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Successful induction of severe destructive arthritis by the transfer of *in vitro*-activated synovial fluid T cells from patients with rheumatoid arthritis (RA) in severe combined immunodeficient (SCID) mice

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SUMMARY

In order to investigate the role of pathogenic T cells in RA, the establishment of an RA model using patients' T cells is thought to be essential. In this study, multiple and severe destructive arthritis was established by transferring *in vitro*-stimulated synovial fluid T (SFT) cells from patients with RA through simultaneous injection into knee joint and peritoneal cavity of SCID mice without causing xenogeneic graft-*versus*-host disease (GVHD). Neither the transfer of unstimulated SFT cells nor sole i.p. injection was sufficient to induce severe arthritis. Interestingly, in contrast with SFT cells, *in vitro*-activated peripheral blood lymphocytes from RA patients failed to trigger such arthritis, suggesting that pathogenic T cells might be concentrated in synovial fluid of RA patients. This, the first severe arthritis model mimicking RA induced by RA patients' T cells, is expected to provide important information about RA pathogenesis and a possible therapeutic approach.

Keywords SCID mice arthritis model synovial fluid T cells rheumatoid arthritis

INTRODUCTION

RA is a chronic multiple joint disease causing synovial membrane hyperplasia, aggressive synovial cell proliferation and destructive damage of bone and cartilage [1]. This illness has been understood to be an autoimmune disease involving specific T cells through research on several RA-like animal models such as type II collagen (CII)-induced arthritis (CIA) [2,3] and adjuvant arthritis [4,5]. However, the pathogenic mechanisms of RA are still unknown.

For the study of the human immune system and autoimmune disease *in vivo*, many approaches [6] to transfer human immunocytes into SCID mice have recently been employed. However, while success with some SCID human disease models, including systemic lupus erythematosus (SLE) [7], multiple sclerosis [8], and Sjögren's syndrome (SS) [9], has been reported, the induction of overt severe chronic arthritis using T cells from RA patients has never been accomplished. Our previous attempts to induce arthritis through the transfer of T cells of synovial fluid (SFT cells) from RA patients to SCID mice both by i.p. injection and by injection into knee joint failed; Sack *et al.* also reported that due to early cell emigration from the injected joint space, injection of synovial mononuclear cells to SCID mice knee joints did not cause cartilage destruction [10].

In this study, we present our success in the induction of multiple destructive arthritis with high incidence through the transfer of *in vitro*-activated SFT cells by the simultaneous injection into both knee joint and peritoneal cavity of SCID mice. In addition, we found that in contrast to SFT cells, peripheral blood mononuclear cells (PBMC) from RA patients, even if activated *in vitro*, did not trigger arthritis.

PATIENTS AND METHODS

Patients

Patients were staged according to the American Rheumatism Association criteria. Twelve RA patients (11 females, one male, average age $57{\cdot}3 \pm 12{\cdot}0$ years) and two osteoarthritis (OA) patients (two females, $66{\cdot}0 \pm 5{\cdot}0$ years) as the control patients with non-rheumatic arthritis were used in these experiments.

Cells

SFT cells were purified by the following method. Heparinized synovial fluid (SF; 30–50 ml) from patients with RA or OA was incubated with 100 U/ml hyaluronidase (Wako Pure Chemical Ind., Osaka, Japan) at 37°C for 30 min. SF cells were washed with Hanks' solution (Nissui Pharmaceutical Co., Tokyo, Japan)

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and then incubated with 2-aminoethylisothiouronium bromide hydrobromide (Nacalai Tesque, Kyoto, Japan)-treated sheep erythrocytes on ice for 1 h at a ratio of 1:200. The rosetting (ER^+) populations were separated on Lymphoprep (density 1·077) (Nycomed Pharma AS, Oslo, Norway) gradients by centrifugation at 400 ***g*** for 30 min at room temperature. SFT cells ($0·5–2 \times 10^7$ cells) were enriched after lysis of the sheep erythrocytes and washed. They were analysed by FACScan (Becton Dickinson, Mountain View, CA) after being incubated with MoAbs anti-CD3 (OKT3), anti-MHC class II (L203) (ATCC, Rockville, MD), anti-CD20 (NuB2) (Nichirei Co., Tokyo, Japan), mouse IgG (mIgG) as control and FITC-conjugated sheep anti-mIgG as a second antibody (Cappel, West Chester, PA).

