

# Mature Form of Interleukin 18 Is Expressed in Rheumatoid Arthritis Synovial Tissue and Contributes to Interferon- $\gamma$ Production by Synovial T Cells

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**ABSTRACT. Objective.** To investigate the expression and function of interleukin 18 (IL-18) in synovial tissue (ST) of patients with rheumatoid arthritis (RA).

**Methods.** The localization of IL-18 in ST was analyzed by immunohistochemistry. IL-18 and IL-18 receptor (IL-18R) mRNA were detected by RT-PCR. Expression of IL-18 at the protein level was analyzed by Western blotting. Cytokines in culture supernatants were measured by ELISA.

**Results.** From immunohistochemical analysis, IL-18-producing cells were localized in the lining layer and sublining region of RA ST. Most of them coexpressed CD68 antigen. In ST from patients with osteoarthritis (OA), IL-18-producing cells were localized only in the sublining region and the numbers of these cells were small. From RT-PCR, RA ST expressed mRNA of IL-18, as well as  $\alpha$ - and  $\beta$ -chains of IL-18R. OA ST did not express or very weakly expressed mRNA of  $\alpha$ - and  $\beta$ -chains of IL-18R. ST from RA patients produced significantly larger amounts of IL-18 *in vitro* than OA ST. Western blotting revealed that RA ST expressed mature IL-18 more abundantly than OA ST. IL-12 alone stimulates interferon- $\gamma$  (IFN- $\gamma$ ) production by RA synovial tissue cells, but IL-18 alone could not. In the presence of IL-12, however, IL-18 could synergistically stimulate IFN- $\gamma$  production by RA synovial tissue cells. OA synovial tissue cells responded to neither IL-12 nor IL-12 + IL-18. IL-18 showed synergistic effects with IL-12 on promoting the ability of synovial T cells from RA patients to produce IFN- $\gamma$ .

**Conclusion.** These findings suggest that mature IL-18 is expressed in RA synovia and contributes to the production of IFN- $\gamma$  by infiltrating T cells. (J Rheumatol 2001;28:1779–87)

## Key Indexing Terms:

RHEUMATOID ARTHRITIS  
INTERFERON- $\gamma$

INTERLEUKIN 18

INTERLEUKIN 12  
SYNOVIUM

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of synovium, which leads to polyarticular destruction. In RA synovium, various types of immune competent cells including CD4+ T cells, macrophage derived synovial cells, and fibroblast-like synovial cells accumulate and produce various inflammatory mediators such as cytokines, chemokines, and metalloproteinases that contribute to the development of synovitis and resorption of bone and cartilage. It has been reported that CD4+ T cells play an important role in the synovial inflammation of RA by expressing activation markers<sup>1</sup>,

including HLA-DR antigen<sup>2</sup>, adhesion molecules<sup>1</sup>,  $\alpha$  chain of interleukin 2 (IL-2) receptor<sup>2</sup>, and CD40 ligand<sup>3,4</sup>, and also by interacting with other immune competent cells.

Recently, 2 distinct subsets of T cells, Th1 and Th2, were identified from their cytokine patterns<sup>5</sup>. Th1 cells produce interferon- $\gamma$  (IFN- $\gamma$ ) but little IL-4, and mediate cellular immunity. Th2 cells produce large amounts of IL-4 and IL-5, and mediate humoral immunity<sup>6</sup>. Experiments with animal models of autoimmune diabetes, experimental allergic encephalomyelitis, experimental colitis, and type II collagen induced arthritis<sup>7</sup> suggest that Th1 cells are relevant to the immunopathogenesis of organ-specific autoimmune diseases. It has been reported that CD4+ T cells in RA synovium express IFN- $\gamma$ , but little IL-4<sup>8-12</sup>, suggesting a shift of synovial T cells toward the Th1 phenotype.

