Interferon-γ Enhances Interleukin 12 Production in Rheumatoid Synovial Cells via CD40-CD154 Dependent and Independent Pathways

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ABSTRACT. ***Objective.*** To determine the role of interferon-γ (IFN-γ) in CD40-CD154 dependent production of interleukin 12 (IL-12) by synovial cells of patients with rheumatoid arthritis (RA).

Methods. We examined the effects of IFN-γ, tumor necrosis factor-α (TNF-α), and granulocyte-macrophage colony stimulating factor (GM-CSF) on CD40 expression on CD68+ synovial macrophage-lineage cells (SMC). The effects of IFN-γ and soluble CD154 (sCD154) on IL-12 production by RA synovial cells were determined by ELISA.

Results. CD68+ SMC expressed substantial levels of CD40. IFN-γ, but not TNF-α or GM-CSF, markedly upregulated CD40 expression on CD68+ SMC. IFN-γ also dose dependently increased IL-12 production by synovial cells. The effects of IFN-γ on CD40 expression (EC_{50} = 127.4 U/ml) were observed at a concentration 19 times lower than the effects on IL-12 production (EC_{50} = 6.8 U/ml). Treatment with IFN-γ at a concentration low enough to augment CD40 expression but not IL-12 production enhanced spontaneous IL-12 production synergy with sCD154. The synergistic enhancement of spontaneous IL-12 production was abrogated by CD40-Fc. In contrast, IL-12 production induced by high concentration of IFN-γ was not neutralized by CD40-Fc.

Conclusion. IFN-γ enhanced IL-12 production via both CD40-CD154 dependent and independent pathways in RA synovium. IFN-γ may play a crucial role in the development of RA synovitis through regulation of IL-12 production. (J Rheumatol 2001;28:1764–71)

Key Indexing Terms:
CD40 INTERFERON-γ INTERLEUKIN 12 RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by hyperplasia of the synovium and excessive mononuclear cell infiltration, which leads to progressive joint destruction. Immunohistological studies have shown that the majority of infiltrating mononuclear cells in RA synovium are CD4+ T cells, and these cells may play an important role in the progression of RA[1,2]. Human CD4+ T helper (Th) cells can be classified into distinct subsets according to their cytokine secretion pattern[3]. Th1 cells produce interferon-γ (IFN-γ) and interleukin 2 (IL-2), whereas Th2 cells produce IL-4 and IL-5. T cells from RA synovium produce IFN-γ, but not IL-4, indicating that Th1 cells predominate in RA synovium[4,5]. The etiology of RA remains obscure; however, the cytokine balance skewed towards Th1 predominance suggests the role of the latter in controlling the disease process and resultant chronic inflammation.

IL-12 is recognized as a critical cytokine in the generation of Th1 cells. In murine collagen induced arthritis (CIA), IL-12 markedly enhances disease expression and severity when administered during immunization or at the time of onset, whereas anti-IL-12 treatment prevents the onset of arthritis[6,7]. Increased production of IL-12 in RA synovium and dissociated synovial cells has been also reported[8,9]. IL-12 is thought to be responsible for Th1 predominance in chronic RA inflammation[10,11]. IL-12 is a heterodimeric 70 kDa (p70) cytokine composed of 2 covalently linked subunits: a 40 kDa (p40) subunit and a 35 kDa (p35) subunit[12]. It is mainly produced from monocytes, macrophages, and dendritic cells following activation by bacterial stimuli and interaction of activated T cell surface protein CD154 with its counter-receptor, CD40[13-16]. We have shown that IL-12 production in RA synovium is predominantly mediated by interaction between CD40 on CD68+ synovial macrophage-lineage cells (SMC) and CD154 on infiltrated CD4+ T cells[9]. In addition to IL-12 production, *in vitro* studies revealed that CD40-CD154

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interaction induces the production of various proinflammatory cytokines [tumor necrosis factor-α (TNF-α), IL-1ß, IL-6, and IL-8] and matrix metalloproteinases (MMP) by synovial macrophages and fibroblasts[17-19]. The relevance of CD40-CD154 interaction in the immunopathogenesis of RA is well accepted.