Mononuclear cell-rich SF cells from OA patients were used for cell transfer experiments without purification due to the limitations of total cell numbers available ($\approx 10^6$ cells per patient). PBMC were isolated from 20 ml heparinized peripheral blood of RA patients on density gradients using Lymphoprep (1·077). Normal peripheral blood T (PBT) cells were isolated from healthy volunteers' peripheral blood by the density gradient steps described above and passed through a nylon wool column. PBT cells were $> 95\%$ $CD3^+$.

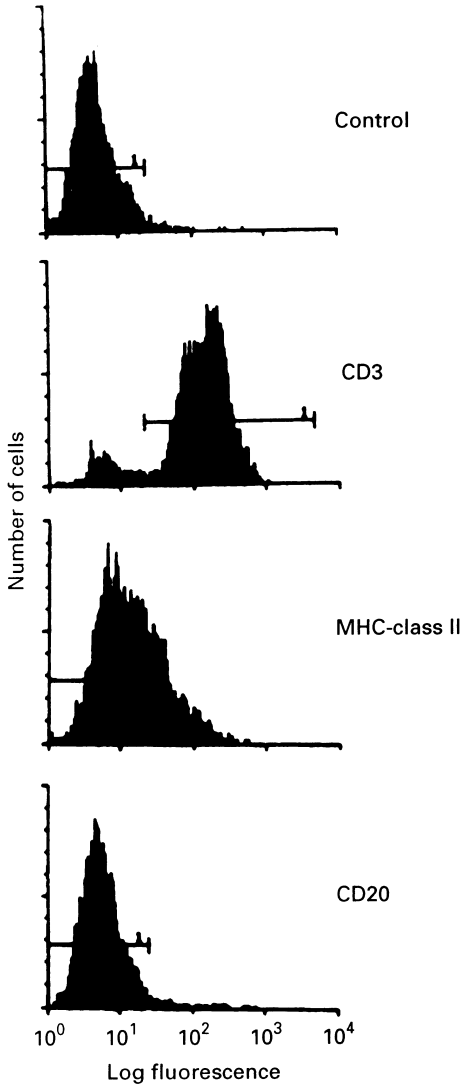


Fig. 1. Expression of surface molecules on synovial fluid T (SFT) cells. SFT cells (2×10^6) were incubated with mIgG (control), MoAb OKT3 (anti-CD3), L203 (anti-MHC class II) and NuB2 (anti-CD20), respectively, and stained with FITC-conjugated sheep anti-mIgG.

Activation of SFT cells in culture

In vitro activation of SFT cells was performed as follows. The SFT cells (5×10^6 cells/ml) were cultured in six-well plates (Falcon 3090; Becton Dickinson) (2 ml/well) with 10 μg/ml phytohaemagglutinin (PHA; GIBCO Labs, Chagrin Falls, OH), 5 μg/ml soluble OKT3 or 40 μg/ml human CII [2] in RPMI 1640 medium (Cosmo Bio., Tokyo, Japan) supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol (2-ME) and 10% fetal calf serum (FCS; Whittaker, MA Bioproducts, Walkersville, MD) in a CO_2 incubator under 5% CO_2 in a humidified atmosphere at 37°C. Three days later, cells were collected by pipetting and after washing twice with Hanks' solution, cells were used for transfer.

Type II collagen

CII was purified from human costal cartilage according to Miller's method [11]. CII preparation without contamination as assessed by SDS–PAGE was used for the study. Lipopolysaccharide (LPS) content contaminated in CII preparation was negligible (< 10 ng/ml) by Limutester (Funakoshi, Tokyo, Japan).

