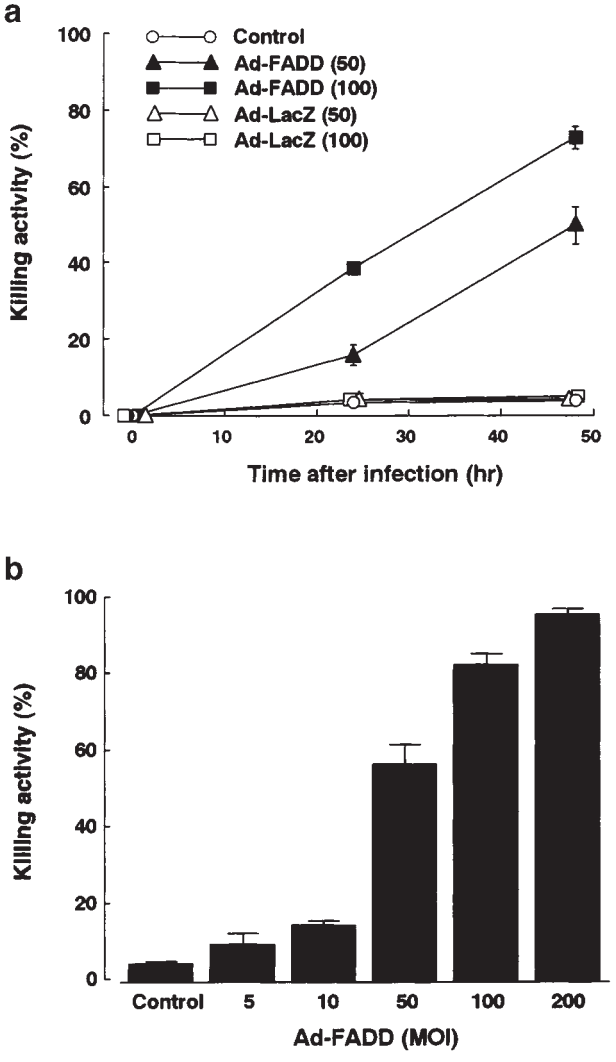

Figure 1 *Killing activity of Ad-FADD in cultured RA synoviocytes. Time-dependent effect of Ad-FADD and Ad-LacZ at MOIs of 50 and 100 on cell viability of cultured RA synoviocytes (a). Dose-dependent effect of Ad-FADD 48 h after infection at various MOIs (b). Data are expressed as mean ± s.e.m. of four synoviocytes obtained from individual RA patients.*

Effect of Ad-FADD injection on rheumatoid synovium engrafted in SCID-RA mice

To evaluate directly the effects of FADD gene transfer on the rheumatoid synovium *in vivo*, we locally injected Ad-FADD into proliferating rheumatoid synovia engrafted in severe combined immunodeficiency mice (SCID-RA), a model of RA.[10,17] The distribution of locally injected adenoviral vector was examined by the expression of β-gal in rheumatoid synovium injected with Ad-LacZ by staining with 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal). β-Gal-positive cells were observed in synovial tissues injected with Ad-LacZ (Figure 4). Importantly, β-gal-positive cells were not detected in cartilage tissues (Figure 4b).

Histopathological analyses of the engrafted rheumatoid synovia in SCID-RA mice injected with Ad-LacZ (a control adenoviral vector) and uninfected control revealed morphological features similar to the rheumatoid synovium of arthritic joints, including proliferation of synoviocytes, infiltration of various inflammatory cells, pannus-like formation and bone and cartilage erosion (Figure 5a and b). In sharp contrast, injection of Ad-FADD resulted in the disappearance of most synoviocytes and mononuclear cells after 7 days (Figure 5c). These results indicate that FADD gene transfer by adenoviral vector induced a preferential cell death in the rheumatoid synovium *in vivo*.

Injection of Ad-FADD induces apoptosis of rheumatoid synovium engrafted in SCID-RA mice

Finally, to confirm that the cell death induced by infection with Ad-FADD in the above studies was due to apoptosis, we examined the presence of DNA fragmentation in engrafted rheumatoid synovium by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) method. As shown in Figure 6, TUNEL-positive cells were frequently observed in engrafted rheumatoid synovium injected with Ad-FADD (Figure 6b). Quantitative analysis showed that the percentage of TUNEL-positive cells was 39 ± 7% (mean ± s.e.m.) in engrafted synovium injected with Ad-FADD. In contrast, no such cells were observed in engrafted synovium injected with Ad-LacZ (Figure 6a). These results confirmed that synoviocytes cell death induced by Ad-FADD was due to the induction of apoptosis.

