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FAS Antigen Expression on Synovial Cells Was Down-Regulated by Interleukin 1β

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Recent reports revealed that Fas antigen is functionally expressed on human synovial cells and apoptosis can be induced in these cells by anti-Fas antibody. We examined the effect of interleukin 1β (IL-1β) on Fas antigen-mediated apoptosis on human synovial cells in vitro. Using flowcytometric analysis, IL-1β inhibited Fas antigen expression on synovial cells in a dose-dependent fashion. No significant difference of Fas antigen gene expression between IL-1β-tretated and untreated synovial cells was ovserved by RT-PCR analysis, suggesting that the inhibitory effect of Fas antigen expression by IL-1β is at posttranscriptinal level. Apoptosis of synovial cells was easily induced by treatment of these cells with anti-Fas antibody. In contrast, pretreatment of synovial cells with IL-1β protected these cells against Fas antigen-mediated apoptosis. The expression of bcl-2 on synovial cells, known to interfere with the apoptotic process mediated by the Fas antigen, was not influenced by IL-1β. Our results suggest that IL-1β inhibits Fas antigen-mediated apoptosis of synovial cells and may perpetuate the hyperplasia of the synovium in patients with rheumatoid arthritis.

Interleukin 1β (IL-1β) has a wide variety of biological activities, such as stimulation of fibroblast proliferation (1), acute phase reactant production (2,3), bone resorption (4,5), and increment of adhesion molecules on endothelial and synovial cells (6–8). In patients with rheumatoid arthritis (RA), the serum concentration of IL-1β reflects the activity of RA (9). Furthermore, the production of IL-1β by RA synovium in vitro correlates well with the degree of inflammation determined on arthroscopy (10). These data suggest that IL-1β plays a central role in the pathogenesis of RA. Recent studies demonstrated that Fas antigen is expressed on the synovial cells and that Fas antigen-mediated apoptosis can be determined on synovial cells (11). In the present study, we examined the effect of IL-1β on Fas antigen-mediated apoptosis of human synovial cells. Using flowcytometric analysis, Fas antigen expression on synovial cells was suppressed by IL-1β in a dose-dependent fashion. Pretreatment of synovial cells with IL-1β significantly inhibited Fas antigen-mediated apoptosis of these cells. In addition, treatment with IL-1β failed to influence the expression of protooncogene bcl-2, which is suspected of interfering with Fas antigen-mediated apoptosis (12). Our results suggest that IL-1β can inhibit Fas antigen-mediated apoptosis of synovial cells by down-regulating Fas antigen expression on these cells and may perpetuate the pronounced hyperplasia of the synovium in patients with RA.

MATERIALS AND METHODS

Preparation of synovial cells. Synovial tissue samples were obtained, after consent, from patients with RA and osteoarthritis (OA) during a corrective surgery. The method used for the isolation of synovial cells was described previously in detail (7). The resultant synovial cell preparations were less then 1% reactive with the monoclonal antibodies (mAbs), CD3 (Coulter Immunology, Hialeah, FL), Leu M3 (Becton Dickinson, CA), CD20 (Coulter Immunology), and anti-human von Willebrand factor (Immunotech S.A., Marseille, France), which, respectively, define an antigen on all mature T cells, on monocytes/macrophages, on pan-B cells and on vascular endothelial cells.

Flowcytometric analysis of Fas-antigen and bcl-2 on synovial cells. Synovial cells (3×10^5/well) were cultured in RPMI

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Abbreviations: IL-1β, interleukin 1β; mAb, monoclonal antibody; RA, rheumatoid arthritis; OA, osteoarthritis.

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1640 containing 2% bovine serum albumin (Sigma Chemical Co., St. Louis, MO) in the presence or absence of various concentrations of recombinant interleukin 1β (rIL-1β, Otsuka Pharmaceutical Co., Tokushima, Japan) for indicated times, in a 6-well plate (Costar, Cambridge, MA), then detached from the plate with 0.265 mM EDTA. The detached cells were washed with PBS three times and incubated with anti-Fas mAb (MBL, Nagoya, Japan). The cells were then washed with PBS and reacted with PE-conjugated goat anti-mouse IgG (MBL, Nagoya). The expression of Fas antigen on synovial cells was analyzed using a flowcytometer (EPICS PROFILE-II, Coulter Immunology).

