Expression and Function of the Co-Stimulator H4/ICOS on Activated T Cells of Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To investigate the expression and function of the inducible co-stimulator H4/ICOS in rheumatoid arthritis (RA) patients. H4/ICOS is the newest member of the CD28/CTLA-4 family to have been found to be expressed on activated T cells, and it participates in a variety of important immunoregulatory functions.

Methods. The levels of H4/ICOS expression on T cells among peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC) from 28 patients with RA were analyzed by flow cytometry. To explore the role of H4/ICOS function in the inflammation of rheumatoid joints, lymphokine production by SF CD4+ T cells co-stimulated by H4/ICOS was assayed. Expression of H4/ICOS ligand (B7RP-1) mRNA in synovial tissues from patients with RA was examined by reverse transcription polymerase chain reaction (RT-PCR).

Results. H4/ICOS-positive cells were increased significantly in whole, CD4+, and CD8+ T-cell fractions of SFMC compared with control PBMC. Comparison between control PB and PB from patients with active RA showed that H4/ICOS-positive whole and CD8+ T-cell fractions were increased significantly in the PB of RA patients. H4/ICOS costimulation clearly increased interferon-γ, interleukin 4 (IL-4), and IL-10 production by SF CD4+ T cells. By RT-PCR, RA synovial tissue was shown to express mRNA of B7RP-1.

Conclusion. Our results suggest that local immune responses may be modulated by H4/ICOS expressed on T cells in the joints of patients with RA, and thus H4/ICOS may be involved in the pathogenetic mechanism of RA. (J Rheumatol 2003;30:1157–63)

Key Indexing Terms:
T LYMPHOCYTES CO-STIMULATORY MOLECULE RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease and is classified as an autoimmune disorder. The observation that specific class II MHC polymorphisms confer a risk of RA has suggested that T cells have a pivotal role in the development of RA[1,2], and aggregation of CD4+ cells has been observed around synovial capillaries during the very early stage of RA[3]. T cells isolated from the synovial tissue and synovial fluid (SF) of RA patients express CD45RO surface protein isoforms characteristic of terminally differentiated memory T cells[4]. Monocyte-derived pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1), have been reported to play an important role in the development of the abnormal changes seen in RA patients[5-7], and production of these cytokines is regulated by 2 functional subsets, type 1 helper T (Th1) cells and type 2 helper T (Th2) cells[8]. Th1 cells are thought to promote joint destruction by activating TNF-α and IL-1-producing monocytes, whereas Th2 cells have a protective function by inhibiting production of these cytokines[9]. It has been reported that a molecule designated H4 co-stimulates murine T cells in physical association with T cell receptors (TCR)[10], is highly expressed on a newly discovered Vα14+ T cell subpopulation of murine thymocytes[11], and is expressed on activated human peripheral blood (PB) T cells[12,13]. H4 has recently been shown to be identical to a newly identified human CD28-related co-stimulator, inducible co-stimulator (ICOS), which is induced after activation and regulates the immune response[14], since monoclonal anti-H4 antibody reacts with cells transfected with ICOS cDNA[15]. Accumulating evidence has indicated that H4/ICOS is an important determinant of the develop-

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Supported in part by grants from the Ministry of Education, Science, Sports and Culture of Japan, and the Ministry of Public Welfare of Japan.

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Submitted February 26, 2002; revision accepted December 19, 2002.

ment of Th cells and antibody class switching[16-19], delivering specific signals, as shown in our recent report[20]. However, little work has been done on the expression and role of H4/ICOS in the pathogenesis of RA.

We examined the expression of the H4/ICOS molecule on the SF and PB T cells of patients with RA and its co-stimulatory activity on *in vitro* cytokine production. In addition, we discuss the possible role of H4/ICOS in the pathogenesis of RA.

MATERIALS AND METHODS

Patient samples. After obtaining informed consent, samples of SF and PB were obtained from 28 patients with active RA (11 men and 17 women, age 33 to 88 years) at the Institute of Rheumatology and Department of Oral and Maxillofacial Surgery of Tokyo Women's Medical University. Patients satisfied the 1987 criteria for RA of the American College of Rheumatology (ACR, formerly, the American Rheumatism Association)[21]. Active RA was diagnosed according to the criteria for remission of the American College of Rheumatology[22]. Osteoarthritis (OA) (n = 9) was also diagnosed according to ACR criteria[23]. SF samples were collected into heparinized tubes by needle aspiration of inflamed knee joints. Synovial tissues of inflamed joints in RA patients were obtained immediately after surgery. PB samples from 21 healthy volunteers (11 men and 10 women, age 22 to 58 years) were used as controls.

