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# Inhibitory effects of interleukin-10 on synovial cells of rheumatoid arthritis

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## SUMMARY

This paper describes the immunoregulatory effects of interleukin-10 (IL-10) on synovial cells *in vitro*. Synovial cells were cultured with IL-10 in the presence or absence of various cytokines. Following incubation, the costimulatory molecule expression on synovial cells and cytokine production in culture supernatants were analysed by an indirect immunofluorescence method and enzyme-linked immunosorbent assay, respectively. We also examined the effect of IL-10 on the function of synovial cells as antigen-presenting cells (APC). Synovial cells spontaneously express several kinds of costimulatory molecule and produce various kinds of cytokines. Stimulation of synovial cells with interferon-$\gamma$ (IFN-$\gamma$), IL-1$\beta$, or 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) markedly enhanced the expression of costimulatory molecules and cytokine production of these cells. Both spontaneous and up-regulated costimulatory molecule expression and cytokine production were significantly suppressed by the addition of IL-10. Autologous T-cell proliferation was stimulated by purified protein derivative (PPD) in IFN-$\gamma$-treated synovial cells and treatment of these synovial cells with IL-10 also suppressed T-cell proliferation. Our results suggest that IL-10 has an inhibitory effect on synovial cells and is an important immunoregulatory component of the cytokine network in rheumatoid arthritis.

## INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by chronic synovitis which often leads to joint destruction.[1] Various cytokines, such as tumour necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-1 (IL-1), IL-6, IL-8, and granulocyte–macrophage colony-stimulating factor (GM-CSF) are present in the synovial fluid and synovial membrane in patients with RA,[2–5] and are considered important participants in the pathophysiology of the disease. However, in addition to the proinflammatory cytokines, a compensatory anti-inflammatory response is also observed in the RA synovial membrane. Thus, the expression of high levels of IL-1 receptor antagonist (IL-1ra) and soluble TNF receptor are present in the RA synovium,[6–8] suggesting that homeostatic mechanisms limiting joint destruction exist in the rheumatoid joint.

IL-10 is a polyfunctional cytokine produced by T cells, macrophages/monocytes and B cells. It was originally described as an inhibitor of cytokine synthesis by Th1 clones and subsequently shown to mediate its inhibitory effects on Th1 clones by acting on the antigen-presenting cells (APC).[9–11] In addition, IL-10 was also reported to suppress directly T-cell proliferation and IL-2 production.[12] The serum and synovial fluid of RA patients contain significantly elevated IL-10 levels compared with the level in normal subjects or in control patients.[13] In addition, IL-10 protein and mRNA are expressed in the synovial membranes of RA and osteoarthritis (OA).[14] Treatment of synovial membrane cultures with anti-IL-10 enhances cytokine production, including TNF-$\alpha$ and IL-1$\beta$ in the culture medium, while the addition of exogenous IL-10 decreases cytokine production by cultured synovial membranes,[14] suggesting anti-inflammatory effects for IL-10 on the synovial membrane of patients with RA.

In the present study, we investigated the effect of IL-10 on costimulatory molecule expression, cytokine production, and antigen-specific T-cell proliferation by synovial cells *in vitro*.

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Abbreviations: APC, antigen-presenting cells; mAb, monoclonal antibody; PPD, purified protein derivative; RA, rheumatoid arthritis; TPA, 12-*O*-tetradecanoyl phorbol 13-acetate.

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## MATERIALS AND METHODS

### *Synovial cell culture*

Synovial tissue was obtained from 12 patients with RA at the time of surgery for total knee replacement. All patients fulfilled the criteria of the American Rheumatism Association for RA. The experimental protocol was approved by the Hospital Human Ethics Review Committee and a signed consent was obtained from all patients. Isolation of synovial cells was performed according to the method described previously by



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our laboratory.[15] In brief, the synovial tissue was trimmed of fat with scissors and minced, then added to a mixture of collagenase (Sigma Chemical Co., St. Louis, MO) and dispase (Godo Shusei Co., Tokyo, Japan). The tissue mixture was digested for 45 min during gentle stirring at 37°. The suspension was then passed through a nylon mesh, and the resulting cells were allowed to adhere to Petri dishes (Falcon 3003, Becton Dickinson Co., Oxnard, CA) in RPMI-1640 supplemented with 10% fetal bovine serum (FBS; Gibco Laboratories, Grand Island, NY). To eliminate non-adherent cells, the plated cells were extensively washed with phosphate-buffered saline (PBS). The synovial cells were then cultured in RPMI-1640 supplemented with 10% FBS and used for the following experiments between 7 and 10 days after surgical removal of rheumatoid synovium.