Discussion

RA is characterized by abnormal synovial hyperplasia and in its final stages by cartilage and bone destruction.[1] Although proliferative synoviocytes express functional Fas antigen and FasL-positive infiltrating cells are present in rheumatoid synovium,[6–8] the Fas/FasL system does not appear to function optimally because synovial hyper-

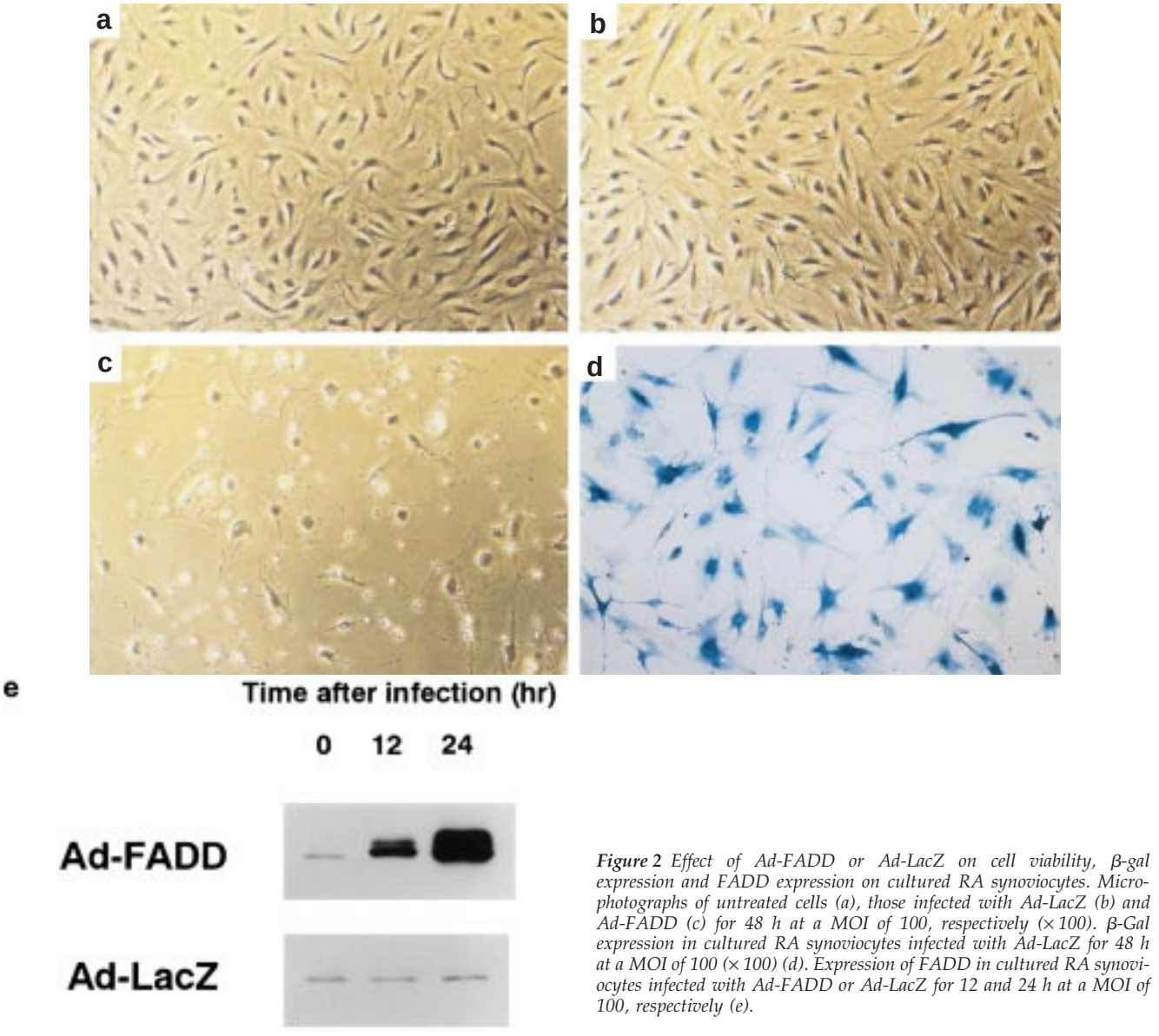

Figure 2 *Effect of Ad-FADD or Ad-LacZ on cell viability, β-gal expression and FADD expression on cultured RA synoviocytes. Microphotographs of untreated cells (a), those infected with Ad-LacZ (b) and Ad-FADD (c) for 48 h at a MOI of 100, respectively (×100). β-Gal expression in cultured RA synoviocytes infected with Ad-LacZ for 48 h at a MOI of 100 (×100) (d). Expression of FADD in cultured RA synoviocytes infected with Ad-FADD or Ad-LacZ for 12 and 24 h at a MOI of 100, respectively (e).*

plasia remains the main feature of RA. Thus, it is very attractive that active induction of apoptosis might be effective in the control of RA. Using the same strategy, we have recently demonstrated that *ex vivo* gene transfer of FasL was also a potentially effective mode of controlling synovial hyperplasia in RA.[10] However, at present, preparation of autologous transfected cells that avoid host immune responses is difficult. Using an alternative approach, in the present study, we examined the effects of *in vivo* gene transfer of FADD using adenoviral vector to induce apoptosis in the rheumatoid synovium directly.