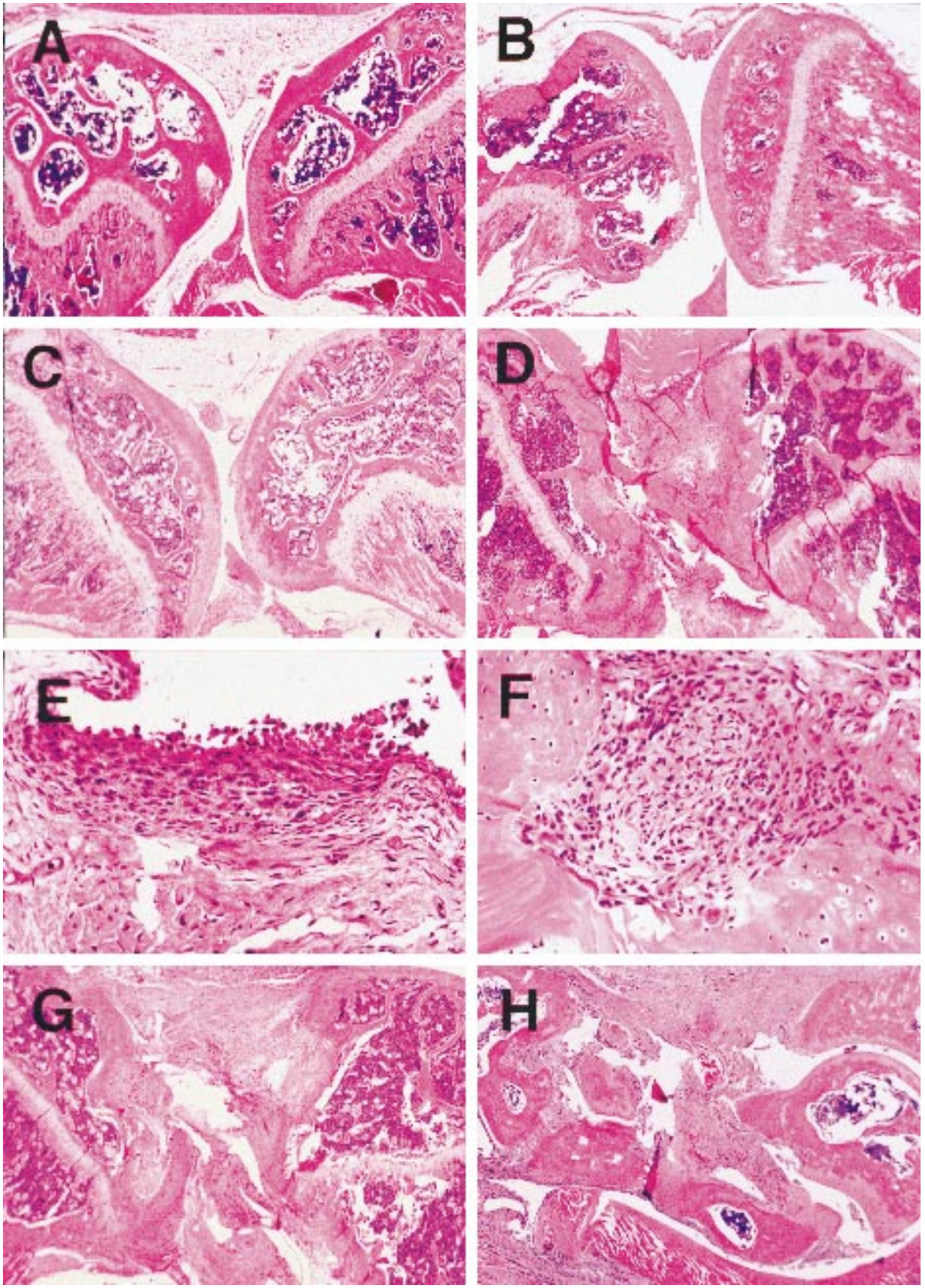
Cell transfer

Stimulated or unstimulated SFT cells ($1–6 \times 10^6$) were injected into the right knee joint and peritoneal cavity (the ratio of cell numbers 1:4) of 5–6-week-old SCID mice (Clea Japan, Inc., Osaka, Japan). After 6 weeks, joints and organs of the SCID mice were histologically investigated.

Histological examination

After 6 weeks from the cell transfer into SCID mice, knee and foot joints were harvested, fixed in paraformaldehyde, decalcified with 0·5 M EDTA pH 7·4, sectioned and stained with haematoxylin and eosin (H–E). Synovial hyperplasia was graded by the average cell layers of the synovial lining cells: –, no lesion (single cell layer); ±, minimal (two to three cells); +, mild (four to five cells); ++, moderate (six to seven cells); +++, severe ($\geqslant 8$ cells layer). Fibrosis and bone or cartilage erosion were also graded on five scales: –, no lesion; ±, minimal (lesion $\leqslant 10\%$); +, mild ($10\% <$ lesion $\leqslant 30\%$); ++, moderate ($30\% <$ lesion $\leqslant 60\%$); +++,