*Costimulatory molecule expression on synovial cells*

Synovial cells ($3 \times 10^5$/well in six-well tissue culture clusters; Costar, Cambridge, MA) were cultured in RPMI-1640 supplemented with 2% FBS in the presence or absence of recombinant interferon-$\gamma$ (rIFN-$\gamma$; 500 IU/ml, Shionogi Co., Osaka Japan), rIL-1$\beta$ (10 IU/ml, Otsuka Pharmaceuticals Co., Tokushima, Japan), rGM-CSF (500 pM, Kirin Co., Tokyo, Japan), or 12-*O*-tetradecanoyl phorbol 13-acetate (TPA; 10 ng/ml, Sigma) for 48 hr. To examine the effect of IL-10 on the costimulatory molecule expression of synovial cells, rIL-10 (Genzyme, Cambridge, MA) was added in the presence or absence of various reagents described above and cultured for 48 hr. After incubation, synovial cells were detached by the addition of 0·265 mM ethylenediaminetetraacetic acid (EDTA) and detached cells were washed twice with PBS containing 1% FBS.

The costimulatory molecules and human leucocyte antigen (HLA)-DR expression on synovial cells were determined by an indirect immunofluorescence method. The cells were first incubated with murine monoclonal antibody (mAb) specific for intercellular adhesion molecule-1 (ICAM-1, Immunotech S.A., Marseille, France), lymphocyte function-associated antigen-3 (LFA-3, Immunotech), vascular cell adhesion molecule-1 (VCAM-1, Immunotech), HLA-DR (Immunotech), B7-1, or B7-2 for 30 min on ice. Anti-B7-1 and B7-2 mAb were kindly provided by Dr Miyuki Azuma and Prof. Ko Okumura, Juntendo University School of Medicine, Tokyo, Japan. After washing, the synovial cells were further incubated with phycoerythrin (PE)-conjugated anti-mouse IgG (MBL, Nagoya, Japan) and analysed by a flowcytometer (EPICS-PROFILE-II, Coulter Immunology, Hialeah, FL).

*Cytokine measurement*

Synovial cells ($3 \times 10^5$/well) in six-well tissue culture clusters (Costar) were cultured in RPMI-1640 containing 2% FBS in the presence or absence of various reagents described above, including IL-10, for 48 hr. After incubation, the culture supernatants were collected and used for assay. IL-1$\beta$, IL-6, IL-8, granulocyte colony-stimulating factor (G-CSF), and GM-CSF were measured by immunoenzymetric assay. Briefly, plates precoated with monoclonal antibody to each cytokine were incubated with the samples, incubated further with polyclonal rabbit anti-cytokine antibody and then reacted with goat anti-rabbit immunoglobulin conjugated to horseradish peroxidase. Between each step, excess reactants were removed by washing three times with 0·01 M phosphate buffer (pH 7·4) containing 1% bovine serum albumin. Absorbance of the chromogenic compound produced by the addition of the enzyme substrate, was measured at 490 nm. The sensitivity of cytokine determination was 20 pg/ml.

*T-cell proliferation assay*

To examine whether synovial cells act as APC to T cells and whether IL-10 could modulate this function, we investigated the autologous T-cell proliferative response toward synovial cells pulsed with purified protein derivative (PPD; Japan BCG Co., Tokyo, Japan). The method of T-cell isolation from peripheral blood (PB) has been described in detail elsewhere.[15] All patients experienced positive tuberculin reactions or Bacillus Calmette–Guérin (BCG). Briefly, mononuclear cells were isolated from PB by Ficoll–Conray gradient centrifugation (Daiichi Pharmaceutical Co., Tokyo, Japan). The cells were depleted of adherent cells by incubating the cell suspension in Petri dishes for 2 hr at 37°. A T cell-enriched population was prepared from the non-adherent cells by rosetting with 5% sheep red blood cells (SRBC, Nippon Biotest Laboratory, Tokyo, Japan) and passed through a nylon wool column at 37° for 1 hr. T cell-rich populations containing more than 95% T cells, less than 1% B cells and less than 1% monocytes were designated as T cells.