In this study, we found that overexpression of FADD through its gene transfer induced apoptosis of RA synoviocytes. FADD is identified as an adapter molecule of Fas antigen containing the death domain (DD) and death effector domain (DED).[18,19] It can associate with cytoplasmic DD of Fas antigen under Fas stimulation, caspase-8 is then recruited to FADD, a process mediated through DED.[20,21] After its recruitment of caspase-8 to FADD, it is activated, leading to activation of the caspase cascade, a process that results in the transmission of apoptotic signal to the nucleus. We have recently identified the specific signaling pathways of Fas-mediated apoptosis in synoviocytes. In particular, FADD/caspase-8/caspase-3/poly(ADP-ribose) polymerase pathway is essential for signal transduction of Fas-mediated apoptosis in RA synoviocytes.[14] Thus, FADD is a key molecule for Fas-mediated apoptosis in RA synoviocytes. In this respect, previous studies have reported that overexpression of FADD can induce apoptosis of various cells.[12,13,18,19] Interestingly, deletion mutants of FADD, which contain DED but not DD, can also induce apoptosis, suggesting that overexpression of FADD can induce apoptosis without involving the DD of Fas antigen.[18,19] We found that overexpression of FADD induced apoptotic cell death of cultured RA synoviocytes accompanied with DNA fragmentation and nuclear condensation. In contrast, a control adenoviral vector (Ad-LacZ) failed to induce cell death of RA synoviocytes. In addition, apoptosis of cultured RA