Reagents and culture medium. A bacterial superantigen, toxic shock syndrome toxin-1 (TSST-1), was obtained from Toxin Tec. Inc. (Sarasota, FL, USA). Human recombinant IL-2 (rIL-2) was provided by Shionogi Co. (Osaka, Japan). The RPMI 1640 culture medium used contained 100 µg of streptomycin/ml, 100 U of penicillin/ml, 10% fetal calf serum, and 5×10^{-5} M 2-ME.

Monoclonal antibodies (Mab). The C398.4A Mab specific for H4/ICOS was produced in Armenian hamsters by immunization with the murine T cell clone D10.G4.1, as described[10] (kindly provided by Dr. U. Dianzoni, Avogadro University, Novara, Italy). A Mab to CD28 (KOLT-2) was a generous gift from Dr. K. Sagawa (Kurume University, Kurume, Japan). A hybridoma cell line producing a Mab specific for CD3 (OKT3) was purchased from American Type Culture Collection (Rockville, MD, USA). Mab specific for HLA-DR/DP (I2C3) and CD8 (Nu Ts/c) have been described[24]. Goat anti-hamster IgG (anti-HIgG) was obtained from ICN Pharmaceuticals (Aurora, OH, USA). The following conjugated Mab were used: FITC-conjugated-anti-CD4 (SK3), FITC-conjugated-anti-CD8 (SK1), FITC-conjugated-anti-CD3 (UCHT1) and PE-conjugated-anti-CD3 (UCHT1) (Coulter Corporation, Miami, FL, USA), PE-conjugated-anti-CD45RO (UCHL-1), and PE-streptavidin (Becton Dickinson, San Jose, CA, USA), PE-conjugated-anti-HLA-DR (I2) (Coulter Immunology, Hialeah, FL), PE-conjugated-anti-CD25 (B1.499.9) (Immunotech S.A., Cedex, France). Anti-H4/ICOS Mab was purified from culture supernatant by protein G-sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden), and was conjugated with biotin in our laboratory. Anti-CD3 Mab were used in the form of dialyzed ammonium sulfate precipitates of ascitic fluid.

Cells. PB mononuclear cells (PBMC) and SF mononuclear cells (SFMC) were isolated by Ficoll-Conray density gradient centrifugation[25]. Whole T cell preparations were obtained by the sheep red blood cell (SRBC) rosette method[26]. Briefly, PBMC or SFMC in 10 ml RPMI 1640 culture medium were mixed with 100 × their cell number of S-2-aminoethyl isothiouronium-treated SRBC. The mixtures were centrifuged at 1500 rpm for 10 min at 4˚C, kept on ice for 1 h, gently resuspended, and fractionated into SRBC-rosetted and nonrosetted cells by Ficoll-Conray density centrifugation. To obtain CD4+ T cell preparations, whole T cell fractions were treated with anti-HLA-DR/DP and anti-CD8 Mab, washed, and mixed with anti-mouse IgG antibody-coupled magnetic beads (Dynabeads, Dynal, Oslo, Norway). After placing the mixtures on ice for 20 min, Mab-coated cells were depleted with a magnet (CD4+ T cells, > 95%). Human T leukemia cell line Jurkat cells were provided from Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan. Human B cell lymphoma cell line KM3 was a kind gift from Dr K. Oshimi, Juntendo University, Tokyo, Japan.

Preparation of TSST-1-induced CD4+ T-cell blasts. Superantigen TSST-1-induced T cell blasts were obtained as described[27,28]. Briefly, PB CD4+ T cells were stimulated for 3 days with 10 ng of TSST-1/ml in the presence of DR+ L cells (8124) as accessory cells (AC). Recovered cells were separated by Percoll (density: 1.068) centrifugation. The large lymphoblasts at the interface between the culture medium and Percoll were expanded by incubation with 100 units of rIL-2/ml for 2 days in 2 cycles. Preparations of TSST-1-induced CD4+ T cell blasts expressed TCR Vß2 and Vß4[29], and H4/ICOS on a expression level comparable to CD28 (data not shown). AC were irradiated with 3000 rad with an x-ray source and treated with 50 µg/ml of mitomycin C for 30 min at 37˚C before used as AC.