CD40 is a 45–50 kDa glycoprotein member of the TNF receptor family, and has been identified on many cell types including B cells, macrophages, dendritic cells, and endothelial cells. Although CD40 is constitutively expressed on those cells, *in vitro* stimulation with IFN-γ, granulocyte-macrophage colony stimulating factor (GM-CSF), IL-3, or TNF-α greatly upregulates CD40 expression on monocytes, dendritic cells, endothelial cells, and fibroblasts and results in increased responses of these cells following CD154 ligation[20,21]. Cultured RA synovial fibroblast cells also show enhanced CD40 expression in response to IFN-γ[17,18]. IFN-γ also exhibits synergistic effects on synovial fibroblast proliferation induced by CD154 ligation[17].

We investigated the role of IFN-γ in CD40-CD154 dependent IL-12 production by RA synovial cells. We found that IFN-γ was a potent inducer of CD40 on CD68+ SMC, and that upregulation of CD40 by IFN-γ consequently enhanced IL-12 production induced by CD154 ligation. These data suggest an important role of IFN-γ in regulating CD40 mediated IL-12 production in RA synovium.

MATERIALS AND METHODS

Subjects. Synovial tissues were obtained at the time of surgical treatment from patients with RA diagnosed according to American College of Rheumatology criteria[22]. The study group consisted of 8 patients aged 52 to 80 years. The experimental protocol was approved by the hospital human ethics review committee, and signed consent was obtained from each patient.

Reagents. For flow cytometry, the following monoclonal antibodies (Mab) conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), and allophycocyanin (APC) were used: anti-human CD40 (EA-5 with PE; Ancell, Bayport, MN, USA), anti-human CD68 (KP1 with FITC; Dako, Glostrup, Denmark), anti-human CD3 (UCHT1 with APC; Immunotech, Marseille, France), and anti-murine CD8a (53-6.7 with PE; PharMingen, San Diego, CA, USA). Isotype matched control Mab (mouse IgG1; MOPC-21 with FITC or PE, and rat IgG2a; R35-95 with PE) were purchased from PharMingen. Human recombinant cytokines were purchased from the following sources: IFN-γ from Pharma Biotechnologie Hannover (Hannover, Germany), TNF-α and GM-CSF from PeproTech (London, England). IFN-γ, TNF-α, and GM-CSF were used at 10^0–10^4 U/ml, 1–1000 ng/ml, and 1–1000 ng/ml, respectively.

The fusion protein sCD154, consisting of the extracellular domain of murine CD8a tagged with FLAG and the extracellular domain of human CD154, was produced by constitutive transfection of HEK293 cells as described[23], with minor modifications. CD40-Fc, which fused the extracellular domain of human CD40 and the human IgG1 Fc region, was also prepared by constitutive transfection of HEK293 as described[24]. sCD154 could bind to CD40+, CD68+ SMC and the binding was abrogated by excessive amounts of CD40-Fc. Bioactivity of sCD154 was also checked by the induction of CD95 on Daudi lymphoma cells, which constitutively express functional CD40.

Preparation and culture of synovial cells. Synovial cells were prepared by collagenase and DNase digestion of small minced membranes as described[25], with minor modifications. In brief, synovial tissues were digested in RPMI 1640 medium containing 5% fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS, USA), 1 mg/ml collagenase A (Roche Diagnostics, Sandhoferstr, Germany), and 0.15 mg/ml DNase I (Roche) for 2 h at 37°C. After incubation, the tissue was pipetted through sterile 108 μm^2 nylon mesh into a sterile centrifuge tube. Cells were then washed 3 times with RPMI 1640 plus 5% FBS. Cells were cultured in 24 well plates (Sumitomo Bakelite, Tokyo, Japan) at 2×10^6 cells/well in RPMI 1640 containing 5% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin with or without various types of stimuli, at 37°C. Supernatants were harvested at 48 h and stored at –80°C until measurement for the production of IL-12 p40. Cells were recovered by trypsin-EDTA (Gibco, Rockville, MD, USA), washed, and then used for flow cytometry.