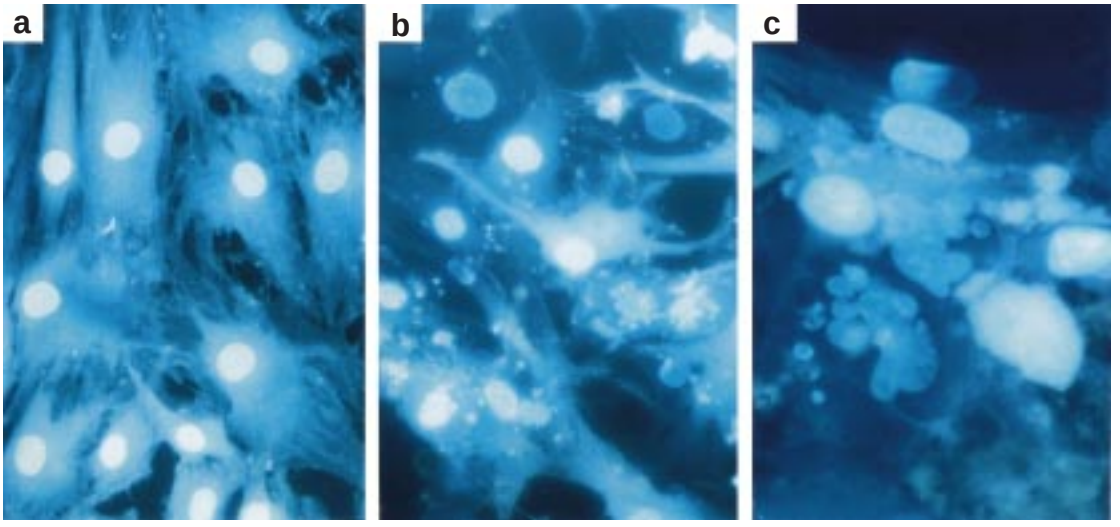

Figure 3 *Induction of DNA fragmentation and nuclear condensation by Ad-FADD in cultured RA synoviocytes. Fluorescence microphotographs of cells stained with Hoechst 33258. Cultured RA synoviocytes infected with Ad-LacZ (a, × 400) and Ad-FADD (b, × 400; c, × 1000) for 48 h at a MOI of 100, respectively.*

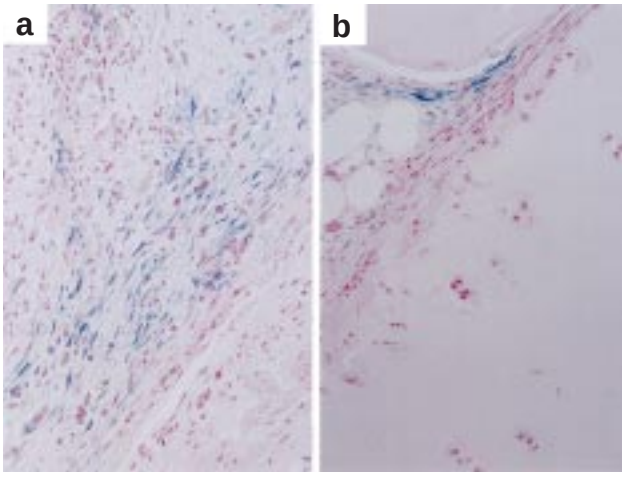

Figure 4 *β-Gal expression in SCID-RA mice injected with Ad-LacZ. Microphotographs of engrafted rheumatoid synovium tissues without (a, × 200) or with cartilage tissues (b, × 200). Seven days after injection of Ad-LacZ, tissues were removed and stained with X-gal, then prepared and stained with nuclear fast red. Data are representative of three individual experiments.*

synoviocytes induced by FADD gene transfer was inhibited by inhibitors of caspase-8 or caspase-3 (data not shown). These results strongly suggest that overexpression of FADD potently induced apoptosis of cultured RA synoviocytes by activating the caspase cascade independent of Fas antigen.

In the next series of experiments, we evaluated the effect of local injection of Ad-FADD on proliferating rheumatoid synovium using SCID-RA mice. This mouse is considered a suitable model of RA, because engrafted human rheumatoid synovium into SCID mice maintains the histologic components of rheumatoid synovia.[10,17] In this system, we showed the disappearance of synoviocytes in engrafted synovia injected with Ad-FADD but not Ad-LacZ. Moreover, TUNEL-positive cells were observed only in tissues injected with Ad-FADD. These results clearly indicated that elimination of synoviocytes in the engrafted rheumatoid synovium was due to the induction of apoptosis by local injection of Ad-FADD.

Other alternative approaches of gene therapy for RA have been recently tested using *ex vivo* or *in vivo* gene transfer of several cytokine-related genes, such as interleukin-1 (IL-1) receptor antagonist and tumor necrosis factor α (TNFα) receptor.[22] Transfer of IL-1 receptor antagonist gene inhibited the inflammatory effects induced by local injection of IL-1β.[23] Moreover, TNFα receptor gene transfer demonstrated an anti-arthritic effect.[24] In these studies, however, long-term gene expression is required to maintain the cytokine balance in the joints. In contrast, this is not necessary in our approach since the goal of our method is the transient induction of sufficient apoptosis against cells transfected with FADD gene and thereby the elimination of synovial hyperplasia. Although the adenoviral vector cannot integrate foreign genes into the host's chromosomal DNA, this vector has a high efficiency for gene delivery not only to cultured RA synoviocytes *in vitro* but also those in the rheumatoid synovium *in vivo* as previously reported.[15,16] Interestingly, our study showed that gene expression mediated via adenoviral vector was limited to the cells of synovium tissue, FADD gene expression not being observed in chondrocytes of the cartilage tissue *in vivo*. Furthermore, Ad-FADD did not induce apoptosis of chondrocytes. Therefore, adenoviral vector may be advantageous for the treatment of RA by specifically inducing apoptosis of synoviocytes, through FADD gene transfer. Furthermore, such specificity suggests that FADD gene transfer is likely to induce apoptosis of synovial cells without leading to destruction of the cartilage tissue. Thus, FADD gene transfer by adenoviral vector seems to be potentially suitable for gene therapy of RA against synovial cells.