Synovial cells stimulated with or without IFN-$\gamma$ in the presence or absence of IL-10 for 48 hr were pulsed with an optimal dose of PPD (10 $\mu$g/ml) for 4 hr at 37°. Non-pulsed cells were handled in a similar fashion. During the next 60 min, all cells were incubated with mitomycin C at a concentration of 50 $\mu$g/ml, and subsequently washed four times. Autologous T cells ($1 \times 10^5$) were then cultured in quadruplicate in 96-well flat-bottomed microtitre plates (Costar) with antigen pulsed or non-pulsed synovial cells ($1 \times 10^4$) in 0·2 ml of RPMI-1640 containing 10% autologous serum for 7 days. In some experiments, mAb against ICAM-1, VCAM-1, LFA-3, B7-1, B7-2 and HLA-DR were added. The proliferative response of T cells was measured by adding 0·5 $\mu$Ci/well of [$^3$H]thymidine (New England Nuclear, Boston, MA) 24 hr prior to harvesting of the cells onto glass filters. The newly synthesized DNA was measured using a semiautomatic cell harvester (Labo Mash, Labo Science, Tokyo, Japan).

*Statistical analysis*

Statistical analysis was performed using the Student's *t*-test and *P* values less than 0·05 were selected as the level of significance.

## RESULTS

**Inhibitory effect of IL-10 on costimulatory molecule expression on synovial cells**

We initially characterized the adherent RA synovial cells obtained as described in the Materials and Methods. Although the preparation of synovial cells obtained was less than 1% reactive with mAb, CD3 (Coulter Immunology), CD20 (Coulter Immunology), and anti-von Willebrand factor (Immunotech) which define all mature T cells, pan-B cells and vascular endothelial cells, about 10% of the cells were reacted with CD14 (macrophages/monocytes, Coulter Immunology) and a small percentage of the cells was also clearly positive

with B7-1 and B7-2(Fig. 1). Fibroblast-like synovial cells lack the expression of CD14, B7-1,and B7-2[16] and only macrophage-like synovial cells express B7-2,[17] suggesting that the synovial cell preparation used in our experiments was a mixture of fibroblast-like synovial cells and macrophage-like synovial cells. We next analysed the expression of other costimulatory molecules on the surface of synovial cells. As shown in Fig. 2, ICAM-1, VCAM-1 and LFA-3 were constitutively expressed on unstimulated synovial cells. HLA-DR was only dimly expressed on unstimulated synovial cells (Fig. 2). Previous studies from our laboratory indicated that stimulation of synovial cells with IL-1$\beta$ or IFN-$\gamma$ enhanced the adhesion of T cells to synovial cells.[15] Thus, we examined the effect of stimulation of synovial cells by various cytokines on the expression of costimulatory molecules. As shown in Fig. 2, stimulation of synovial cells with IFN-$\gamma$ or IL-1$\beta$, significantly increased the expression of ICAM-1 and VCAM-1 (mean fluorescence intensity of ICAM-1, both percentage of positive cells and the mean fluorescence intensity of VCAM-1) although neither the percentage of positive cells nor the mean fluorescence intensity of LFA-3-positive cells was unchanged by the treatment with either IFN-$\gamma$ or IL-1$\beta$. The expression of B7-1 and B7-2 was not changed by IFN-$\gamma$ or IL-1$\beta$ (data not shown). HLA-DR was markedly expressed after stimulation with IFN-$\gamma$ (Fig. 2). The effect of TPA on costimulatory molecule expression on synovial cells was similar to IL-1$\beta$ (data not shown).

Previous studies demonstrated that major histocompatibility complex (MHC) Class II and costimulatory molecule expression, including cytokine-induced ICAM-1 on the cells of the macrophage lineage, are inhibited by IL-10.[18–21] Therefore, in the next step, we examined the effect of IL-10 on costimulatory molecule and HLA-DR expression on synovial cells. Figure 3 shows the effect of IL-10 on IFN-$\gamma$-induced ICAM-1 expression on synovial cells. As shown in Fig. 3, IFN-$\gamma$-induced ICAM-1 expression on synovial cells was dose-dependently inhibited by IL-10. The maximum suppression was observed at a concentration of 10 ng/ml. VCAM-1 and HLA-DR expression induced by IFN-$\gamma$ and ICAM-1 and VCAM-1 expression induced by IL-1$\beta$ or TPA were also inhibited by IL-10 (Table 1). In addition, IL-10 diminished the basal expression of ICAM-1 and VCAM-1 (Table 1) although LFA-3, B7-1, and B7-2 expression of synovial cells was not influenced by IL-10 (data not shown).

### IL-10 inhibits cytokine production by synovial cells

Various cytokines, including IL-10, are present in high concentrations in the RA synovium and synovial fluid.[2–5,13] Furthermore, IL-10 inhibits cytokine synthesis by human cultured monocytes.[18] Thus, we next examined the effect of IL-10 on cytokine production by synovial cells. Table 2 shows the profile of cytokine production by synovial cells. Synovial cells spontaneously produced various kinds of cytokines and both IL-1$\beta$ and TPA caused a profound increase of production of all these cytokines. As expected, IL-10 markedly suppressed both spontaneous and enhanced production of these cytokines.