Total IL-12 measurement. Human total IL-12 (p40) was measured by ELISA (DuoSeT™; Genzyme, Cambridge, MA, USA) according to the protocol recommended by the manufacturer. In brief, a 96 well microtiter plate (MaxiSorp™; Nunc, Roskilde, Denmark) was coated with anti-IL-12 p40 antibody (10 μg/ml) in Dulbecco's phosphate buffered saline (PBS) at 4°C overnight. After decanting the antibody from wells, the plate was washed 3 times with PBS containing 0.05 % Tween-20. The plate was blocked with PBS supplemented with 1% bovine serum albumin (BSA) and 0.05% Tween-20 for 2 h, and then the samples were added and incubated for 2 h at 37°C. After incubation, the samples were discarded and the plate was washed and treated with biotinylated anti-human IL-12 p40 antibody (0.2 μg/ml) for 1 h, followed by streptavidin-peroxidase incubation (30 min, 37°C). The plate was incubated with TMB peroxidase substrate mixture (Sumitomo Bakelite) and the absorbance was measured at 450 nm by microplate reader (Spectramax 190; Molecular Devices, Sunnyvale, CA, USA).

Flow cytometric analysis of CD40 on SMC. Cultured synovial cells were stained with PE labeled anti-CD40 Mab and APC labeled anti-CD3 Mab in PBS containing 1% FBS for 30 min at 4°C, and then washed twice with PBS. After fixation with 1% paraformaldehyde for 20 min, cells were preincubated 10 min with 1% FBS and 0.5% saponin (Sigma, St. Louis, MO, USA) in PBS, and then stained with FITC labeled anti-CD68 for 30 min. After washing twice with PBS containing 1% FBS and 0.5% saponin, cells were resuspended in PBS with 1% FBS and analyzed on FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA). All treatments were carried out at room temperature. Data were expressed as mean fluorescence intensity (MFI). For the sCD154 binding experiment, synovial cells were incubated with 10 μg/ml sCD154 in PBS for 30 min at 37°C. After washing, cells were stained with PE labeled anti-murine CD8a Mab and then co-stained with CD68 as described.

Statistical analysis. All data were expressed as mean ± SEM of individual experiments derived from RA synovial cells of 8 different patients of triplicate wells. Fifty percent effective concentration (EC_{50}) values were calculated by linear regression (maximum likelihood method). Differences between groups were examined for statistical significance by 2 tailed Student t test. A p value < 0.05 denoted a statistically significant difference.

RESULTS

CD40 expression on RA synovial macrophage-lineage cells. The proportion of CD3+ and CD68+ cells among synovial cells was 10.1 ± 8.4% and 24.8 ± 16.1%, respectively. CD68+ SMC expressed CD40 (MFI 35.9 ± 4.7, Figure 1). Although other CD40+ cells (CD3– and CD68–) were also observed (data not shown), we focused on CD68+ SMC in the following experiments since the major source of CD40 mediated IL-12 production in rheumatoid synovial cells has been shown to be CD68+ SMC[9].

Upregulation of CD40 expression on SMC by cytokines. CD40 expression on monocytes, macrophages, and