Fig. 2. Histological examination of knee and foot joints in SCID mice which developed severe arthritis through the transfer of stimulated synovial fluid T (SFT) cells of RA patients. Cells (1×10^6 and 4×10^6) were injected into the right knee joint and peritoneal cavity of SCID mice, respectively. Histological examination was done 6 weeks after the injection of cells. An intact knee joint of SCID mice (A), right knee joints of SCID mice transferred with phytohaemagglutinin (PHA)-stimulated peripheral blood T (PBT) cells (5×10^6 cells) from normal donors (B) and with unstimulated SFT cells (5×10^6 cells) from RA patients (C). The joints of SCID mice transferred with OKT3-stimulated SFT cells (5×10^6 cells) from the same patient as C (D–H); a joint of a right knee (D,E,F), a left knee (G) and a right foot (H). (Mag. ×100 (A–D, G and H); ×800 (E,F).)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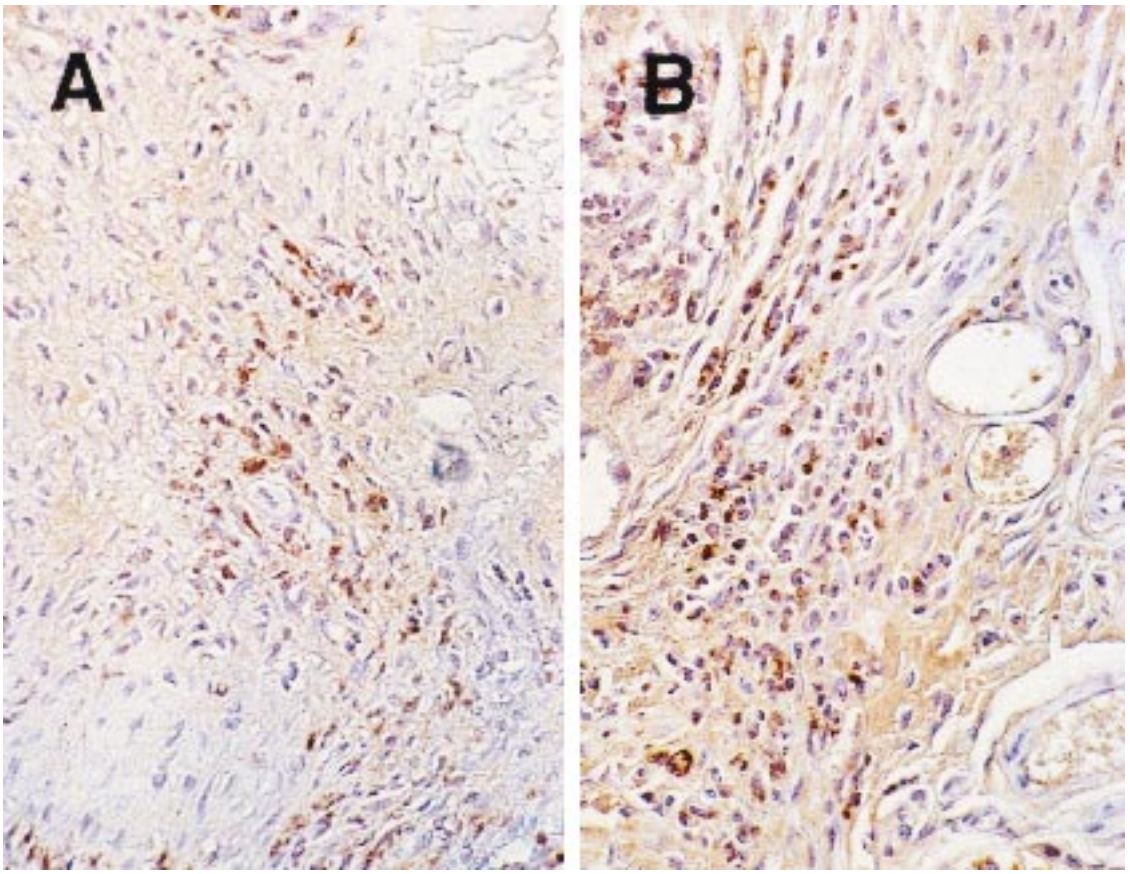

Fig. 3. MT-1 staining of SCID mice synovium causing severe destructive arthritis by transferring synovial fluid T (SFT) cells of RA patients. The same tissue sections of knee joints as D–G of Fig. 2 were immunohistochemically stained with anti-human T cell MoAb, MT-1. Both lesions of the right knee which was SFT-injected (A) and the uninjected left knee (B) were MT-1^{+}. (Mag. ×1600.)