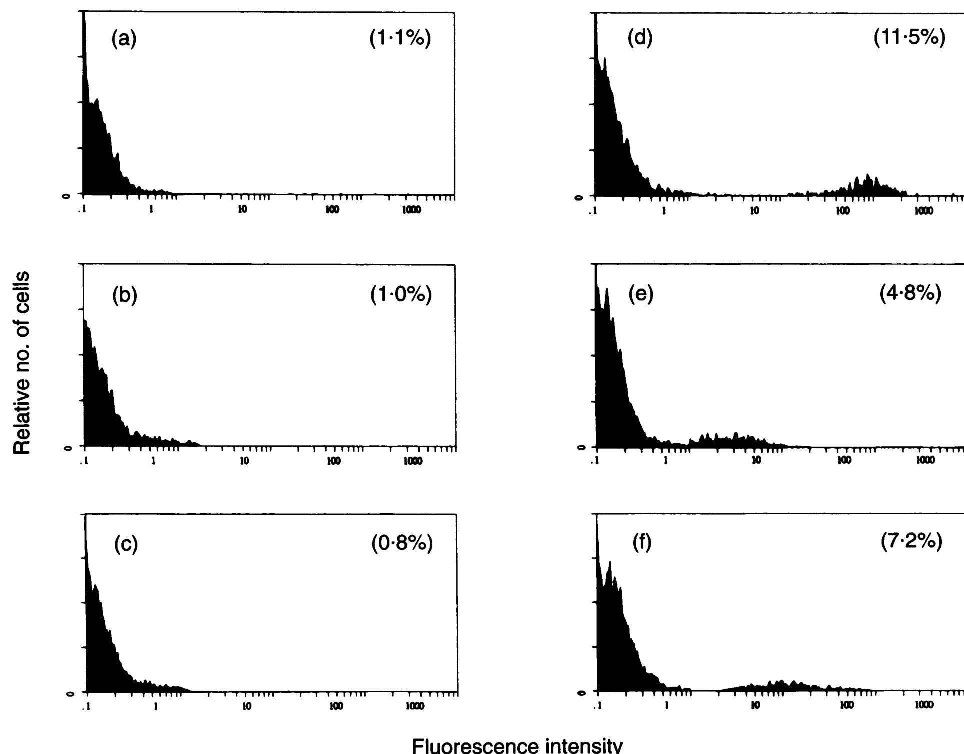


**Figure 1.** Flow cytometric analysis of synovial cells from patients with RA. Synovial cells from patients with RA were cultured in the absence of IL-10 and the expression of each molecule was examined as described in the text; (a) isotype-matched control, (b) CD3, (c) CD20, (d) CD14, (e) B7-1, (f) B7-2. Numbers in parenthesis represent the percentage of positive cells. Data represent an example of five experiments.