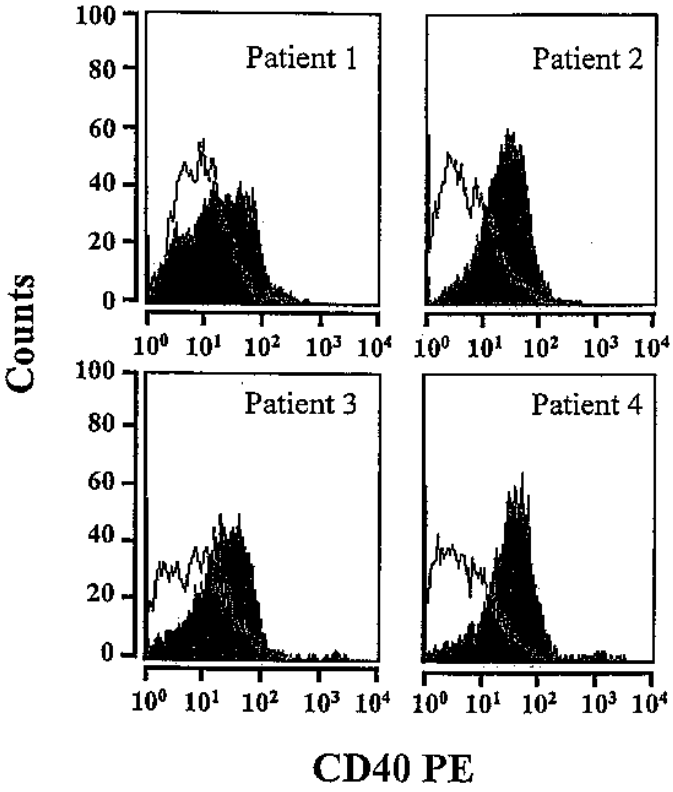

Figure 1. CD40 expression on CD68+ synovial macrophage-lineage cells from patients with RA. Synovial cells were isolated and stained with PE labeled anti-CD40 Mab. After fixation and permeabilization, cells were stained with FITC labeled anti-CD68 Mab. An analysis gate was set on CD68+ cells. Histograms represent staining with anti-CD40 Mab (shaded area) or control mouse IgG1 (open area) on CD68+ cells. Histograms represent staining from 8 different samples.

dendritic cells from peripheral blood of healthy subjects is greatly enhanced by IFN-γ, GM-CSF, and TNF-α[20,21]. Therefore, we examined the effects of these cytokines on CD40 expression on RA SMC. IFN-γ (10^0–10^4 U/ml) significantly enhanced CD40 expression on SMC in a concentration dependent manner (Figure 2). TNF-α slightly increased CD40 expression only at higher concentrations (> 100 ng/ml) (MFI 84.1 ± 10.8), while GM-CSF had no effect on CD40 expression at concentrations up to 1000 ng/ml (Figure 2). The maximum CD40 expression level induced by IFN-α was 8.2 times basal expression (MFI 292.5 ± 23.7), and the EC_{50} value of IFN-γ for the enhancement of CD40 expression was 6.8 U/ml (95% CI 4.8–9.8; Figure 3).

Enhancement of IL-12 p40 production by IFN-γ. IFN-γ is known to induce IL-12 p40 production by peripheral blood mononuclear cells. Thus, in the next series of experiments we examined the effect of IFN-γ on IL-12 p40 production by synovial cells. Spontaneous IL-12 p40 production by synovial cells (21.8 ± 7.8 pg/ml) was markedly enhanced by IFN-γ alone in a concentration dependent manner (704.6 ± 69.0 pg/ml at 10^4 U/ml; $p < 0.05$; Figure 3). The EC_{50} value of IFN-γ for IL-12 p40 production, 127.4 U/ml (95% CI

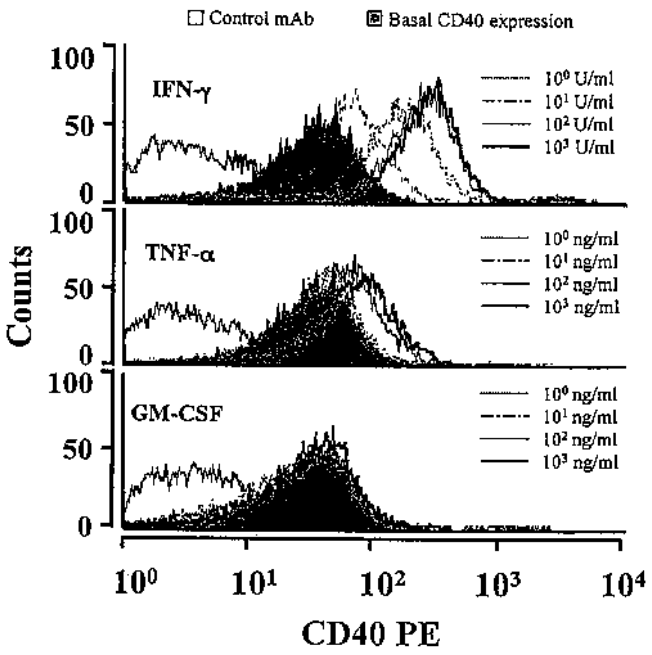

Figure 2. Effects of IFN-γ, TNF-α, and GM-CSF on CD40 expression on SMC. RA synovial cells (2×10^6 cells) were cultured with IFN-γ (10^0–10^4 U/ml), TNF-α (1–1000 ng/ml), and GM-CSF (1–1000 ng/ml) for 48 h. Cells were stained with PE labeled anti-CD40 Mab and FITC labeled anti-CD68 Mab as described in Figure 1. Histograms represent staining with anti-CD40 Mab or control mouse IgG1 on CD68+ cells, in synovial cells from 8 different samples.