In conclusion, we have shown here that overexpression of FADD on synovial cells, by its gene transfer mediated via adenovirus vector, induces apoptosis of these cells both *in vitro* and *in vivo*. These results indicate that *in vivo*

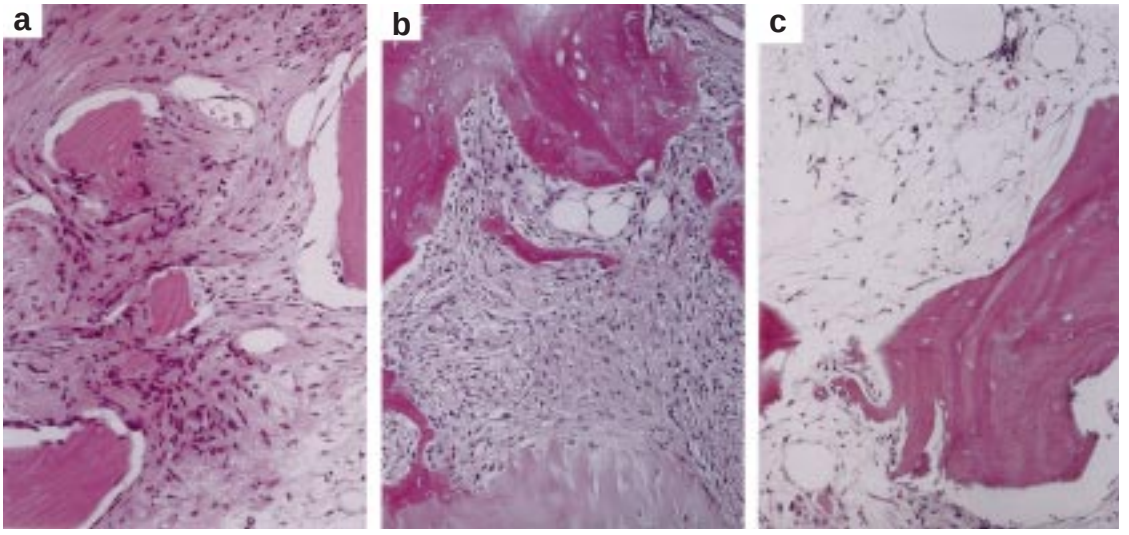

Figure 5 *Effects of Ad-FADD or Ad-LacZ on rheumatoid synovium engrafted in SCID-RA mice. Microphotographs of engrafted rheumatoid synovial tissue sections of untreated control (a, × 200), injected with Ad-LacZ (b, × 200) or Ad-FADD (c, × 200). Seven days after injection with adenovirus, tissues were removed and prepared and stained with H-E. Data are representative of three individual experiments.*