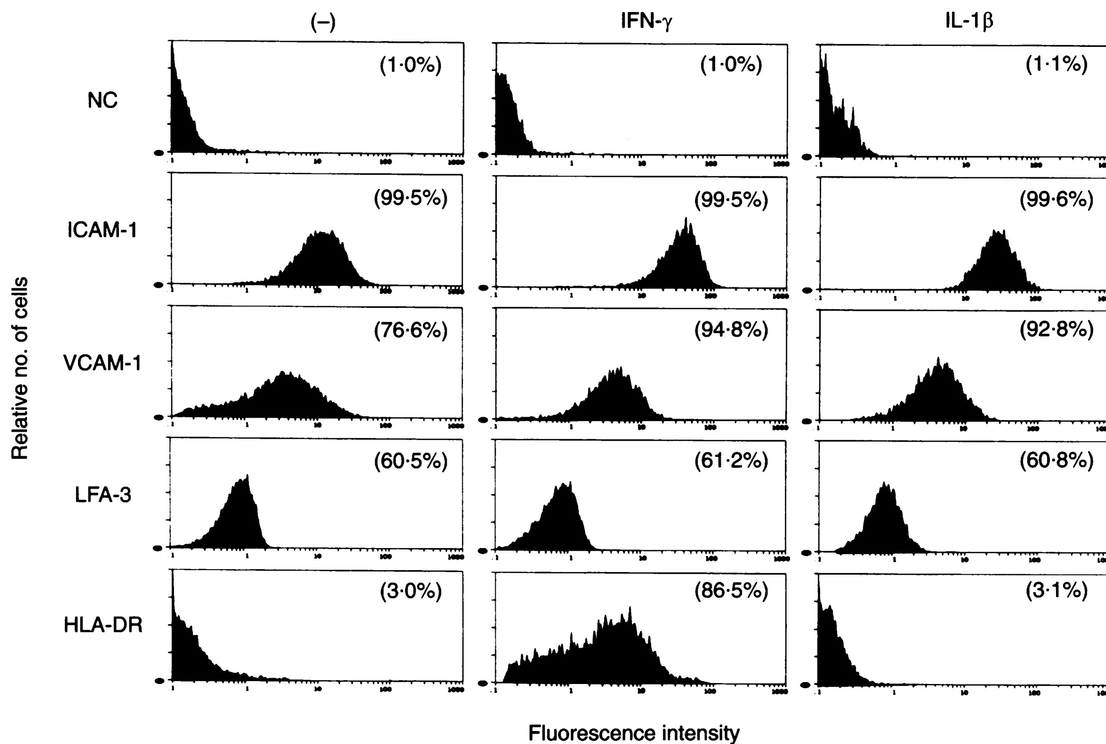


**Figure 2.** Costimulatory molecule and HLA-DR expression of synovial cells stimulated by IL-1$\beta$ or IFN-$\gamma$. Synovial cells from patients with RA were stimulated by rIL-1$\beta$ (10 IU/ml) or rIFN-$\gamma$ (500 IU/ml) for 48 hr. The expression of each molecule was determined as described in the text. Numbers in parenthesis represent the percentage of positive cells; (-), unstimulated synovial cells. IL-1$\beta$, rIL-1$\beta$-stimulated synovial cells; IFN-$\gamma$, rIFN-$\gamma$-stimulated synovial cells. Data represent an example of five experiments.

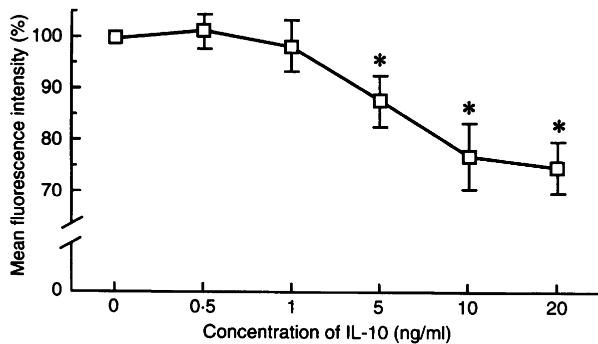


**Figure 3.** Inhibitory effect of IL-10 on IFN-$\gamma$-induced ICAM-1 expression on synovial cells. Synovial cells from patients with RA were cultured with rIFN-$\gamma$ (500 IU/ml) in the presence of various concentrations of rIL-10 for 48 hr and ICAM-1 expression on synovial cells was determined as described in the text. Mean fluorescence intensity of ICAM-1 expression on unstimulated synovial cells was calculated as 100%. Data shown are the mean ±SD of mean fluorescence intensity from five separate experiments. *, $P<0.01$ versus data in the absence of IL-10.

**Table 1.** Inhibitory effect of IL-10 on costimulatory molecules and HLA-DR expression of synovial cells

| Stimulus | IL10 | ICAM-I (%) | VCAM-I (%) | HLA-DR (%) |
|---|---|---|---|---|
| (−) | (−) | 100 | 100 | NT |
| (−) | (+) | 80·0±4·9* | 83·5±6·9* | NT |
| IFN-γ | (−) | 100 | 100 | 100 |
| IFN-γ | (+) | 78·5±10·0* | 77·5±5·9* | 81·0±5·0* |
| IL-1β | (−) | 100 | 100 | NT |
| IL-1β | (+) | 76·5±7·5* | 77·2±8·9* | NT |
| TPA | (−) | 100 | 100 | NT |
| TPA | (+) | 81·0±5·0* | 72·0±7·9* | NT |

Synovial cells from patients with RA were cultured with rIFN-$\gamma$ (500 IU/ml), rIL-1$\beta$ (10 IU/ml), or TPA (10 ng/ml) in the presence or absence of rIL-10 (10 ng/ml) for 48 h. The expression of each molecule was determined as described in the text. Mean fluorescence intensity of each molecule in the absence of IL-10 was calculated as 100%. Data shown are the mean ± SD of mean fluorescence intensity from five separate experiments. NT, not tested; *, $P<0.01$ versus control (mean fluorescence intensity in the absence of IL-10).