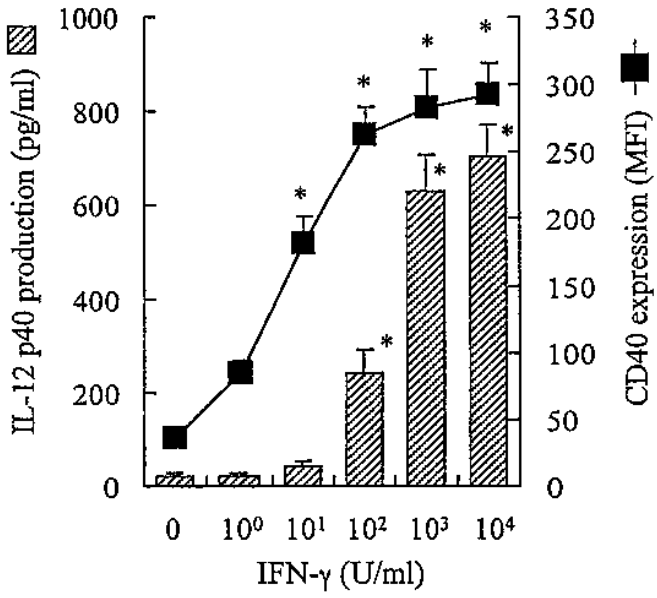

Figure 3. Effects of IFN-γ on CD40 expression and IL-12 production by synovial cells. RA synovial cells (2×10^6 cells) were cultured with IFN-γ (10^0–10^4 U/ml). Cells and supernatants were recovered after 48 h. Cells were stained and analyzed for CD40 expression by flow cytometry as described in Figure 1. Supernatants were analyzed for IL-12 p40 production by specific ELISA. CD40 expression shown by mean fluorescence intensity (MFI). Values represent mean ± SEM for synovial cells from 8 patients with RA. *$p < 0.05$ versus control medium.

72.9–233.2), was 19 times higher than that for CD40 expression.

Synergistic effect of IFN-γ and sCD154 on IL-12 production by synovial cells. We have shown that IL-12 production in RA synovium is predominantly mediated by interaction between CD40 on CD68+ SMC and CD154 on infiltrated CD4+ T cells[9]. To determine whether the level of CD40 on SMC could influence IL-12 production by synovial cells, we examined the effects of IFN-γ and sCD154 on IL-12 production. sCD154 at > 10 μg/ml significantly enhanced IL-12 p40 production: 102.3 ± 30.2 and 112.4 ± 44.5 pg/ml in cultures with 10 and 30 μg/ml sCD154, respectively, compared with 21.8 ± 7.6 pg/ml in untreated cultures ($p < 0.05$) (Figure 4).

Although IFN-γ alone at 10 U/ml had no significant effect on IL-12 p40 production (41.6 ± 10.4 pg/ml, Figure 5A), CD40 expression on SMC was significantly enhanced by 10 U/ml IFN-γ (Figure 3). Thus 10 U/ml IFN-γ was used for subsequent experiments. At this concentration, IFN-γ synergistically increased IL-12 p40 production with sCD154 (1–30 μg/ml, Figures 4 and 5A). The synergistic effect of IFN-γ and sCD154 on IL-12 p40 production (255.6 ± 44.1 pg/ml) was almost abrogated by treatment with CD40-Fc (12.6 ± 10.7 pg/ml, Figure 5A). In contrast, enhanced IL-12 p40 production by high concentration of IFN-γ (10^3 U/ml) had no synergistic effects with sCD154 (Figure 5B). Further, increased production of IL-12 p40 by high concentrations of IFN-γ was not neutralized by CD40-Fc (Figure 5B). The amount of IL-12 p40 induced by high IFN-γ (10^3 U/ml) was 2.8 times higher than that induced by low IFN-γ (10 U/ml) plus sCD154 (10 μg/ml).