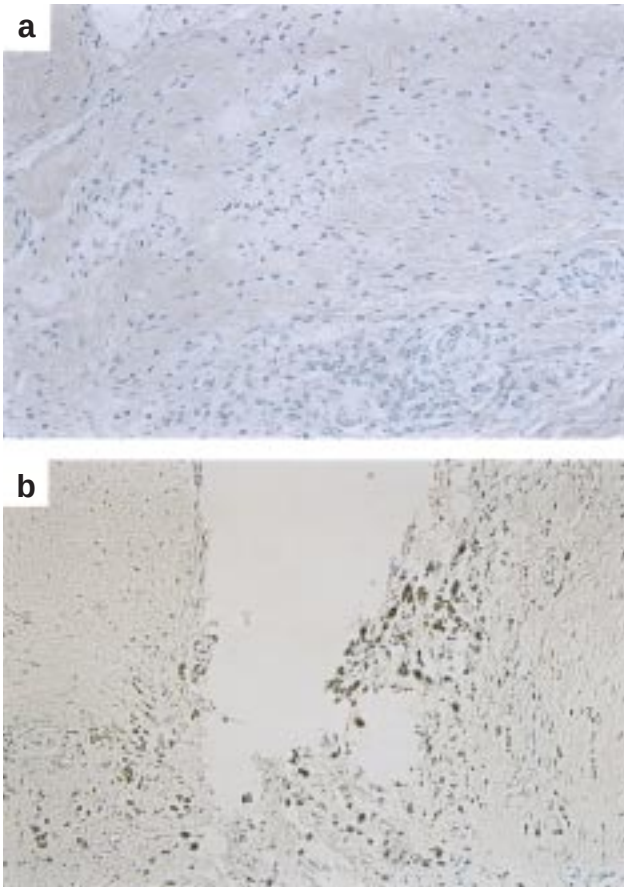

Figure 6 *Induction of apoptosis by Ad-FADD in rheumatoid synovial tissues engrafted in SCID-RA mice. Microphotographs of engrafted rheumatoid synovial tissue sections injected with Ad-LacZ (a, × 200) and Ad-FADD (b, × 200). Seven days after injection with adenovirus, tissues were removed and prepared by the TUNEL method. Data are representative of three individual experiments.*

gene transfer of FADD gene might be potentially effective for RA. Before such use is clinically feasible, however, there is a need for the development of specific gene delivery systems for proliferative synovial cells and/or development of a new adenoviral vector that does not induce adverse effects. This is an important refinement process, particularly since the adenoviral vector is known to induce adverse effects associated with cellular and humoral immune responses.[25,26]

Materials and methods

Preparation of synovial cells

Synovial tissues were obtained from patients with RA during arthroplasty. All patients gave informed consent. Cultured synovial cells were established according to the standard method described by our laboratory[27] and from five to 10 passages were used. Cells were cultured in Dulbecco's modified Eagle's medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal calf serum (PAA laboratories, Linz, Austria) until used in the experiments.

Construction of replication-defective recombinant adenoviral vector containing FADD or LacZ gene

A recombinant adenovirus containing FADD or *LacZ* gene was established as described previously.[10] The 0.7 kb *Xho*I/*Hin*dII fragment of human FADD cDNA was ligated to the *Eco*RI site of pCAcc[28] to generate pCAcc-FADD. The 3.0 kb *Cla*I/*Cla*I expression cassette from pCAcc-FADD was inserted into the *Cla*I site of pAxcw[29] to generate pAxCA-FADD. We used LacZ adenovirus as the control vector.[28]

Cytotoxic assay

Synovial cells were seeded in a six-well plate at 1×10^5 cells per well. After 24 h, cells were infected with various MOIs of Ad-FADD or Ad-LacZ. The cells were then stained with trypan blue at 0, 24 and 48 h after infection

and the number of dead cells was counted under a microscope (Olympus, Tokyo, Japan).

X-gal staining of cultured RA synoviocytes

Forty-eight hours after infection with Ad-LacZ, cultured RA synoviocytes were fixed in 0.5% glutaraldehyde in PBS for 10 min at room temperature. The cells were then washed with PBS containing 1 mM $MgCl_2$ and incubated with a solution of 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 1 mM $MgCl_2$ and 1 mg/ml X-gal (Gibco BRL) in PBS (pH 7.8) at room temperature for 4 h.

Detection of DNA fragmentation and nuclear condensation by Hoechst 33258 staining

DNA fragmentation and nuclear condensation were detected using the chromatin dye Hoechst 33258 (Wako Pure Chemical Industries, Osaka, Japan). Forty-eight hours after infection with Ad-FADD, cells were fixed for 30 min in PBS containing 1% glutaraldehyde (Wako). After fixation at room temperature, the cells were treated with 5 mg/ml Hoechst 33258 in PBS for 10 min and then examined using a fluorescence microscope (Olympus).

Immunoblot analysis

Cell lysates were prepared from RA synoviocytes at 0, 12 and 24 h after infection with Ad-FADD or Ad-LacZ at a MOI of 100. The cells (4×10^5) were harvested and centrifuged at 500 *g* for 5 min. Then, the pellets were lysed in a lysis buffer containing 10 mM Tris, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 10 μg/ml of leupeptin and 10 μg/ml of aprotinin. Insoluble material was removed by centrifugation at 12 000 *g* for 5 min and lysates were subjected to 10% polyacrylamide/sodium dodecylsulfate (SDS) gel electrophoresis before electrophoretic transfer to a nitrocellulose membrane. The membrane was blocked with 2.5% bovine serum albumin (Nacalaitesque, Kyoto, Japan) and 2.5% skim milk (Gibco BRL) in PBS and for 60 min at room temperature. After washing with PBS containing 0.1% Tween, the membrane was incubated for 60 min with specific antibodies for FADD (Transduction Laboratories, Lexington, KY, USA). After washing, the membrane was incubated for 60 min with rabbit anti-mouse IgG (Zymed Laboratories, San Francisco, CA, USA). After washing, the immunoreactive proteins were visualized using an enhanced chemiluminescence detection system (Amersham, Arlington Heights, IL, USA).