Assay for co-stimulatory activity of H4/ICOS on lymphokine production. To assay lymphokine production, 2.5×10^5 TSST-1-induced control PB CD4+ T cell blasts or SF CD4+ T cells were stimulated in 48-well culture plates (Becton Dickinson) coated with antibody, as follows. The plate was first coated with anti-HIgG (10 µg/ml) for 1 h, bound with a fixed amount (3 µg/ml) of anti-CD3 Mab at 37˚C for 1 h, and then with a fixed amount (3 µg/ml) of anti-CD28, anti-H4/ICOS, or control HIgG at 37˚C for 1 h. Culture supernatants were examined for IL-2 activity using IL-2-dependent CTLL-2 cells, as reported[30]. Data are presented as units of IL-2/ml. IL-4, IL-10, and interferon-γ activity in the culture supernatants was determined with sandwich enzyme-linked immunosorbent assay according to the manufacturer's instructions (BD PharMingen, San Diego, CA, USA). Data are presented as pg of IL-4 and IL-10/ml or ng of interferon-γ (IFN-γ)/ml. The detection level of IL-2, IL-4, IL-10, and IFN-γ, was 0.1 units/ml, 10 pg/ml, 10 pg/ml and 50 pg/ml, respectively.

Flow cytometric analysis. To detect expression of H4/ICOS and various activation molecules on PBMC and SFMC, cells were stained with several combinations of the appropriate PE- and FITC-conjugated Mab and examined by 2-color flow cytometric analysis. Samples of 1×10^4 viable cells were analyzed with an EPICS XL flow cytometer (Coulter).

Reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted from RA synovial tissues, KM3 cells and Jarkat cells with Isogen (Nippon Gene, Tokyo, Japan). One microgram of heat denatured total RNA was reverse transcribed into cDNA at 42˚C for 2 h using RAV-2 reverse transcriptase (Takara Biomedicals, Osaka, Japan) and random hexamer primers (Takara Biomedicals) with oligo(dT)-latex (Nippon Roche, Tokyo, Japan). cDNA (0.5 µl) was used as PCR templates and amplified by Taq DNA polymerase (Takara Biomedicals). PCR primers used were as follows: hB7RP-1 forward 5' -CTCACCTTCACGTGTA-CATC-3'; hB7RP-1 reverse 5'-CAAACGTGGCCAGTGAGCTC-3'; ß-actin forward 5'-TGAAGCTGTGCTATGTTGCT-3'; ß-actin reverse 5'-TCAGTAACAGTCCGCCTAGA-3'. PCR amplification using hB7RP-1 primers was performed for 35 cycles of 94˚C for 30 s, 62˚C for 30 s, and 72˚C for 30 s, and ß-actin PCR was performed for 30 cycles of 94˚C for 30 s, 50˚C for 30 s, and 72˚C for 30 s. PCR products were separated by electrophoresis on a 2.5% agarose gel with ethidium bromide and visualized by UV light. The length of PCR products of hB7RP-1 and ß-actin cDNA was 514 and 503 bp, respectively.

Statistical analysis. To account for the significant skew in levels of expression of cell surface molecules, the nonparametric Mann-Whitney's U test was employed to analyze differences between healthy PBMC, active RA PBMC, and RA SFMC groups.

RESULTS

Expression of various surface molecules on T cells from patients with active RA. We first analyzed the expression of H4/ICOS and several other molecules, i.e., CD45RO, HLA-

DR, and CD25 in the whole, CD4+, and CD8+ T cell fractions from PB and SF of patients with active RA. In SF, there was an increased proportion of H4/ICOS+ T cells in all 3 T cell fractions (61.9 ± 3.4% in the whole T cell fraction, 82.9 ± 2.1% in the CD4+ T cell fraction, and 30.6 ± 4.2% in the CD8+ T cell fraction), as compared with the control PB T cells (Table 1). Expression of H4/ICOS in SF ranged from 35.4% to 92.7% in the whole T cell fraction, from 62.8% to 96.7% in the CD4+ T cell fraction and from 0.0% to 62.5% in the CD8+ T cell fraction (Figure 1). There were also increases in the proportions of CD45RO+, HLA-DR+, and CD25+ T cells in the whole and CD4+ T cell fractions, and of CD45RO+ and HLA-DR+ T cells in the CD8+ T cell fraction. In PB, H4/ICOS+ T cells were increased in the whole T cell fraction (21.5 ± 3.8%) and the CD8+ T cell fraction (11.8 ± 2.7%). There were also increases in the proportions of HLA-DR+ cells and CD25+ cells in several combinations (HLA-DR+ T cells among CD8+ PB T cells, CD25+ T cells among whole PB T cell fraction, and CD8+ PB T cells). It is noteworthy that markedly higher levels of these molecules, except CD25, were expressed in the SF T cells than in the PB T cells (Table 1). The proportions of H4/ICOS+ T cells in the whole, CD4+, and CD8+ T cell fractions in paired PB and SF from individual RA patients

Table 1. Expression of various surface markers on whole, CD4+, and CD8+ T cell fractions from the synovial fluid and peripheral blood of RA patients and controls. Cells were stained with several combinations of appropriate PE- and FITC-conjugated mab and examined for the expression of H4/ICOS, CD45RO, HLA-DR, and CD25 by 2 color flow cytometric analysis.