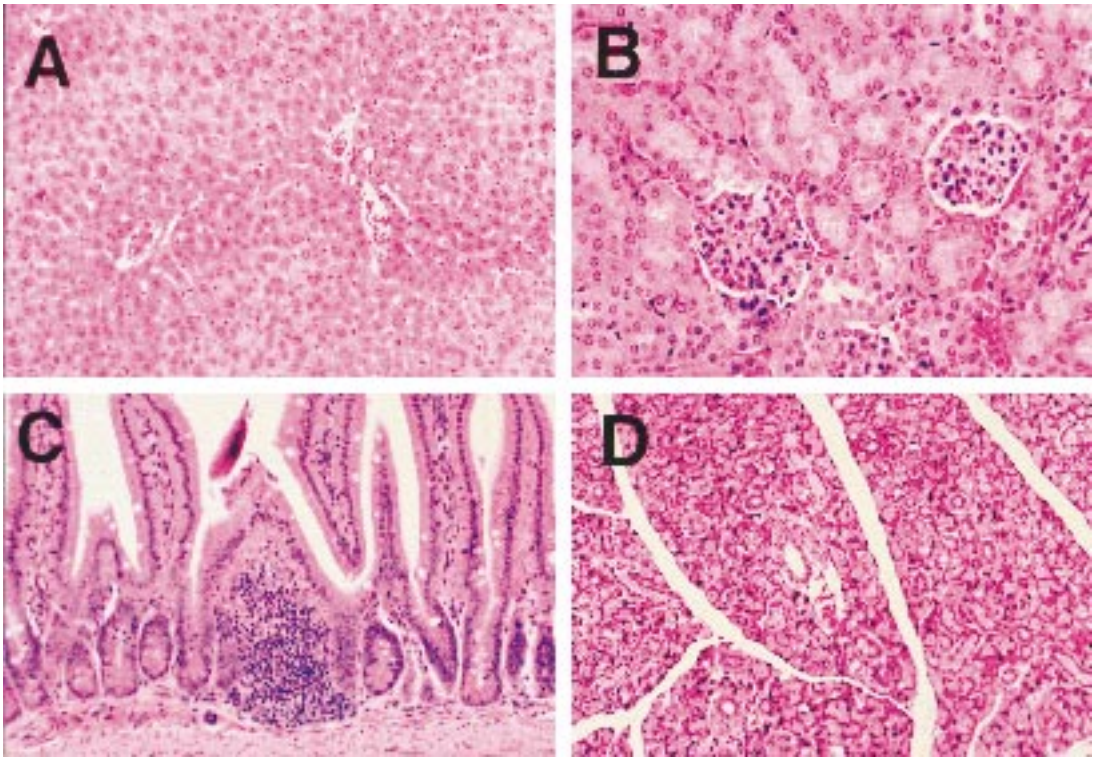

Fig. 4. Examination for graft-*versus*-host disease (GVHD) in a SCID mouse developing severe destructive arthritis. Paraformaldehyde-fixed and HE-stained liver ×500 (A), kidney ×1600 (B), intestine ×660 (C) and submaxillary gland ×400 (D) were examined for GVHD. All features were closely similar to those of intact SCID mice.

severe (60% < lesion). Evaluation of synovial hyperplasia, fibrosis, and bone/cartilage erosion were read in a blind fashion.

Additionally, some sections were stained immunohistochemically with a MoAb specific for human T cells, MT-1 (Bio-Science Products AG, Emmenbrücke, Switzerland), by an indirect immunoperoxidase method and Vectastain ABC Kit (Vector Labs, Burlingame, CA).

RESULTS

Surface molecule expression of SFT cells

The SFT cells separated from RA synovial fluid expressed $CD3^+ > 90\%$, MHC class $II^+ \leqslant 25\%$, $CD20^+$ (B cell) < 3% as shown in Fig. 1; $CD3^+$ SFT cells consisted of about 80% $CD4^+$ cells and 20% $CD8^+$ cells (data not shown).