To investigate the expression of bcl-2 on synovial cells, cells were permeabilized with digitonin, as described previously (13). After confirming the adequacy of permeabilization by trypan blue uptake, the permeabilized cells were incubated with anti-bcl-2 mAb, kindly provided by Dr. Yoshihide Tsujimoto, Biomedical Research Center, Osaka University Medical School, Osaka, Japan. After incubation, the cells were further reacted with FITC-conjugated goat anti-mouse IgG (Tago, Burlingame, CA) and the expression of bcl-2 on synovial cells was analyzed on a flowcytometer (EPICS PROFILE-II). To check the adequacy of permeabilization on flowcytometric analysis, we used the anti-α-tubulin mAb (Cedarlane Laboratories, Ontario, Canada) as a positive control.

RNA preparation and Fas antigen reverse transcriptase polymerase chain reactions(RT-PCR). The PCR reactions were based on the RACE protocol (27). Total cellular RNA was extracted from the synovial cells cultured with or without IL-1β and peripheral blood mononuclear cells (PBMC) obtained from healthy donors using guanidium thiocyanate and phenol (RNAzol B, Cinna/Biotek Labs Intl. Inc., Friendswood, Texas) and complementary DNA (cDNA) transcripts were prepared from 10μg of cellular RNA using oligo-dT priming (1μg/20μl volume) and avian reverse transcriptase (30U/20μl) (Life Science Inc) in the presence of RNAsin (40U/20μl) (Promega Inc, Madison, WI). The reactions were diluted to 100μl with sterile water. For the PCR reactions, 5μl of denatured cDNA was amplified in a 20μl final volume with 1U Taq DNA polymerase (Promega), 0.5 μg of both primers (5′-CTC ACC AGC AAC ACC AAG TGC -3′,3′-TTC GGC TTT TAT ATC CTC GAT -5′), Taq polymerase buffer containing 1.5mM $MgCl_2$ and with 1.5 mM of each dNTP (Promega Inc). A 35 cycle step program (95°C for 1 min, 56°C for 2 min, and 72°C for 3 min) was followed by a 10 minute extension at 72°C (Model PJ2000 DNA Thermal cycler, PERKIN ELMER, USA). The amplified products were subjected to electrophoresis on 1.5% agarose gels. The predicted size of fragment was 311 bp.

Induction of synovial cells apoptosis by anti-Fas antibody. Cultured synovial cells were examined for anti-Fas antibody-induced apoptosis. Synovial cells were cultured in RPMI 1640 containing 2% BSA in the presence or absence of rIL-1β, then treated with anti-Fas antibody (1000 ng/ml) or control mouse IgM (1000 ng/ml, Seikagaku Co., Tokyo) for 12 hours. Following detachment from the plate, the synovial cells were fixed with 70% ethanol and treated with RNAase (100 μg/ml, Sigma), and then stained with propidium iodide (100 μg/ml, Sigma) for 30 minutes on ice. Stained cells were analyzed using a flowcytometer (EPICS PROFILE-II). Apoptosis was quantified by flowcytometric determination of the proportion of cells with hypodiploid DNA (14). We also determined the degree of DNA fragmentation on agarose gel, a process characteristic of apoptosis, using the method described previously (15,16).

RESULTS

Effect of IL-1β on Fas antigen expression on synovial cells. Incubation with various concentrations of rIL-1β for different time intervals demonstrated that the expression of Fas antigen on synovial cells was inhibited by rIL-1β. The inhibition was dose-dependent and the maximum suppression was noted after incubation for 48 hours at a concentration of approximately 10 IU/ml (Figure 1).

RT-PCR of Fas antigen gene. In order to approach the mechanisms of the inhibitory effect of IL-1 β on Fas antigen expression by synovial cells, a quantitative RT-PCR of Fas antigen gene was performed. The amplification of cDNA synthesized from a range of 125-1000 ng of PBMC cellular RNA for 35 cycles resulted in a linear visualized signals on agarose gel by ethidium bromide (Figure 2f–i), therefore, 500 ng of each synovial cellular RNA was amplified. There was no significant difference in Fas antigen gene expression between IL-1β-treated and -untreated synovial cells (Figure 2a–e).