SCID-RA mice

SCID mice engrafted with human rheumatoid synovium were prepared as described previously.[10,17] After joint surgery, the human tissue specimen was quickly engrafted into female SCID mice aged 6 to 7 weeks. All mice were handled under specific pathogen-free conditions. Mice were anesthetized with inhalation of diethylether. A 1-cm incision was made in the middle of the back and paravertebral muscles were exteriorized. The back muscles were incised and 1 cm^3 mixture of synovium and joint cartilage cut into small pieces was mixed together and implanted. After 1 month, Ad-FADD (1×10^8 p.f.u.) was injected into the site containing the engrafted material. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation.

X-gal staining of synovial tissue sections

Seven days after injection of Ad-LacZ into SCID-RA mice, synovial tissues including the joint cartilage were fixed in 0.5% glutaraldehyde in PBS for 1 h at room temperature. The tissues were then incubated overnight at room temperature in a solution of 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 2 mM $MgCl_2$ and 1 mg/ml X-gal in PBS (pH 7.8). After staining for β-galactosidase activity, tissues were post-fixed in 4% paraformaldehyde at room temperature for 4 h and decalcified in 10% EDTA solution (pH 7.4). The tissues were then embedded in paraffin, sectioned, mounted on to glass slides, and stained with nuclear fast red (Wako). Experiments were performed using SCID-RA mice engrafted with synovial tissue from three patients with RA.

Histopathological and TUNEL analyses

Seven days after injection of Ad-FADD into SCID-RA mice, synovial tissues including joint cartilage were fixed in 4% paraformaldehyde at room temperature for 4 h and decalcified in 10% EDTA solution (pH 7.4). Tissues were then embedded in paraffin, sectioned, mounted on to glass slides, and used for further experiments. For histopathological examination, sections were stained with hematoxylin and eosin (Wako) (H-E). Fragmented DNA was detected by the TUNEL method as described previously.[10] Sections were immersed in TdT buffer containing 30 mM Tris-HCl (pH 7.2), 140 mM sodium cacodylate and 1 mM cobalt chloride. TdT (Gibco BRL) and biotinylated dUTP (Sigma Chemical, St Louis, MO, USA) were added to the buffer solution. The reaction was terminated by immersing in TB buffer containing 300 mM sodium chloride and 30 mM sodium citrate. Then, sections were treated with peroxidase-labeled streptavidin and visualized by Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA). Finally, sections were stained with methylgreen (Wako). The experiments were performed using SCID-RA mice engrafted with synovial tissues from three patients with RA.

Acknowledgements

We thank Dr Toshifumi Takakuwa for preparation of paraffin sections, and Miss Hideko Morita for technical assistance. This work was supported by grants from the Ministry of Education, Science, Sports and Culture of Japan, and the Ministry and Welfare of Japan, and Rheumatology and Genetic Research Found from Santen Pharmaceutical Co. Ltd.

References

1 Harris ED Jr. Rheumatoid arthritis. Pathophysiology and implications for therapy. *New Engl J Med* 1990; **322**: 1277–1289.
2 Weinblatt ME. Treatment of rheumatoid arthritis. In: Koopman WJ (ed). *Arthritis and Allied Conditions: a Textbook of Rheumatology*. Williams & Wilkins: Baltimore, 1997, pp 1131–1141.
3 Nishioka K *et al*. Apoptosis in rheumatoid arthritis: a novel pathway in the regulation of synovial tissue. *Arthritis Rheum* 1998; **41**: 1–9.
4 O'Sullivan JB, Cathcart ES. The prevalence of rheumatoid arthritis: follow-up evaluation of the effect of criteria on rates in Sudbury, Massachusetts. *Ann Intern Med* 1972; **76**: 573–577.
5 Feigenbaum SL, Masi AT, Kaplan SB. Prognosis in rheumatoid arthritis: a longitudinal study of newly diagnosed younger adult patients. *Am J Med* 1979; **66**: 377–384.