Transfer of SFT cells into SCID mice

While the transfer of PHA-stimulated PBT cells from normal donors (Fig. 2B), and of unstimulated SFT cells from RA patients (Fig. 2C) into knee joint and peritoneal cavity of SCID mice triggered minimal synovial hyperplasia and villus, it did not produce pannus formation or bone or cartilage erosion; the resulting appearance was similar to intact joint of control mice (Fig. 2A). In contrast, as shown in Fig. 2D, OKT3-stimulated SFT cells (the same patient as Fig. 2C) induced severe destructive arthritis involving aggressive synovial cell proliferation to form synovial hyperplasia with villus (Fig. 2E), erosion of cartilage and bone by infiltration of violently proliferative fibroblasts to form pannus (Fig. 2F), and serious joint damage (Fig. 2D).

It is noteworthy that this arthritis was hardly accompanied by macroscopic clinical signs such as joint swelling or redness. However, histological examination revealed that synovitis started about 4 weeks after injection and continued for more than 3 months, and subsided gradually thereafter. These severe features of arthritis were also observed in other joints which were not used for injection with cells, including left knee (Fig. 2G), foot (Fig. 2H), and elbow and hand (data not shown), suggesting that this experimental arthritis was not induced by injury caused by cell injection into right knee joint and has an RA-like polyarthritic nature.

Immunohistochemical staining with MoAb anti-human T cells in arthritis lesions

To examine whether the injected SFT cells had migrated to the arthritic lesions of SCID mice, two sections of right and left knee joint (Fig. 2D,G) were immunohistochemically stained with MoAb MT-1, which specifically recognizes human T cells. $MT\text{-}1^+$ cells were detected in both sections (Fig. 3A,B).

Investigation for xenogeneic graft-versus-*host disease*

It is known that the transfer of human immunocytes into animals sometimes induces xenogeneic graft-*versus*-host disease (GVHD), which could cause arthritis. To eliminate such a possibility, we investigated several organs histologically: liver, kidney, intestine and submaxillary gland. As shown in Fig. 4, these sections were closely similar to those of control mice (data not shown), except for a slight increase of lymphoid follicles in the intestine. This indicates that GVHD was in fact not induced, and that SFT cells themselves caused the severe joint inflammation.

Transfer of T cells or mononuclear cells from RA and OA patients, and normal donors

Table 1 shows the experimental results of the transfer of SFT cells and PBMC from RA patients, of SF cells from patients with OA, and of PBT cells from normal donors in SCID mice. As a control experiment, PBT cells from healthy donors failed to induce obvious arthritis (nos 32–34) or triggered only faint synovial hyperplasia (nos 35 and 36) or fibrosis (no. 36) with mild or less grade ($\leqslant$ +), but not obvious bone or cartilage erosion, even if they had been stimulated with PHA or OKT3 *in vitro*.

Unstimulated SFT cells from RA patients were also insufficient to induce arthritis, as illustrated by experiments 2, 4, 6, 10 and 13. However, those cells cultured with stimulators, e.g. OKT3, PHA/concanavalin A (Con A) or CII *in vitro*, triggered an increase of synovial hyperplasia, fibrosis, or erosion of bone or cartilage and caused severe arthritis by transfer (nos 1–26 in Table 1). Table 2 summarizes the frequency of arthritis induction by transfer of activated SFT cells. SFT cells, activated with OKT3, PHA/Con A or CII *in vitro*, efficiently induced obvious arthritis, including moderate or severe synovial hyperplasia ($\geqslant$ ++), or mild or more severe fibrosis or bone/cartilage erosion ($\geqslant$ +). Among stimulants, PHA/Con A and CII were more effective in induction of arthritis than OKT3 (Table 2).

On the other hand, PHA-activated PBMC ($CD3^+ > 70\%$) from RA patients (nos 27 and 28) did not cause obvious arthritis, as shown in Table 1. Also, PHA-activated SF cells ($CD3^+ > 40\%$) from OA patients (nos 30 and 31) failed to trigger serious arthritis (Table 1).

DISCUSSION

In this study, we succeeded in inducing severe arthritis by transferring *in vitro*-activated SFT cells from RA patients into knee joint and peritoneal cavity of SCID mice. This arthritis involved synovial hyperplasia, pannus formation, and bone and cartilage erosion, and occurred in other joints as well as the right knee joint which was used for cell injection, including left knee, foot, elbow and hand, suggesting a polyarthritic nature mimicking RA.