### Inhibitory effect of IL-10 on autologous T-cell proliferation by synovial cells in response to PPD

In the final set of experiments, we investigated the effect of IL-10 on the capacity of synovial cells for T-cell proliferation in response to PPD. Autologous T cells were cultured with IFN-$\gamma$-treated or untreated synovial cells in the presence or absence of PPD and T-cell proliferative response was measured as described in the text. As shown in Fig. 4(a), IFN-$\gamma$-treated synovial cells induced remarkable T-cell proliferation in response to PPD, suggesting that synovial cells act as stimulatory APC to T cells. We also tested the blocking capacity of antibodies specific to the costimulatory molecules and HLA-DR. The addition of anti-HLA-DR mAb significantly blocked T-cell proliferation toward synovial cells, indicating that the response was antigen-specific (Fig. 4b). In addition, anti-ICAM-1 mAb, anti-VCAM-1 mAb and anti-LFA-3 mAb

**Table 2.** Effect of IL-10 on cytokine production by synovial cells

| | | Cytokine production (pg/ml) | | | | |
|---|---|---|---|---|---|---|
| Stimuli | IL-10 | IL-1β | IL-6 | IL-8 | G-CSF | GM-CSF |
| Exp. 1 | | | | | | |
| (–) | (–) | 106 | 280 | 2055 | 285 | 25 |
| | (+) | 79 (25·5%) | 240 (14·3%) | 1802 (12·3%) | 211 (26·0%) | LT (>20%) |
| IL-1β | (–) | NT | 3055 | 19 050 | 1580 | 365 |
| | (+) | NT | 2226 (27·1%) | 13 508 (29·1%) | 1100 (30·4%) | 246 (32·6%) |
| TPA | (–) | 508 | 1890 | 14 920 | 1080 | 265 |
| | (+) | 410 (19·3%) | 1422 (24·8%) | 10 250 (31·3%) | 690 (36·1%) | 192 (27·6%) |
| Exp. 2 | | | | | | |
| (–) | (–) | 75 | 785 | 4059 | 925 | LT |
| | (+) | 62 (17·3%) | 525 (33·1%) | 3250 (19·9%) | 711 (23·1%) | LT |
| IL-1β | (–) | NT | 4955 | 20 052 | 9825 | 369 |
| | (+) | NT | 3822 (22·9%) | 13 990 (30·2%) | 6912 (30·0%) | 336 (8·9%) |
| TPA | (–) | 916 | 3252 | 17 226 | 5715 | LT |
| | (+) | 525 (42·7%) | 2142 (34·1%) | 11 922 (30·8%) | 3362 (41·2%) | LT |
| Exp. 3 | | | | | | |
| (–) | (–) | 71 | 492 | 7988 | 1009 | 42 |
| | (+) | 42 (40·8%) | 379 (23·0%) | 6725 (15·8%) | 812 (19·5%) | LT (>52·3%) |
| IL-1β | (–) | NT | 4100 | 27 226 | 13 226 | 192 |
| | (+) | NT | 2912 (29·0%) | 20 022 (26·5%) | 8125 (38·6%) | 102 (46·9%) |
| TPA | (–) | 142 | 3292 | 23 556 | 2022 | 28 |
| | (+) | 100 (29·6%) | 2420 (26·5%) | 16 525 (29·8%) | 1415 (30·0%) | LT (>28·6%) |

Synovial cells from patients with RA were cultured with rIL-1β (10 IU/ml), or TPA (10 ng/ml) in the presence or absence of rIL-10 (10 ng/ml) for 48 h and cytokines in the culture supernatant were detected as described in the text. Numbers in the parenthesis represent per cent inhibition compared with cytokine production in culture supernatant without rIL-10. NT, not tested; LT, lower than 20 pg/ml.

significantly inhibited T-cell proliferation (Fig. 4b). Only a low level of inhibition was observed with the addition of anti-B7-1 mAb and anti-B7-2 mAb caused a moderate suppressive effect for T-cell proliferation (Fig. 4b). Since IL-10 suppressed the IFN-γ-induced expression of HLA-DR, ICAM-1 and VCAM-1 on synovial cells, autologous T-cell proliferation was markedly inhibited by IL-10 (Fig. 4b).

## DISCUSSION

Various cytokines are detected in large concentrations in the synovial fluid and synovial membrane of RA patients.[2–5,13] In addition, previous results demonstrated the expression of several costimulatory molecules in the RA synovium.[22–24] Furthermore, IL-β and IFN-γ stimulate synovial cells *in vitro* and augment the adhesion of T cells to those cells,[15] indicating that the expression of costimulatory molecule and its regulation by cytokines play an important role in the pathophysiology of RA.