6 Nakajima T *et al*. Apoptosis and functional Fas antigen in rheumatoid arthritis synoviocytes. *Arthritis Rheum* 1995; **38**: 485–491.
7 Asahara H *et al*. Expression of Fas antigen and Fas ligand in the rheumatoid synovial tissue. *Clin Immunol Immunopathol* 1996; **81**: 27–34.
8 Firestein GS, Yeo M, Zvaifler NJ. Apoptosis in rheumatoid arthritis synovium. *J Clin Invest* 1995; **96**: 1631–1638.
9 Fujisawa K *et al*. Therapeutic effect of the anti-Fas antibody on arthritis in HTLV-I *tax* transgenic mice. *J Clin Invest* 1996; **98**: 271–278.
10 Okamoto K *et al*. Induction of apoptosis in the rheumatoid synovium by Fas ligand gene transfer. *Gene Therapy* 1998; **5**: 331–338.
11 Mulligan RC. The basic science of gene therapy. *Science* 1993; **260**: 926–932.
12 Kondo S *et al*. FADD gene therapy for malignant gliomas *in vitro* and *in vivo*. *Hum Gene Ther* 1998; **9**: 1599–1608.
13 Shinoura N *et al*. Apoptosis by retrovirus- and adenovirus-mediated gene transfer of Fas ligand to glioma cells: implications for gene therapy. *Hum Gene Ther* 1998; **9**: 1983–1993.
14 Okamoto K *et al*. FADD is Fas-mediated apoptosis modulator in synoviocytes. *Rheumatology* (in press).
15 Nita I *et al*. Direct gene delivery to synovium. An evaluation of potential vectors *in vitro* and *in vivo*. *Arthritis Rheum* 1996; **39**: 820–828.
16 Roessler BJ *et al*. Adenoviral-mediated gene transfer to rabbit synovium *in vivo*. *J Clin Invest* 1993; **92**: 1085–1092.
17 Sakai K *et al*. Potential withdrawal of rheumatoid synovium by the induction of apoptosis using a novel *in vivo* model of rheumatoid arthritis. *Arthritis Rheum* 1998; **41**: 1251–1257.
18 Boldin MP *et al*. A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. *J Biol Chem* 1995; **270**: 7795–7798.
19 Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 1995; **81**: 505–512.
20 Boldin MP, Goncharov TM, Goltsev YV, Wallach D. Involvement of MACH, a novel MORT-1/FADD-interacting protease, in Fas/APO-1- and TNF receptor-induced cell death. *Cell* 1996; **85**: 803–815.
21 Muzio M *et al*. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. *Cell* 1996; **85**: 817–827.
22 Evans CH *et al*. Gene therapy for rheumatic diseases. *Arthritis Rheum* 1999; **42**: 1–16.
23 Bandara G *et al*. Intraarticular expression of biologically active interleukin 1-receptor- antagonist protein by *ex vivo* gene transfer. *Proc Natl Acad Sci USA* 1993; **90**: 10764–10768.
24 Le CH, Nicolson AG, Morales A, Sewell KL. Suppression of collagen-induced arthritis through adenovirus-mediated transfer of a modified tumor necrosis factor a receptor gene. *Arthritis Rheum* 1997; **40**: 1662–1669.
25 Yang Y *et al*. Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proc Natl Acad Sci USA* 1994; **91**: 4407–4411.
26 Dai Y *et al*. Cellular and humoral immune responses to adenoviral vectors containing factor IX gene: tolerization of factor IX and vector antigens allows for long-term expression. *Proc Natl Acad Sci USA* 1995; **92**: 1401–1405.
27 Fujisawa K *et al*. Activation of transcription factor NF-κB in human synovial cells in response to tumor necrosis factor α. *Arthritis Rheum* 1996; **39**: 197–203.
28 Yoshida Y, Hamada H. Adenovirus-mediated inducible gene expression through tetracycline-controllable transactivator with nuclear localization signal. *Biochem Biophys Res Commun* 1997; **230**: 426–430.
29 Miyake S *et al*. Efficient generation of recombinant adenoviruses using adenovirus DNA-treatment protein complex and a cosmid bearing the full-length virus genome. *Proc Natl Acad Sci USA* 1996; **93**: 1320–1324.