IL-18 has been cloned as a novel cytokine that induces IFN- $\gamma$  production of T cells<sup>13</sup>. The molecular structure of IL-18 is related to IL-1 family proteins<sup>14</sup>. Both IL-18 and IL-1 $\beta$  are folded similarly to all  $\beta$ -sheet molecules, and cleaved into mature proteins by IL-1 $\beta$ -converting enzyme (ICE/caspase-1)<sup>15</sup>. The IL-18 receptor (IL-18R) complex is made up of IL-1 receptor related protein (IL-1Rrp)<sup>16</sup> with an IL-1 receptor accessory-like protein (AcPL)<sup>17</sup>. IL-1Rrp and

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AcPL are termed the  $\alpha$  and  $\beta$  chains of IL-18R (IL-18R $\alpha$  and IL-18R $\beta$ ), respectively. IL-18 binds to IL-18R $\alpha$ , and then IL-18R $\beta$  binds to the complex. This heterodimeric complex is required for IL-18 signaling in terms of nuclear factor  $\kappa$ B (NF $\kappa$ B) induction and c-Jun N-terminal kinase activation<sup>15</sup>. In addition to IFN- $\gamma$  induction, IL-18 stimulates the synthesis of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1 $\beta$ , IL-8<sup>18</sup>, granulocyte-macrophage colony stimulating factor (GM-CSF)<sup>19</sup>, and Fas ligand<sup>20</sup>. It has been reported that synovial tissue (ST) from patients with RA expresses both IL-18 and IL-18R $\alpha$ , that IL-18 could stimulate RA synovial cells to produce IFN- $\gamma$ , and that IL-18 could exacerbate arthritis in a murine collagen induced arthritis model<sup>21,22</sup>. However, which form of IL-18, pro-IL-18 or mature IL-18, is produced by RA ST and whether the response of synovial cells to IL-18 differs between RA and other arthritides has not been investigated. In this study, we investigated (1) localization of IL-18 in RA synovium, (2) expression of not only IL-18R $\alpha$  but also IL-18R $\beta$  mRNA in RA synovium, (3) augmented expression of the mature form of IL-18 in RA synovium, (4) different responsiveness of synovial T cells to IL-18 between RA and osteoarthritis (OA), and (5) the effect of IL-18 on promoting the ability of RA synovial T cells to produce IFN- $\gamma$ .

## MATERIALS AND METHODS

**Subjects.** Subjects consisted of patients with RA (n = 24) and OA (n = 14). Diagnosis of RA was in accordance with the 1987 criteria of the American College of Rheumatology<sup>23</sup>. OA was also diagnosed according to ACR criteria<sup>24</sup>.

**Reagents.** Recombinant human IL-2, IL-12, and IL-18 were purchased from R&D Systems (Minneapolis, MN, USA). Goat anti-human IL-18 polyclonal antibody and control mouse IgG were purchased from Dako Japan (Tokyo, Japan). Mouse anti-human IL-18 monoclonal antibody (R&D Systems) was biotinylated using a Mini-biotin-XX protein labeling kit (Molecular Probes, Eugene, OR, USA). Mouse anti-human  $\beta$ -actin monoclonal antibody (Mab) was purchased from Sigma (St. Louis, MO, USA).

**Immunohistochemical staining of frozen sections.** Frozen sections of ST were fixed in cold acetone and internal peroxidase activity was blocked by incubating in 0.03% H<sub>2</sub>O<sub>2</sub>-phosphate buffered saline for 15 min at room temperature (RT). IL-18-producing cells were detected using mouse anti-human IL-18 polyclonal antibody (Dako) and the TSA-indirect kit (NEN Life Science Products, Boston, MA, USA) according to the manufacturer's instructions. For double labeled staining, frozen sections were initially stained for IL-18 and then stained for either CD3 or CD68 antigen using mouse anti-human CD3 or anti-human CD68 Mab (Dako), respectively, and a Vectastain ABC-alkaline phosphatase kit (Vector, Burlingame, CA, USA).