	Percentages of Positive T cells[a]				
	H4/ICOS	CD45RO	HLA-DR	CD25	CD4/CD8 [c]
Whole T-cell fraction					
Control PBMC (n = 21)	14.2 ± 1.9[b]	31.2 ± 2.4	12.0 ± 1.1	7.6 ± 0.7	57.9 ± 5.0/47.8 ± 4.2
RA PBMC (n = 15)	21.5 ± 3.8**	35.6 ± 2.8	13.7 ± 2.1	14.3 ± 2.2**	62.6 ± 4.9/37.3 ± 4.6
RA SFMC (n = 22)	61.9 ± 3.4*	82.1 ± 2.3*	72.5 ± 4.5*	12.5 ± 1.4**	50.8 ± 3.6/46.6 ± 3.5
$CD4^+$ T cell					
Control PBMC (n = 21)	37.5 ± 3.0	42.8 ± 2.6	10.6 ± 1.0	20.4 ± 1.5	
RA PBMC (n = 15)	44.7 ± 4.1	51.0 ± 4.8	10.5 ± 1.6	24.7 ± 3.1	
RA SFMC (n = 22)	82.9 ± 2.1*	94.8 ± 1.2*	74.3 ± 4.6*	30.0 ± 2.6**	
$CD8^+$ T cells					
Control PBMC (n = 20)	5.3 ± 1.0	17.9 ± 2.0	13.8 ± 1.4	1.4 ± 0.3	
RA PBMC (n = 15)	11.8 ± 2.7**	18.3 ± 2.2	23.3 ± 3.1**	5.3 ± 1.4*	
RA SFMC (n = 21)	30.6 ± 4.2*	59.8 ± 3.0*	76.6 ± 5.7*	3.3 ± 1.6	

(a) Percentages of positive T cells in the whole and CD4+ and CD8+ T cell fractions. (b) mean ± SEM. (c) Percentages of CD4+ and CD8+ T cells in whole CD3+ T cells. ** $p < 0.05$, * $p < 0.01$, when compared with control PB cells by Mann-Whitney's U test.