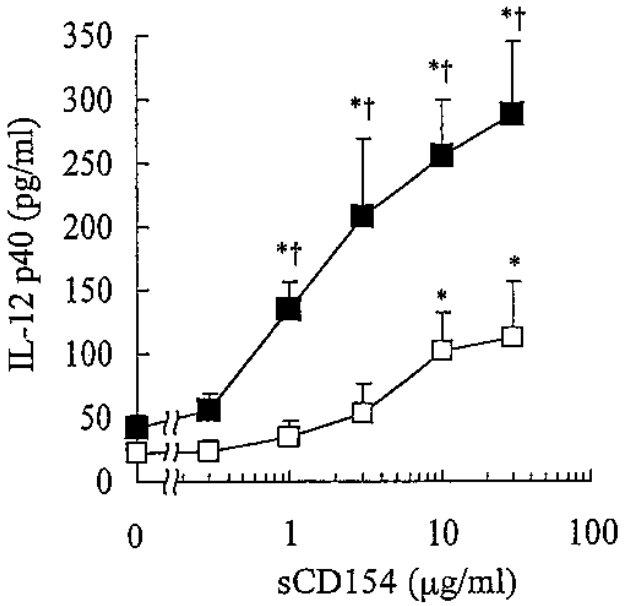

Figure 4. Synergistic effects of IFN-γ and sCD154 on IL-12 production by synovial cells. RA synovial cells (2×10^6 cells) were stimulated by various concentrations of sCD154 with (■) or without (□) IFN-γ (10^1 U/ml) for 48 h. IL-12 p40 in culture supernatants was determined by specific ELISA. Values represent mean ± SEM for synovial cells from 8 patients with RA. *$p < 0.05$ versus control medium, †$p < 0.05$ versus sCD154 alone.

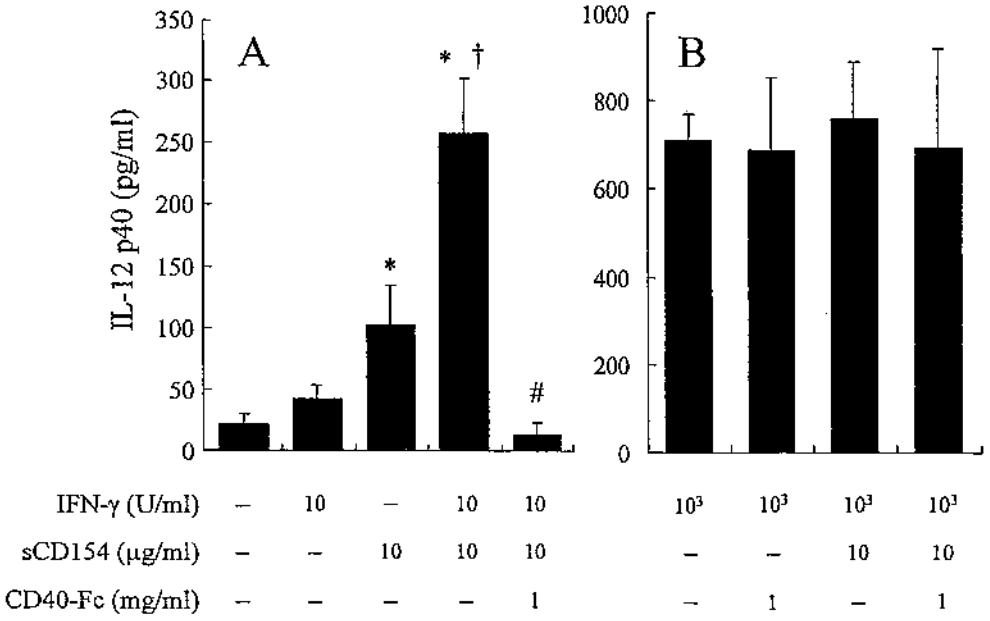

Figure 5. Effects of CD40-Fc on synergistic IL-12 production by IFN-γ and sCD154. RA synovial cells (2×10^6 cells) were stimulated by sCD154 (10 μg/ml) plus IFN-γ (10^1 U/ml) (A), or IFN-γ (10^3 U/ml) (B) with or without CD40-Fc (1 mg/ml) for 48 h. IL-12 p40 in the culture supernatants was determined by specific ELISA. Bars represent mean ± SEM for synovial cells from 8 patients with RA. *$p < 0.05$ versus control medium, †$p < 0.05$ versus sCD154 or IFN-γ alone, #$p < 0.05$ versus sCD154 plus IFN-γ treatment.