**Reverse transcription-polymerase chain reaction (RT-PCR).** Total RNA was extracted using TriZol (Gibco BRL, Grand Island, NY, USA). One microgram of heat denatured total RNA was reverse transcribed with oligo-dT primer and Superscript II (Gibco BRL) in a volume of 20  $\mu$ l. Two microliters of the cDNA were used as PCR templates and amplified by Taq DNA-polymerase (Roche Diagnostics, Basel, Switzerland). PCR primers were as follows: IL-18 forward 5'-GCT TGA ATC TAA ATT ATC AGT C-3'; IL-18 reverse 5'-GAA GAT TCA AAT TGC ATC TTA T-3'; IL-18R $\alpha$  forward 5'-CCC AAC GAT AAA GAA GAA CGC C-3'; IL-18R $\alpha$  reverse 5'-TGT CTG TGC CTC CCG TGC TGG C-3'; IL-18R $\beta$  forward 5'-AAC ACA ACC CAG TCC GTC CAA-3'; IL-18R $\beta$  reverse 5'-AAC ATC AGG AAA TAG GCT CAG-3';  $\beta$ -actin forward 5'-TAC ATG GCT GGG GTG

TTG AA-3';  $\beta$ -actin reverse 5'-AAG AGA GGC ATC CTC ACC CT-3'. The lengths of PCR products of IL-18, IL-18R $\alpha$ , IL-18R $\beta$ , and  $\beta$ -actin cDNA were 342, 419, 291, and 218 bp, respectively.

**Western blotting.** One hundred milligrams of ST was minced and homogenized in RIPA buffer [25 mM Tris-HCl, pH 8.0; 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 10% glycerol, 2 mM EDTA, 1% NP40, 1% sodium deoxycholate] containing protease inhibitors (1 mM phenylmethylsulfonylfluoride, 5  $\mu$ g/ml aprotinin, 50  $\mu$ g/ml leupeptin, 0.5  $\mu$ g/ml pepstatin A) using a homogenizer (IKA Labortechnik; Janke & Kunkel GmbH, Staufen, Germany) at 4°C for 5 min. Supernatants were collected by centrifugation at 14,000 rpm for 30 min at 4°C and the protein concentration of lysates was determined using a BCA protein assay reagent (Pierce; Rockford, IL, USA). Lysates were separated by electrophoresis in 15% SDS-polyacrylamide gels and transferred to a nitrocellulose membrane (0.45  $\mu$ m; Bio-Rad). IL-18 and  $\beta$ -actin were detected using anti-human IL-18 polyclonal antibody (Dako) and anti-human  $\beta$ -actin Mab, respectively, and the signal was visualized using the Immune-Star chemiluminescent protein detection system (Bio-Rad). The intensity of the bands was quantified using NIH image 1.33 software.

**Synovial organ culture.** ST was obtained at the time of joint replacement. The tissues were dissected into 3 mm diameter fragments and washed 3 times with 50 ml of RPMI 1640 (Nikken Kagaku, Tokyo, Japan) supplemented with penicillin (100 U/ml) and streptomycin (100 mg/ml) (all from Gibco). Tissue fragments weighing 100 mg were selected and cultured in 1 ml of RPMI 1640-10% fetal calf serum (FCS; Filtron Pty, Brooklyn, Australia) in 24 well culture plates (Costar, Cambridge, MA, USA). After 48 h, fluids from duplicate synovial organ cultures were collected and stored at -80°C.

**ELISA for human IL-18.** Microtiter plate wells (Costar) were coated with 50  $\mu$ l of goat anti-human IL-18 polyclonal antibody (1.0  $\mu$ g/ml; Dako) in monocarbonate buffer (100 mM NaHCO<sub>3</sub>, pH 8.2) at 4°C overnight. After discarding the antibody, the plates were washed 4 times with phosphate buffered saline (PBS)-Tween 20 (137 mM NaCl, 2.68 mM KCl, 7.04 mM NaH<sub>2</sub>PO<sub>4</sub>, 1.15 mM KH<sub>2</sub>PO<sub>4</sub>, 0.05% Tween). Subsequent additions of the reagents with 4 PBS-Tween washes were as follows: (1) 150  $\mu$ l of PBS-10% FCS (RT, 2 h); (2) 100  $\mu$ l of recombinant human IL-18 or samples diluted in RPMI 1640-10% FCS (4°C overnight); (3) 100  $\mu$ l of biotinylated mouse anti-human IL-18 Mab (1.0  $\mu$ g/ml) diluted in PBS-10% FCS (RT, 2 h); (4) 100  $\mu$ l of avidin-peroxidase diluted in PBS-10% FCS (2.5 mg/ml, RT, 30 min); (5) 100  $\mu$ l of ABTS peroxidase substrate mixture (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). The absorbance at 414 nm and 490 nm was measured using an ELISA plate reader (Immuno Mini NJ-2300, Nippon Intermed, Tokyo, Japan). The detection limit of this assay system was 7.8 pg/ml.