First, we found that the transfer of SF mononuclear cells, which include SFT cells and SF monocytes, induced arthritis, if activated with T cell mitogens *in vitro* (data not shown). Therefore, SFT cells were purified from SF mononuclear cell preparation and used for transfer into SCID mice, to define whether SFT cells actually work in triggering arthritis. The isolated SFT cell fraction, which was $> 90\%$ $CD3^+$, as shown in Fig. 1, contained about 25% MHC class II^+ cells (composed of 20% MHC class II^+ T cells and 5% non-T non-B cells). Therefore, it seems that these class II^+ cells could act as antigen-presenting cells for transferred SFT cells in the SCID joints. However, MHC class II^+ synovial fluid adherent cells ($0{\cdot}5\text{–}1{\cdot}7 \times 10^6$ cells) alone could not induce such severe joint damage (data not shown). Furthermore, through immunohistochemical staining, $MT\text{-}1^+$ cells were detected in arthritic lesions of both knee joints. This indicates that transferred SFT cells migrated to and populated the remote joints and possibly worked to trigger such severe arthritis as shown in Fig. 2.

Unlike RA, this arthritis induced by SFT cells failed to form lymphoid follicles. This might be due to the lack of B cells, as follicular dendritic cells need to trap immune complexes in order

Table 1. Comparison of the ability to induce arthritis in SCID mice by transferring synovial fluid T (SFT) cells and peripheral blood mononuclear cells (PBMC) from RA patients, SF cells from osteoarthritis (OA) patients and peripheral blood T (PBT) cells from normal donors

Experiment no.*	Patients	Age (years)/ sex	Disease duration (years)/stage	Stimulant	Synovial hyperplasia	Fibrosis	Bone or cartilage erosion
SFT cells from RA							
1	T.I.	54/F	0·17/I	PHA	++	++	+++
2	K.N.	32/M	4/III	none	±	+	±
3				OKT3	+++	+++	+++
4	Y.I.	64/F	9/IV	none	–	–	±
5				PHA	++	–	+
6	A.O.	44/F	3/IV	none	+	±	±
7				PHA	+	++	+
8				OKT3	+	–	±
9				Con A	+	+	+
10	I.F.	47/F	26/IV	none	+	–	±
11				PHA	++	±	±
12				Con A	+	+	+
13	T.O.	70/F	14/IV	none	+	±	±
14				PHA	++	±	±
15				OKT3	–	–	±
16				CII	+	++	++
17	T.T.	48/F	14/III	PHA	++	++	+++
18				CII	+	+	++
19	Y.T.	70/F	8/III	PHA	+	+	+
20				OKT3	+	++	++
21				CII	+	–	±
22	T.N.	56/F	24/IV	PHA	+	±	–
23				CII	+	++	+++
24	Y.T.	69/F	39/IV	PHA	+++	+	+
25				CII	++	+	++
26	M.M.	66/F	7/IV	PHA	+	–	–
PBMC from RA							
27	K.N.	32/M	4/III	PHA	–	±	±
28	A.O.	44/F	3/IV	PHA	+	±	±
29	M.S.	68/F	20/IV	PHA	±	–	±
SF cells from OA							
30	M.O.	61/F		PHA	±	+	+
31	T.O.	71/F		PHA	±	–	–
PBT cells from normal donor							
32	Donor 1			PHA	±	–	±
33				OKT3	–	–	–
34	Donor 2			PHA	±	–	±
35				OKT3	+	–	±
36	Donor 3			PHA	+	+	±

* SFT cells ($2{\cdot}5–6 \times 10^6$) and PBMC ($2 \times 10^6–1 \times 10^7$) from RA, SF cells ($0{\cdot}3–0{\cdot}8 \times 10^6$) from OA, and PBT cells ($7 \times 10^6–1 \times 10^7$) from normal donors were transferred per mouse. Arthritis was examined histologically 6 weeks after the injection.

PHA, Phytohaemagglutinin; Con A, concanavalin A; CII, type II collagen.

to form lymphoid follicles [12]. Actually, neither human IgG, IgM, nor rheumatoid factor (RF) was detectable in the peripheral blood serum of SFT-transferred SCID mice with immunonephelometry kits (Nissui Pharmaceutical Co., Tokyo, Japan) (data not shown).