Recently, compensatory anti-inflammatory substances such as IL-ra and IL-10 have also been detected in RA synovium.[6,7,13] IL-10 is reported to act on cells of the macrophage lineage, such as macrophages, microglial cells, astrocytes and epidermal Langerhans' cells and inhibit their MHC class II and costimulatory molecule expression including B7 and ICAM-1.[18–21] IL-10 also suppresses the production of various cytokines by these cells,[18,20] and IL-6 production by synovium monocytes in RA patients.[25] These data suggest that IL-10 has anti-inflammatory properties on the RA synovium.

In the first step, we examined the effect of IL-10 on costimulatory molecule and HLA-DR expression on synovial cells. Our results demonstrated that synovial cells *in vitro* constitutively expressed ICAM-1, VCAM-1 and LFA-3 and that the expression of ICAM-1 and VCAM-1 was significantly augmented by IL-1β, IFN-γ, or TPA. A small percentage of synovial cells was positive with B7-1 and B7-2, although their expression was not changed by the addition of IL-1β, IFN-γ, or TPA. The regulation of B7-1 and B7-2 expression on synovial cells may be different from that on mononuclear cells because B7-1 and B7-2 expression on T cells, B cells and monocytes was clearly augmented after cellular activation.[26] IL-10 inhibited both basal and cytokine-induced costimulatory molecule expression in a dose-dependent fashion, with the maximum suppression observed at a dose close to 10 ng/ml. Since the concentration of IL-10 in the RA synovial fluid ranges between 1 and 5 ng/ml,[14] the concentration used in the present experiment can be achieved *in vivo*. HLA-DR expression was markedly induced in synovial cells stimulated by IFN-γ and was also inhibited by IL-10.

Previous reports revealed that IL-10 suppressed cytokine production of macrophages and astrocytes[18,20] and that synovial cells also produced several types of cytokines.[2,3] Therefore, we next examined the effect of IL-10 on cytokine production by synovial cells. Our results demonstrated that IL-1β, IL-6, IL-8, G-CSF and GM-CSF were produced spontaneously by unstimulated synovial cells and that stimulation of synovial cells by IL-1β or TPA significantly enhanced the production of these cytokines. As IL-10 inhibited costimulatory molecule and HLA-DR expression of synovial cells, production of these cytokines by synovial cells was suppressed by the addition of IL-10. These results confirm those of