**ELISA for IL-4 and IFN- $\gamma$ .** Human IL-4 and IFN- $\gamma$  was measured using an ELISA kit (Endogen, Woburn, MA, USA) according to the manufacturer's instruction. The detection limits of these assay systems were 10.24 pg/ml for IL-4 and 25.6 pg/ml for IFN- $\gamma$ .

**Preparation and culture of synovial tissue cells.** Synovial tissue cells were prepared as described<sup>4</sup>. In brief, the extensively washed ST was minced and then digested by DNase (800 units/ml; Sigma) and collagenase (130 units/ml; Sigma) for 1 h in a 37°C water bath with gentle agitation. The cells were collected by passing through stainless steel mesh and washed 3 times with RPMI 1640-10% FCS. Living cells (1  $\times$  10<sup>5</sup>/well) were cultured in 200  $\mu$ l of RPMI-10% FCS in 96 well flat bottom plates (Costar) with or without various kinds of stimuli for 48 h to collect supernatants. After centrifugation at 2000 rpm for 10 min at 4°C, supernatants were stored at -80°C until assayed for the production of cytokines. To determine the effect of IL-18 on the cytokine profiles of synovial T cells, short term T cell lines were established as described<sup>25</sup>. Briefly, synovial tissue cells were stimulated with anti-CD3 Mab (OKT3; Ortho Diagnostic Systems, Raritan, NJ, USA) and IL-2 in the presence or absence of IL-12 and/or IL-18 for 3 days. Cells were washed and maintained with fresh cytokines but without anti-CD3 Mab for an additional 4 days. At the end of this first week, T cells