Expression of bcl-2 on synovial cells. Permeabilized synovial cells were used in this experiment since bcl-2 is an integral membrane protein which lies within the cells rather than on the surface (17). Following permeabilization with digitonin, almost all synovial cells were positive with anti-α-tubulin mAb (Figure 3). This finding indicated the suitability of the permeabilization technique. The expression of bcl-2 on synovial cells was examined under the above conditions. As shown in Figure 2, bcl-2 was stained in approximately 60% of synovial cells. Treatment with rIL-1β did not influence bcl-2 expression on synovial cells (data no shown).

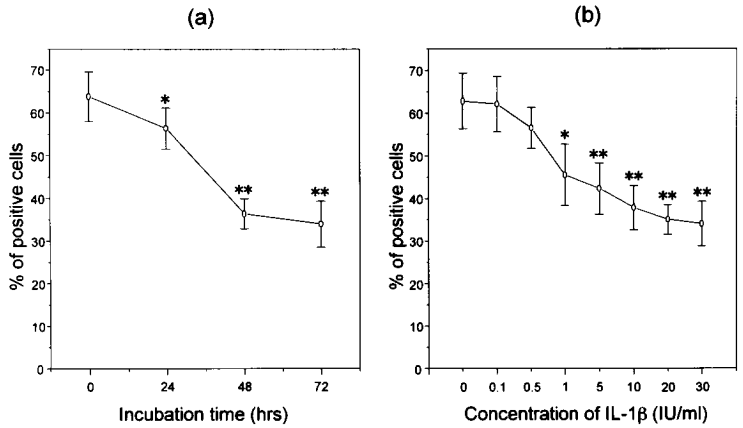

FIG. 1. Inhibition of Fas antigen expression on synovial cells by rIL-1β. (a) Time course study: Synovial cells from patients with RA were incubated with 10 IU/ml of rIL-1β for specified time intervals. After incubation, the expression of Fas antigen on synovial cells was examined by a flowcytometer. (b) Dose-dependent study: Synovial cells from patients with RA were incubated with varying concentrations of rIL-1β for 48 hours and Fas antigen expression on synovial cells was examined by a flowcytometer. Data are mean ± SD of six separate experiments. *$p < 0.05$,**; $p < 0.01$, compared with control.

Permeabilized peripheral blood lymphocytes (PBL) were used as positive control for bcl-2 staining and almost all cells were stained under such conditions (Figure 3).

The regulation of Fas antigen and bcl-2 expression on synovial cells from patients with OA were similar to those of synovial cells from patients with RA (data not shown).

Induction of synovial cells apoptosis by anti-Fas antibody. Finally, we determined the activity of Fas antigen on synovial cells. Treatment of synovial cells from patients with RA with anti-Fas antibody resulted in morphological changes consistent with apoptosis, such as cell shrinkage and detachment from the plate (Figure 4c). This was in contrast to the lack of a remarkable change in synovial cells treated with control mouse IgM (Figure 4a). Treatment with anti-Fas antibody increased significantly the percentage of cells with hypodiploid DNA (Table 1). In contrast, pretreatment of synovial cells with IL-1β protected against Fas antigen-induced apoptosis (Figure 4d and Table 1). Furthermore, DNA fragmentation, characteristic of apoptosis on agarose gel, was