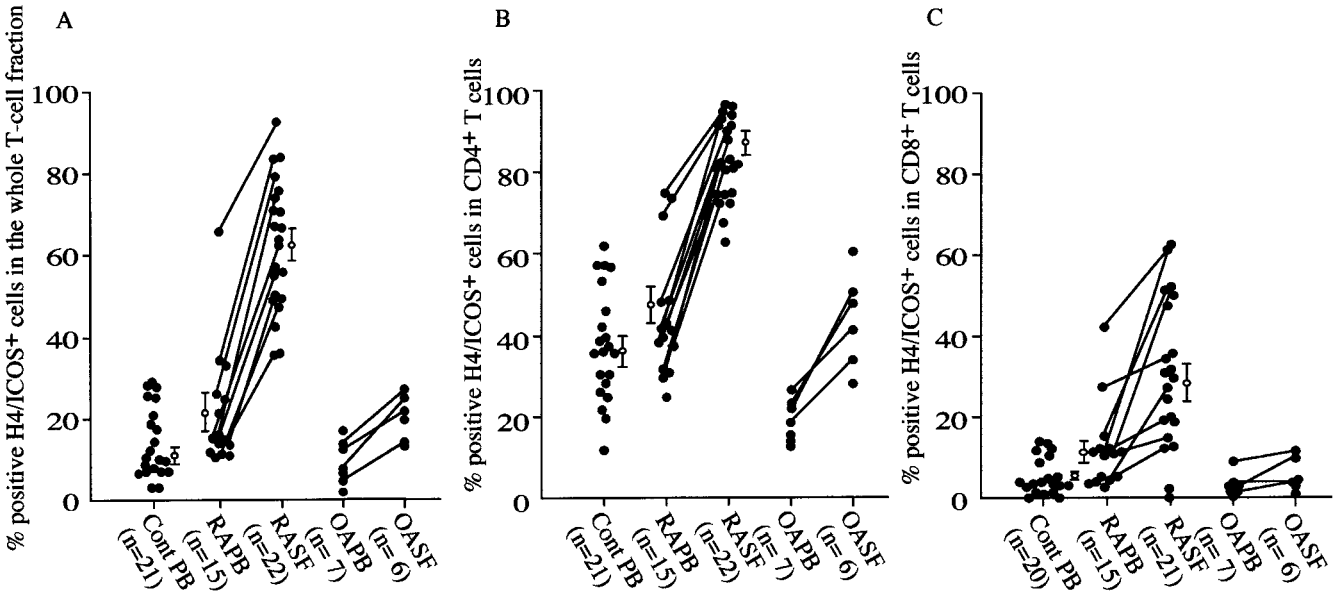


Figure 1. Percentage distribution of H4/ICOS+ cells in the whole, CD4+, and CD8+ T-cell fractions from control PBMC, active RA PBMC and SFMC, and active OA PBMC and SFMC. Cells were stained with appropriate combinations of biotin-conjugated anti-H4/ICOS, avidin-PE, and FITC-conjugated Mab, and 1×10^4 viable cells were analyzed by an Epics XL flow cytometer. Lines connect the values for the PB and SF whole, CD4+, and CD8+ T cell fractions obtained from the same RA patients (n = 9) or OA patients (n = 4).

are connected by lines in Figure 1. The percentage of H4/ICOS+ T cells was quite high in SF in all cases studied, irrespective of the fraction. High levels of expression of H4/ICOS were also seen in the PB T cells of several patients. Representative results of H4/ICOS expression in the whole, CD4+ and CD8+ T cell fractions in control PB T cells and SF T cells are shown in Figure 2. We did not find any significant difference in the percentages of H4/ICOS+ T cells between control PB and inactive RA PB (data not shown). For comparison with another inflammatory joint disease, we analyzed the samples from patients with OA. Proportions of H4/ICOS+ T cells in whole, CD4+, and CD8+ T cell fractions from the PB and SF of patients with active OA were lower than those in corresponding T cell fractions from patients with active RA, although the proportions of H4/ICOS+ T cells in whole and CD4+ T cell fractions were higher in OA SF than in OA PB (Figure 1). These results showed that substantial percentages of the whole, CD4+, and CD8+ T cell fractions in the SF of patients with active RA expressed the H4/ICOS molecule.

Co-stimulatory activity of H4/ICOS on lymphokine production by CD4+ T cells from healthy individuals. Before addressing the question of whether and how H4/ICOS functions in the activation of T cells in RA patients, it is necessary to know the function of H4/ICOS in the activation of normal T cells. ICOS has been found to markedly co-stimulate resting human CD4+ T cells from healthy donors to produce IL-10, but not IL-2[14]. Lymphokine production by resting PB T cells was only marginal in response to costimulation by H4/ICOS in the present study (data not shown), and we think that was because H4/ICOS expression on CD4+ T cells was induced 24–48 h after activation to a comparable level as CD28. We therefore prepared TSST-1-induced CD4+ T cell blasts from the PB T cells of healthy donors, which expressed H4/ICOS at a level comparable to CD28, and examined the ability of H4/ICOS to co-stimulate these T cells. 2.5×10^5 TSST-1-induced CD4+ T cell blasts were stimulated in 48-well culture plates coated with suboptimal doses of anti-CD3 Mab in combination with optimal doses of anti-H4/ICOS Mab, anti-CD28 Mab, or control HIgG, and culture supernatants were assayed for IFN-γ, IL-2, IL-4, and IL-10. Substantial concentrations of these lymphokines were produced in the presence of H4/ICOS or CD28, but not in their absence, indicating effective co-stimulatory activity of H4/ICOS on production of these lymphokines. Peak concentrations of the lymphokines IL-2 and IL-4 were observed 8 h after stimulation, and of IFN-γ and IL-10 at 48 h (Figure 3). H4/ICOS-mediated co-stimulation induced lower production of IL-2 and IL-10 than that induced by CD28 (Figures 3b and d). In contrast, the co-stimulatory effect on IL-4 production was higher in the former than in the latter (Figure 3c). The effect of H4/ICOS-induced co-stimulation on IFN-γ production was comparable to that of CD28-induced co-stimulation (Figure 3a).