When human disease models using SCID mice are established, attention should be paid to the possibility of GVHD. Torbett *et al.* reported that 1×10^7 human peripheral blood lymphocytes (PBL) injected intraperitoneally into SCID mice survived for up to 1 year without causing GVHD [13]. However, Huppes *et al.* recently demonstrated that $CD4^+$ T cells induced acute xenogeneic GVHD in the presence of IL-2 [14]. In this arthritis model, severe inflammation was induced specifically in joints; it was not induced in other organs, including liver, kidney, intestine and submaxillary gland. Furthermore, activated PBT cells from healthy donors failed to induce obvious arthritis, as shown in nos 27 and 28 of Table 1. These facts effectively discount the possibility that this arthritis was due to xenogeneic GVHD.

Among T cells from RA patients, only *in vitro*-activated SFT cells, not unstimulated SFT cells or activated PBMC, induced severe destructive arthritis, as shown in Table 1. This fact suggests

Table 2. Efficiency of arthritis induction by transferring synovial fluid T (SFT) cells from RA patients cultured with T cell stimulants or collagen type II (CII) *in vitro*

Stimulant	Nos causing obvious arthritis*/ nos of experiments	Efficiency
None	1/5	0·20
OKT3	2/4	0·50
PHA/Con A	10/12	0·83
CII	4/5	0·80

* Numbers of obvious arthritis were counted as moderate or severe synovial hyperplasia ($\geqslant$ ++) or mild or more severe fibrosis or bone/cartilage erosion ($\geqslant$ +).

CII, Type II collagen; PHA, phytohaemagglutinin; Con A, concanavalin A.

that SFT cells containing pathogenic T cells were specifically concentrated in the joint and triggered arthritis, reflecting T cell involvement in the pathogenesis of RA. Also, the fact that CII-stimulated SFT cells induced relatively serious arthritis (nos 16, 18, 23 and 25 in Table 1) might support the possible role of CII as a candidate for self-antigen molecules of RA.

It has been reported that successful induction of severe arthritis was achieved through the i.p. transfer to SCID mice of spleen cells from CIA-induced DBA/1 mice together with bovine CII [15]; it has also been reported that the induction of human PBL-induced xenogeneic GVHD in SCID mice required the addition of IL-2 [14]. Considering these, the success in induction of severe arthritis by SFT cells activated *in vitro* with T cell stimulants and CII in this experiment may have been due to two critical factors: first, the activation of pathogenic T cell clones, and concomitant expansion by a growth factor such as IL-2 produced in the culture; second, the injection of activated SFT cells into both knee joint and peritoneal cavity of SCID mice simultaneously. Thus, both when we transferred SFT cells into SCID mice through i.p. injection alone, and when Sack *et al.* injected SCID mice only through knee joint [10], induction of arthritis failed. We also tried the transfer of SFT cells by intrajoint injection alone, but it induced only marginal or no synovitis (data not shown). Therefore, it seems that the combination of i.p. and intrajoint injection is most effective in inducing arthritis. This suggests the possibility that the RA lesion can be developed through the combination of two pathways: T cell-dependent and synoviocyte-dependent [16]. In line with this, we are now studying the possibility that the inflammatory synovial cells, which were activated by cytokine produced by the self antigen-reactive pathogenic T cells injected into the knee joint, and SFT cells continuously recruited into the joint space from the peritoneal cavity, might have worked together to develop severe arthritis in SCID mice.

With regard to SF cells from OA patients, which failed to trigger obvious arthritis in this study, it is presumed that this failure is due to the lack of pathogenic T cells in the synovial fluid of OA. However, we cannot completely eliminate the possibility that this is due to the small number of T cells included in SF cell preparation.

In conclusion, this SCID mouse model is characterized by the following three features: it induces multiple and destructive severe arthritis which mimics RA; it is specific for joint tissues, and not due to xenogeneic GVHD; and it is induced by *in vitro*-activated pathogenic T cells infiltrating into synovial fluid of RA patients. Therefore, this model may be one of the best RA-mimicking animal–human chimaeric models, induced by the transfer of RA patients' T cells into SCID mice, and is expected to provide useful information both for investigating the pathogenesis of RA and for therapeutic approaches.

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