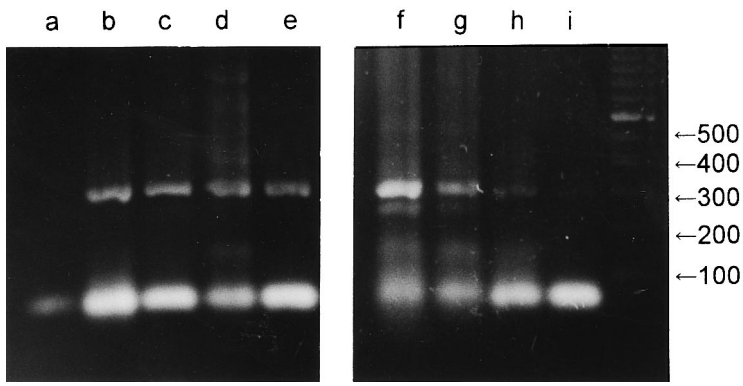

FIG. 2. Fas antigen gene polymerase chain reactions (PCR). The predicted size of fragment was 311bp. 500 ng of each synovial cellular RNA was amplified. a; Blank (Negative control), b; unstimulated SC sample 1, c; IL-1β-stimulated SC sample 1, d; unstimulated SC sample 2, e; IL-1β-stimulated SC sample 2, f-i; RNA concentration study of PBMC (PBMC as a positive control), f; 1000ng, g; 500ng, h; 250ng, I; 125ng of PBMC RNA. Molecular-size markers (in bp) are included at right SC, synovial cells; PBMC, peripheral blood mononuclear cells.

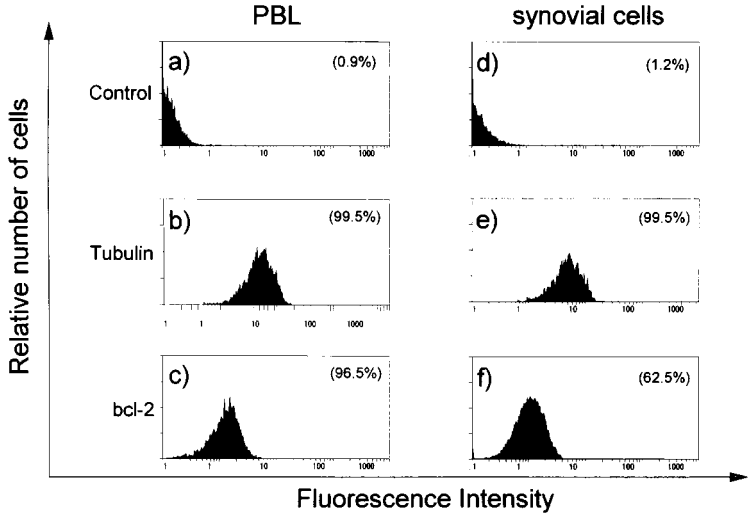

FIG. 3. Flowcytometric analysis of bcl-2 expression on synovial cells and peripheral blood lymphocytes (PBL). Permeabilized cells were reacted with mAbs and further incubated with FITC-conjugated goat anti-mouse IgG. After incubation, flowcytometric analysis was performed to measure the expression of each molecule (a)–(c), Permeabilized peripheral blood lymphocytes reacted with each mAb. (d)–(f), Permeabilized synovial cells from patients with RA reacted with each mAb. Numbers in the parenthesis are percentage of positive cells. Data are a representative example of four experiments.

detected in anti-Fas antibody-treated synovial cells and the treatment with IL-1β significantly inhibited DNA fragmentation in synovial cells (data not shown). Synovial cells from patients with OA were also easily susceptible to apoptosis when treated with anti-Fas antibody and pretreatement with IL-1β suppressed this phenomenon significantly (data not shown).

DISCUSSION

Recent studies have suggested that apoptosis occurs in the synovium of patients with RA both in vivo and in vitro, and that such process can be induced in synovial cells in vitro by treating the cells by anti-Fas antibody (11). In this study, we examined the effect of IL-1β on Fas antigen-mediated apoptosis of synovial cells in vitro. Our results demonstrated that IL-1β inhibited the expression of Fas antigen on synovial cells and suppressed Fas antigen-mediated apoptosis of synovial cells without autoregulation of bcl-2, which is known to interfere with apoptosis mediated by Fas antigen (12). Fas mRNA expression determined by RT-PCR was not changed by IL-1β treatment, suggesting that the reduction of Fas expression by IL-1β is achieved at post-transcriptional level. The T cells in the rheumatoid synovium are activated (18–21) and activated T cells in vitro are reported to express Fas-ligand (22–25). Therefore, in the rheumatoid synovium,

TABLE 1
Percentage of Cells with Hypodiploid DNA

	Added antibody	
Stimulus	Control mouse IgM (%)	IgM class anti-Fas antibody (%)
Control	1.5 ± 0.2	62.8 ± 9.8
rIL-1β	1.8 ± 0.3	12.0 ± 2.0*

* $p < 0.01$, compared with control.
Data are expressed as mean ± SD of five separate experiments.