DISCUSSION

IL-12 is a heterodimeric 70 kDa cytokine composed of 2 subunits, p40 and p35, encoded by separate genes. Studies have shown that p35 transcripts are ubiquitously and constitutively expressed in various cell types, while p40 transcripts are highly regulated[26,27]. Therefore, IL-12 expression is controlled at the level of p40 production. Indeed, it has often been assumed that p40 mRNA or protein production alone is indicative of bioactive IL-12 heterodimer formation[27,28]. In preliminary experiments, we found that spontaneous IL-12 p70 production by synovial cells from RA paralleled p40 production, even though the level of p70 produced was much lower than that of p40 (data not shown). Thus, IL-12 p40 production by synovial cells as observed in this study could be applicable to bioactive IL-12 p70 production. However, it remains to be elucidated whether p35 production or expression in synovial cells is ubiquitous and constitutive.

IL-12 is thought to be responsible for Th1 predominance in chronically inflamed RA joints; however, the mechanisms of production of IL-12 by synovial cells are not fully elucidated. We observed that IL-12 production was induced by CD154 ligation and was markedly enhanced with increments of CD40 expression on SMC. It has been reported that augmentation of CD40 expression on RA synovial cells results in enhancement of CD40 dependent proliferation and cytokine production (IL-6, IL-8, and TNF-α) in these cells[17,18]. Taken together, these data suggest that functional responses induced by CD40-CD154 interaction could be regulated by the level of CD40 expression on CD40-bearing cells. In this study, we also observed that spontaneous IL-12 production by synovial cells was enhanced by exogenous sCD154 in a concentration dependent manner. Indeed, increased proportion of CD154+ CD4+ T cells by stimulation with anti-CD3 antibodies augmented spontaneous IL-12 production by RA synovial cells[9]. These results imply that IL-12 production could be regulated by both the amount of CD40 protein expressed on CD68+ SMC and CD154 on infiltrating CD4+ T cells.

Studies have shown that the proinflammatory and autoimmunity promoting effects of IL-12 are due to the potent positive feedback loop between IFN-γ and IL-12: IL-12 enhances IFN-γ production, and IFN-γ upregulates IL-12 production[29,30]. In this study, IFN-γ upregulated IL-12 production through both CD40-CD154 dependent and independent pathways. Our results indicate that the same positive feedback system between IFN-γ and IL-12 likely exists in inflamed RA synovium, and the effect of IFN-γ on IL-12 production partially depends on CD40-CD154 interaction. Studies have also described close relationships among IFN-γ, IL-12, and CD40-CD154 in the pathogenesis of progressive multiple sclerosis (MS)[31]. MS is an autoimmune disease of the central nervous system, and IL-12 production by microglial cells plays an important role in the pathogenesis of this disease. Becher, *et al*[32] recently reported that IFN-γ enhanced IL-12 production by upregulation of CD40 expression on microglia, a finding consistent with the results of our study.

Although IL-12 plays a crucial role in the generation of Th1 skewed autoimmune disease, under certain conditions it can also inhibit the pathogenesis of disease. This contradictory effect of IL-12 has been reported in a number of autoimmune disease models[33,34]. Kasama, *et al* recently reported that development of CIA was suppressed with high doses of IL-12, whereas at low doses IL-12 exacerbated the development of CIA[35]. In the human immune system, IL-12 also inhibits the Th1 immune response through induction of IL-10[36,37], although the mechanisms that underlie these opposite effects have not been elucidated. In RA synovial cells, IL-12 induced by high concentration of IFN-γ was 2.8 times higher than that synergistically induced by low IFN-γ and sCD154. This extremely large amount of IL-12 induced by high IFN-γ may contribute to the suppressive effects of disease development. In contrast, CD40 expression induced by low concentration of IFN-γ may play an important role in exacerbation of disease caused by a positive feedback loop between IFN-γ and IL-12. Thus, the difference of EC_{50} values of IFN-γ for CD40 expression and IL-12 production may be physiologically important.