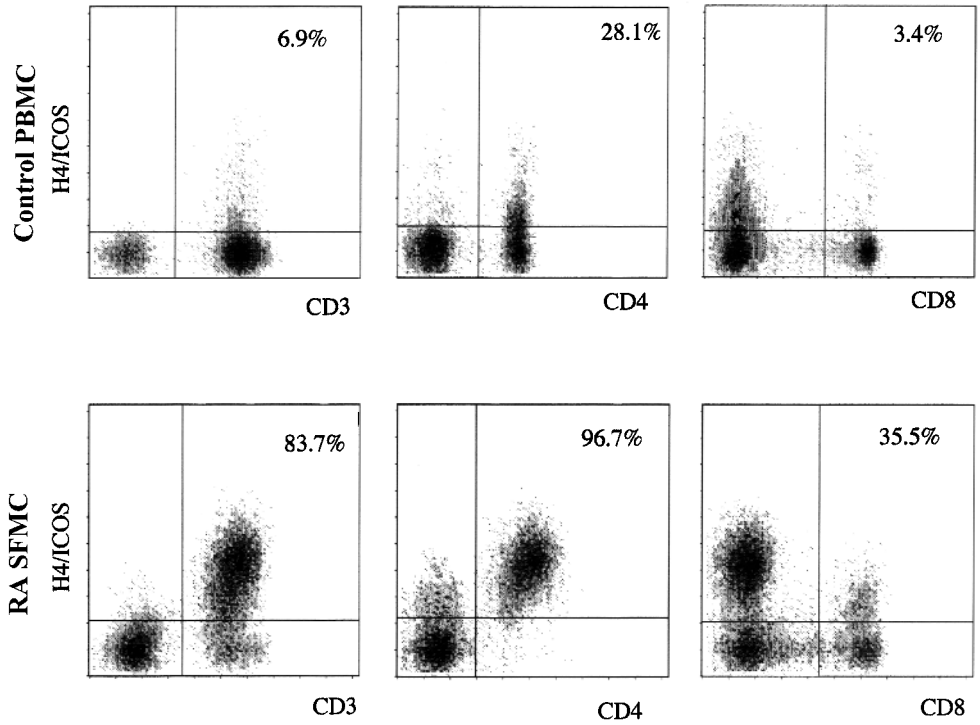
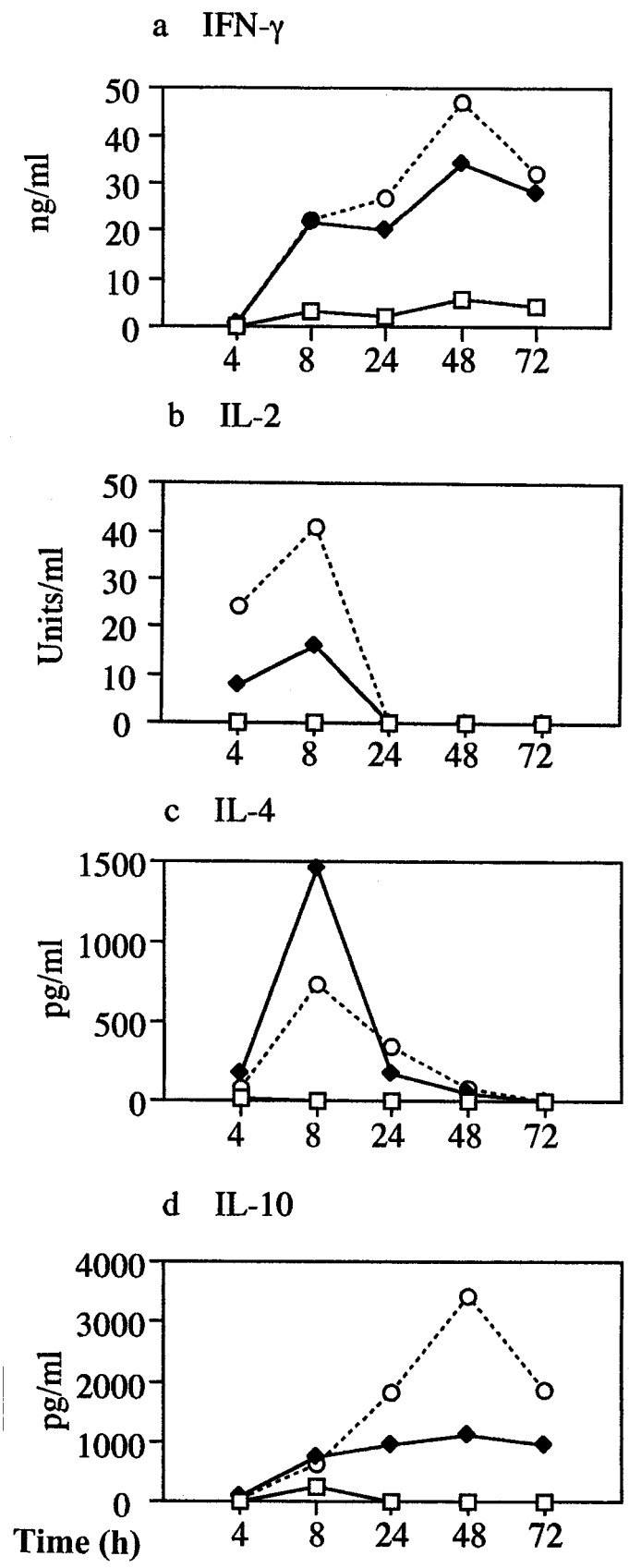