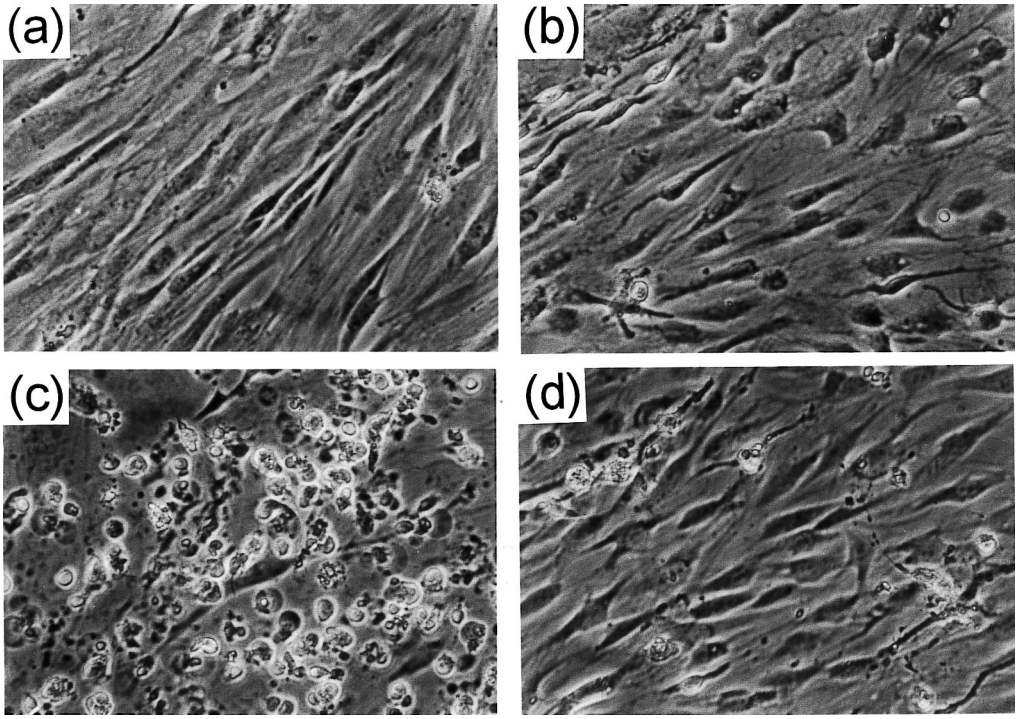

FIG. 4. Phase contrast microscopic analysis demonstrating inhibition of Fas antigen-mediated apoptosis of synovial cells by rIL-1β. Synovial cells from patients with RA were incubated with or without 10 IU/ml rIL-1β for 48 hours. Following incubation, the cells were reacted with either 1000 ng/ml anti-Fas IgM or 1000 ng/ml control mouse IgM for 12 hours. (a), unstimulated synovial cells reacted with control mouse IgM; (b), IL-1β-stimulated synovial cells reacted with control mouse IgM; (c), unstimulated synovial cells reacted with anti-Fas antibody. Note the change in cell morphology typical of apoptosis; (d), IL-1β-stimulated synovial cells reacted with anti-Fas antibody.

the interaction between Fas antigen on synovial cells and Fas-ligand on activated T cells may cause apoptosis of synovial cells and induce regression of proliferation of the synovium which can be seen in patients with RA (26). We were able to demonstrate in this study the ability of IL-1β, which is thought to play a critical role in the pathogenesis of RA, to suppress Fas antigen-mediated apoptosis of synovial cells. This effect supports the notion that IL-1β present in the synovial fluid can inhibit the apoptotic process of synovial cells by suppressing any Fas antigen/Fas-ligand interaction due to the down-regulation of Fas antigen expression on synovial cells. Such process may result in the perpetuation of the hyperplasia of synovium in patients with RA.

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