Synovial cells used in this study consisted of various cell populations, such as synovial macrophage-lineage cells, lymphocytes, fibroblasts, and other inflammatory cells. Accordingly, it is difficult to confirm that the observed effects of IFN-γ represented the direct effects of the cytokine on IL-12-producing cells. However, with regard to CD40 expression, IFN-γ could act directly on CD68+ SMC, which is the major source of CD40-CD154 dependent IL-12 production, because the time course of CD40 expression was similar to that observed in purified monocytes from healthy subjects (data not shown). As for the effect of IFN-γ on IL-12 production, which is CD40-CD154 independent, it was difficult to confirm whether IFN-γ acted directly on IL-12-producing cells, and whether IL-12-producing cells were CD68+ SMC that produce IL-12 dependent upon CD40-CD154. Studies have reported that stimulation of normal human monocytes by IFN-γ alone does not lead to induction of IL-12 p40 mRNA or protein production, although IFN-γ could prime cells for lipopolysaccharide (LPS) induced transcription of the IL-12 p40 gene[38]. This suggests that IFN-γ requires additional stimuli to induce or enhance IL-12 production. Therefore, IFN-γ upregulation of CD40-CD154 independent IL-12 production by synovial cells may occur in concert with additional stimuli such as LPS. In this regard, synovial cells including IL-12-producing cells are persistently exposed to proinflammatory cytokines such as TNF-α, IL-1ß, IL-6, and IL-8[39,40]. Thus, these cytokines may provide additional stimuli for IL-12 production by IFN-γ.

T cell derived cytokines such as IFN-γ and IL-2 are present in relatively low amounts in the inflamed synovium of RA[41,42]. However, it could be postulated that these cytokines are produced within the microenvironment and may be present at functionally active concentrations. An example of this phenomenon is the non-obese diabetic (NOD) mouse model, where systemic administration of antibodies to IFN-γ leads to amelioration of glucose intolerance even though only small amounts of IFN-γ are produced by islet cells[43]. In the microenvironment of RA synovium, infiltrated T cells closely combine with SMC, suggesting the importance of cell-to-cell adhesion and contact[44,45]. These lymphocytes and macrophage-lineage cells express functional costimulatory molecules, CD154/CD40[13-16] and CD28/CD86[46], and the interaction of these molecules is critical for the pathogenesis and progression of RA. T cell derived cytokines probably play a pivotal role in functional regulation of these costimulatory molecules, as shown in our studies by upregulation of CD40 on SMC by IFN-γ. Further, it has been shown that natural soluble CD154 is present in supernatants of activated T cells in 15 and 18 kDa forms, which are functionally active, similar to recombinant sCD154[47-49]. Vakkalanka, *et al*[50] recently reported that sCD154 was significantly elevated in the sera of patients with systemic lupus erythematosus (SLE), and the form of sCD154 represented B cell activation. Thus, it is possible that sCD154 may be present in RA synovium as a soluble mediator like other T cell derived cytokines, and may play a critical role in the synovial microenvironment and inflammation and immune responses. The synergistic effect of sCD154 and IFN-γ on IL-12 production by synovial cells observed in this study may reflect the same phenomenon in inflamed RA synovium. Further experiments are necessary to elucidate whether sCD154 is present and could induce production of cytokines in RA synovium.

Several animal models of autoimmune diseases, including murine CIA, have revealed the beneficial effects of blockade of CD40-CD154 interaction[51-54]. In spite of these encouraging results in animal models, clinical trials of 2 anti-CD154 antibodies, Antova (Biogen Inc., Cambridge, MA, USA) and IDEC-131 (IDEC Pharmaceutical Corp., Sunnyvale, CA, USA), for the treatment of MS, idiopathic thrombocytopenic purpura, and SLE were disappointing due to adverse effects (thromboembolism) or lack of efficacy, respectively (company reports). In this regard, Mach, *et al*[55] demonstrated coexpression of CD154 and CD40 on the vascular endothelium and smooth muscle cells in human atherosclerotic lesions, suggesting that anti-CD154 antibodies may gather and act on the vascular endothelium to enhance the development of emboli. Thus, further studies are necessary to investigate whether such side effects are inherent byproducts of anti-CD154 antibody therapy. As for the lack of efficacy of anti-CD154 antibody, Berner, *et al*[56] recently reported that CD4+ T cells of a particular group of patients with RA expressed high levels of CD154, and the disease activity was significantly higher in this group than in the group expressing low levels of CD154. These results suggest that patients with RA could be divided into responders and nonresponders for anti-CD154 antibody therapy. Although it remains to be evaluated whether the CD154-hyperexpressing RA group would preferentially respond to anti-CD154 antibody treatment, inhibition of IL-12 production by CD40-CD154 blockade may be potentially useful therapy, at least in a limited number of patients with RA.

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