Figure 2. Representative 2-color flow cytometry for CD3 and H4/ICOS expression, CD4 and H4/ICOS expression, and CD8 and H4/ICOS expression in control PBMC and RA SFMC. Cells were stained and analyzed as in Figure 1. The percentage of H4/ICOS-positive cells in the whole, CD4+ and CD8+ T cell fractions are shown.



Similar results of H4/ICOS and CD28 co-stimulation on lymphokine production were observed in repeated experiments. Thus, the results indicated that signaling through H4/ICOS co-stimulates production of various lymphokines by both Th1 and Th2 cells, but that the levels of the co-stimulatory effects differ among the lymphokines.

Co-stimulatory activity of H4/ICOS on the SF CD4+ T cells of patients with active RA. To explore the role of H4/ICOS in the development of the inflammation in RA, SF CD4+ T cells of RA patients were stimulated with suboptimal doses of anti-CD3 Mab in combination with optimal doses of anti-H4/ICOS Mab, anti-CD28 Mab, or control HIgG, and examined for production of IFN-γ, IL-2, IL-4, and IL-10. The range in the percentage of SF CD4+ T cells used in the present study that expressed H4/ICOS was 58.2% to 89.5%. Co-stimulation with H4/ICOS or CD28 enhanced production of IFN-γ, IL-4, and IL-10 (Table 2). Co-stimulatory effect of H4/ICOS on lymphokine production was comparable or a little lower compared to that of CD28. It is noteworthy that the amounts of the 4 lymphokines produced by SF CD4+ T cells in the presence of H4/ICOS or CD28 co-stimulation were quite low compared with the levels produced by fresh preparations of TSST-1-induced T cell blasts (Figure 3, Table 2). Culture of OA SF CD4+ T cells with a combination of anti-CD3 and anti-H4/ICOS did not show any co-stimulatory effect of H4/ICOS on IFN-γ, IL-2, IL-4, and IL-10 production (data not shown).

H4/ICOS ligand expression in the inflamed joints of RA patients. To investigate whether H4/ICOS ligand B7RP-1 mRNA is expressed in the synovial tissues of patients with RA, RT-PCR analysis was performed. A positive band of DNA corresponding to B7RP-1 mRNA was observed at 514 bp in synovial tissues from RA patients and human B cell leukemia cell line KM3 cells, but not in human T cell leukemia cell line Jurkat cells (Figure 4), suggesting that triggering of H4/ICOS+ SF T cells by H4/ICOS ligand expressed in synovial tissue may play a role in RA pathogenesis.

DISCUSSION

In our study SF T cells from patients with RA exhibited markedly higher levels of expression of H4/ICOS than control PB T cells and OA SF T cells. We also observed that H4/ICOS co-stimulation enhanced the production of IFN-γ, IL-4, and IL-10 by SF CD4+ T cells, although the amounts of these lymphokines produced were quite low compared

Figure 3. Co-stimulatory effect of H4/ICOS on lymphokine production by activated control PB CD4+ T cells and its kinetics. TSST-1-induced CD4+ T cell blasts were obtained from the CD4+ T cells of healthy donors as described in Materials and Methods. Data shown are the results of an experiment that was representative of three independent experiments yielding similar results. Symbols: square: HIgG; diamond: anti-H4/ICOS; circle: anti-CD28.

Table 2. Amounts of IFN-γ, IL-2, IL-4, and IL-10 produced by the synovial fluid CD4+ T cells of RA patients. SF CD4+ T cells were stimulated with anti-CD3 mab in combination with anti-H4/ICOS, anti-CD28 mab, or control HIgG Abs as shown in Figure 3. Culture supernatants were collected at 8 h for IL-2 and IL-4 and at 48 h for IFN-γ and IL-10. Expression of H4/ICOS+ CD4+ T cells in patient SF CD4+ T cells were detected as Figure 1.