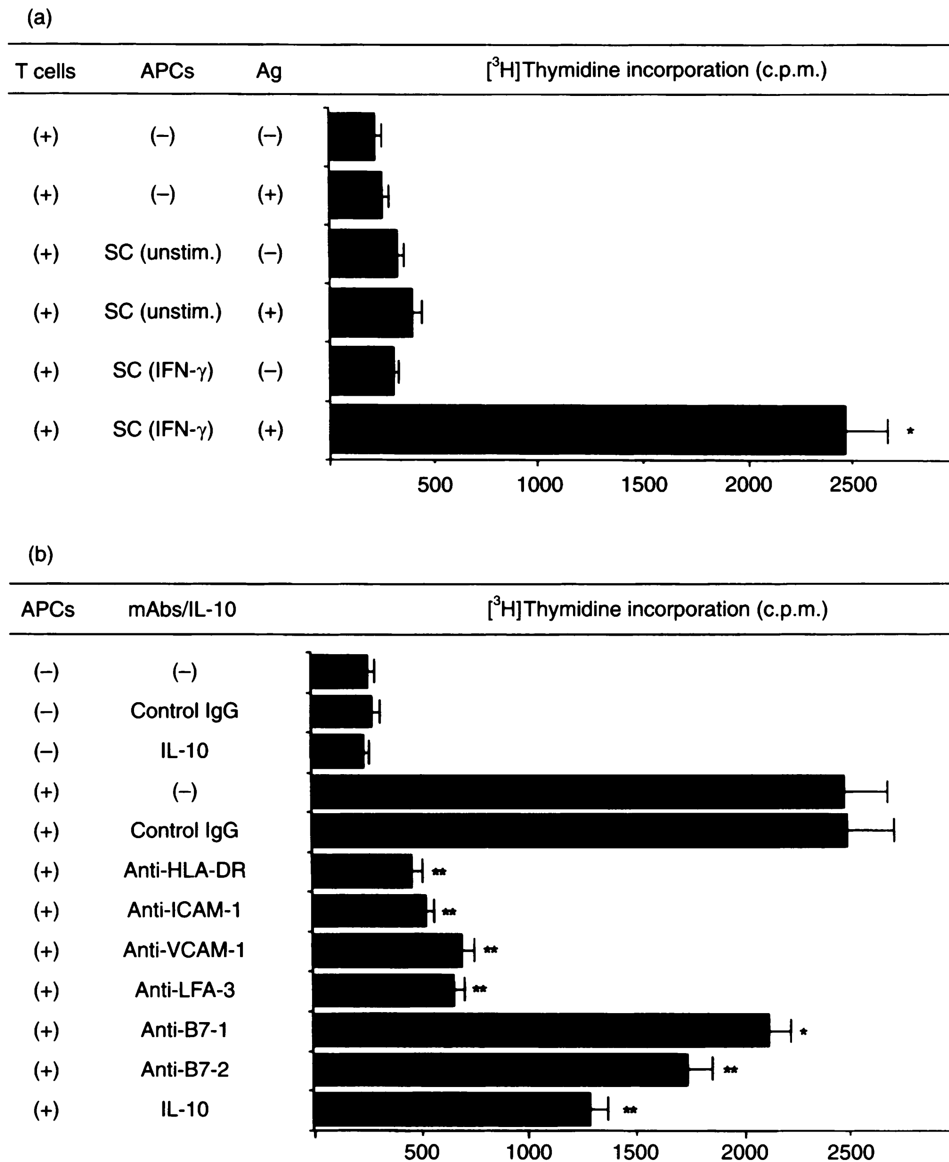


**Figure 4.** Autologous T-cell proliferation against synovial cells in response to PPD. Results shown are the representative data of mean ± SD from four performed. (a) Autologous T cells were cultured with synovial cells (APC) in the presence or absence of PPD (Ag). Newly synthesized DNA of T cells was determined as described in the text. SC(unsti), unstimulated synovial cells; SC(IFN-γ), synovial cells stimulated by rIFN-γ (500 IU/ml) for 48 hr. *, P0·01 versus control (T-cell proliferation without APC or antigen). (b) Autologous T cells were cultured with IFN-γ-stimulated, PPD-pulsed synovial cells (APC) in the presence or absence of various mAb. Autologous T-cell proliferation was also stimulated with PPD-pulsed synovial cells (APC) cultured with rIFN-γ and rIL-10 (10 ng/ml). Newly synthesized DNA of T cells was determined as described in the text. *, $P<0·05$, ** $P<0·01$ versus control (T-cell proliferation induced by IFN-γ-stimulated synovial cells without mAb or IL-10).

Katsikis *et al.*[14] demonstrating the ability of IL-10 to suppress cytokine production by cultured synovial membrane.

Recent studies revealed that interference with either CD28/B7-1, B70,[26–28] CD2/LFA-3, LFA-1/ICAM-1, or VLA-4/VCAM-1[28,29] results in T-cell inactivation toward APC, suggesting that the regulation of the expression of costimulatory molecules on APC is critical in activating T cells. Thus, in the final set of experiments we examined the effect of IL-10 on APC function of synovial cells. We showed in the present study that synovial cells could function as stimulatory APC to T cells. The results of experiments using blocking agents suggested that ICAM-1, VCAM-1 and LFA-3 on synovial cells are the most important costimulatory molecules for T-cell activation. Since IL-10 suppressed IFN-γ-induced ICAM-1, VCAM-1 and HLA-DR expression on synovial cells, this observation indicates that IL-10 inhibits the APC function of synovial cells for T-cell activation.

How does IL-10 suppress the expression of these molecules and production of cytokine by synovial cells? Our results showed a clear inhibitory effect of IL-10 for costimulatory molecule expression and cytokine production of synovial cells. The synovial cell preparations used in our experiments were a

mixture of fibroblast-like synovial cells and macrophage-like synovial cells and previous reports demonstrate that fibroblast-like synovial cells do not respond to IL-10,[14,30] suggesting that IL-10 acts on macrophage-like synovial cells or the cellular interaction between macrophage-like synovial cells and fibroblast-like synovial cells. A recent report showing that IL-10 suppresses cytokine production by freshly isolated synovial cells[31] also supports our results. Signal transduction of IFN-$\gamma$ occurs through both JAK-STAT family (JAK1, JAK2-Stat 1$\alpha$, Stat 1$\beta$) and mitogen-activated kinase (MAP kinase).[32,33] Furthermore, signal transduction of IL-1 occurs through p21 and MAP kinase,[34] while TPA provides a signal to MAP kinase through protein kinase C (PKC).[35] Therefore, although signal transduction by IL-10 receptor is not clear at present, it is possible that IL-10 inhibits common signal pathways of these stimuli, i.e. MAP-kinase activity or early oncogene transcriptions.

In conclusion, our study demonstrated that IL-10 inhibits the expression of different costimulatory molecules and HLA-DR, cytokine production, and APC function of synovial cells. These findings suggest that IL-10 has an important immunoregulatory function in the cytokine network of synovial membrane in patients with RA.

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