Patient		Anti-H4/ICOS				Anti-CD28				HIgG			
	SF H4/ICOS$^+$ CD4$^+$ T cells (%)	IFN-γ (ng/ml)	IL-2 (units/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)	IFN-γ (ng/ml)	IL-2 (units/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)	IFN-γ (ng/ml)	IL-2 (units/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
1	58.2	3.0	2.2	15	560	2.9	2.4	28	910	1.0	1.8	< 10*	70
2	63.0	2.4	1.8	15	105	2.1	2.7	22	220	0.9	1.8	< 10*	64
3	89.5	3.6	1.8	110	205	6.6	2.9	105	385	2.8	1.7	< 10*	100

* Below the detection level.

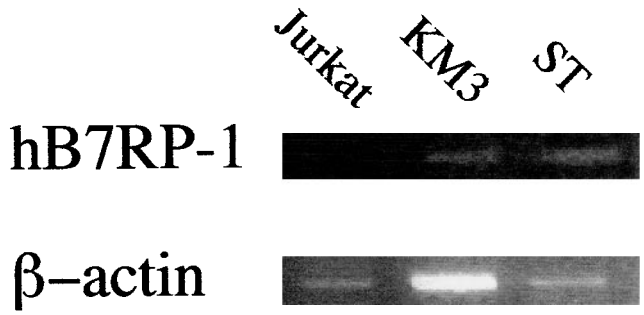


Figure 4. ICOS ligand expression in the synovial tissue of patients with active RA. RT-PCR analysis for hB7RP-1 and ß-actin mRNA in Jurkat, KM3, and synovial tissue (ST) from patients with RA was performed as described in Materials and Methods.

with fresh preparations of TSST-1-induced T cell blasts.

Consistent with previous findings[31,32] a large proportion of SF T cells from RA patients were in an activated state, as shown by the high expression of HLA-DR and CD45RO. Since CD25+ T cells accounted for only around 10% of SF T cells, memory-type T cells rather than newly activated T cells seem to have accumulated in the joints. In agreement with our results, Iannone *et al*[32] showed low expression of CD25 in the SF T cells of RA patients. We found that H4/ICOS was highly expressed in the whole, CD4+, and CD8+ fractions of SF T cells of RA patients (Figure 1), and thus it is quite possible that local immune responses are modulated by H4/ICOS expressed on T cells in the joints of RA patients. Involvement of ICOS in the pathogenesis of experimental Th-mediated diseases in mice has been suggested[33-36], and thus it would be reasonable to speculate that production of Th1- and Th2-type cytokines modulated by H4/ICOS is a factor in accelerating or decelerating the pathogenesis of RA. We found that H4/ICOS co-stimulation enhanced the production of IFN-γ, IL-2, IL-4, and IL-10 by SF CD4+ T cells, although the amounts of lymphokines produced were not high. It is possible that most SF CD4+ T cells are already primed and therefore do not produce higher amounts of cytokines. Based upon these results, H4/ICOS may contribute to joint destruction in the following manner. Expression of a ligand for H4/ICOS, B7RP-1, is upregulated by TNF-α[37]. IFN-γ and IL-17 from activated SF T cells would induce B7RP-1 upregulation on cells working as AC through production of pro-inflammatory cytokines from AC. Expression of B7RP-1 mRNA in synovial tissues from patients with RA was indeed detected using RT-PCR in the present study. B7RP-1 expressed on B cells, monocytes, and fibroblasts in the joints might provide a strong enough signal to SF T cells through H4/ICOS for induction of high production of pro-inflammatory cytokines, thereby leading to destruction of the joints. In contrast to the above scenario, if T cells are co-stimulated by H4/ICOS to produce IL-10, which was shown to be a protective lymphokine by others[38], in the joints, H4/ICOS expression may play a role in suppression of joint inflammation and reversal of joint destruction. Since in our study IL-10 was produced by SF CD4+ T cells in response to H4/ICOS co-stimulation, the progression of the H4/ICOS signaling pathway in joints might be effective in attenuating immune-mediated disease.

In conclusion, we have shown that increased expression of H4/ICOS on SF T cells from patients with RA can provide the signal that enhances the production of IFN-γ along with IL-4 and IL-10 by SF CD4+ T cells. H4/ICOS ligand (B7RP-1) mRNA in synovial tissues from patients with RA was detected by RT-PCR. Our findings suggest that H4/ICOS might contribute to the process of progression by the co-stimulatory effect on the production of IFN-γ or attenuation of inflammation by that on the production of antiinflammatory cytokine IL-10 in rheumatoid joints. We were not able to sufficiently clarify the potential role of H4/ICOS in the pathogenesis of RA in this study. Further investigations into the role of H4/ICOS will provide clues for devising a therapeutic approach to RA.

ACKNOWLEDGMENTS

We are grateful to Dr. K. Sagawa for providing the Mab specific for CD28 (KOLT-2).

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