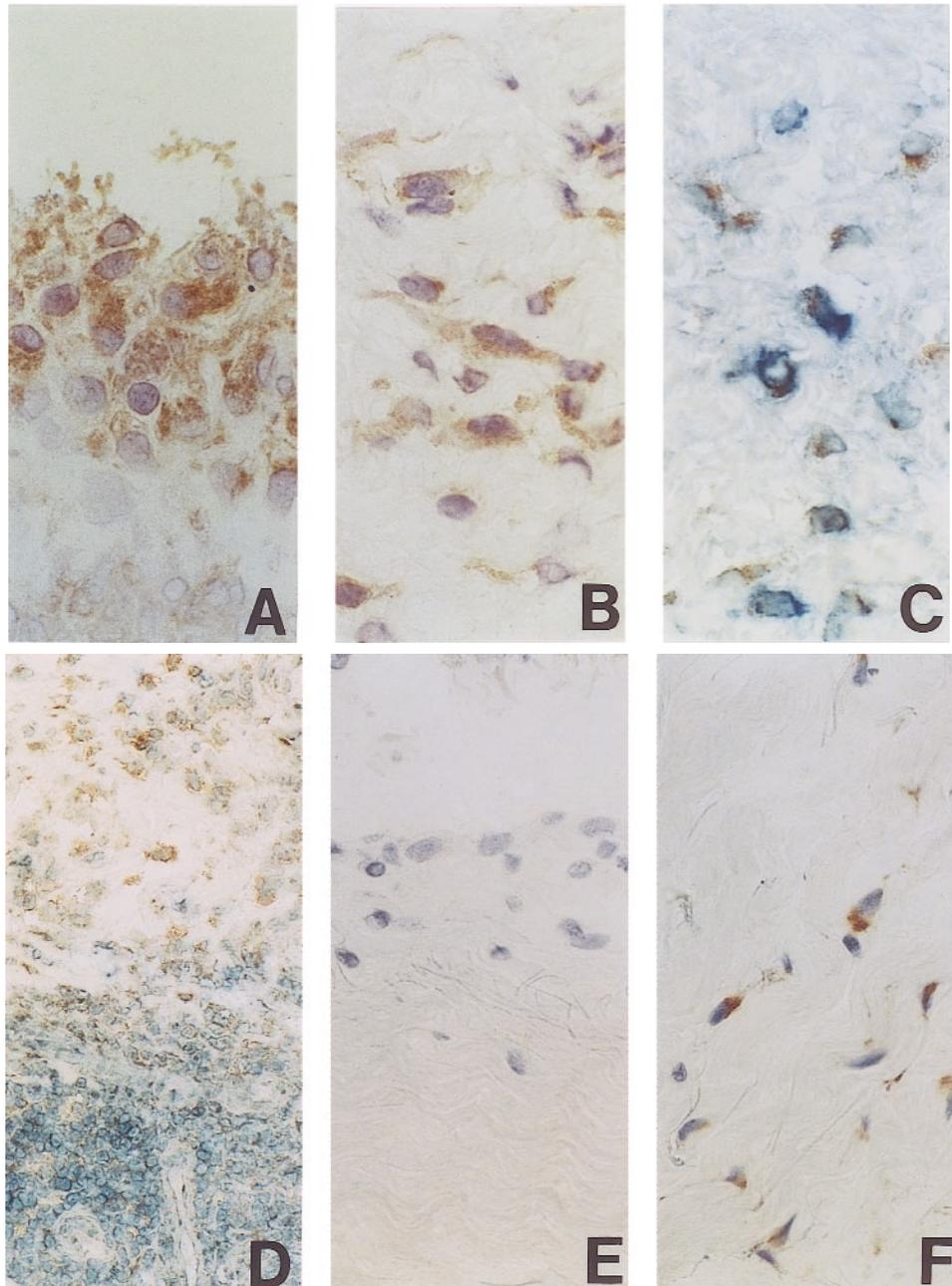
were separated using anti-CD3 Mab coated beads (Miltenyi Biotec, Bergisch Gladbach, Germany), resuspended at  $1 \times 10^5$ /well in 96 well round bottom plates, and restimulated with an optimal concentration of phytohemagglutinin (PHA, 1:1600 dilution; Difco, Detroit, MI, USA) for 24 h to collect the supernatants. IL-4 and IFN- $\gamma$  in the culture supernatants were measured by ELISA.

*Statistical analysis.* Differences were considered significant if  $p < 0.05$ . Mann-Whitney U test was used for comparing the ratio of mature IL-

18/pro-IL-18 and the production of IL-18 between RA ST and OA ST (Figure 3C, Figure 4). Student's t test was used for comparing the production of IFN- $\gamma$  by short term synovial T cell lines (Figure 6).

## RESULTS

*Localization of IL-18-producing cells in RA synovium.* IL-18 positive cells were localized in the lining layer and



*Figure 1.* Immunohistochemistry of synovial tissue (ST). A, B. Immunohistochemical staining of RA ST with anti-IL-18 antibody. IL-18 positive cells were localized in lining layer (A) and sublining region (B) of RA ST. C, D. Double labeled immunohistochemical staining of RA ST with anti-IL-18 antibody and anti-CD-68 Mab (C) or anti-IL-18 antibody and anti-CD3 Mab (D). Most IL-18 positive cells coexpressed CD68 antigen (C), but not CD3 antigen (D). E, F. Immunohistochemical staining of OA ST with anti-IL-18 antibody. IL-18 positive cells were localized in the sublining region (F), but not in the lining layer (E). Original magnification A, B, C, E, F  $\times 400$ ; D  $\times 200$ .

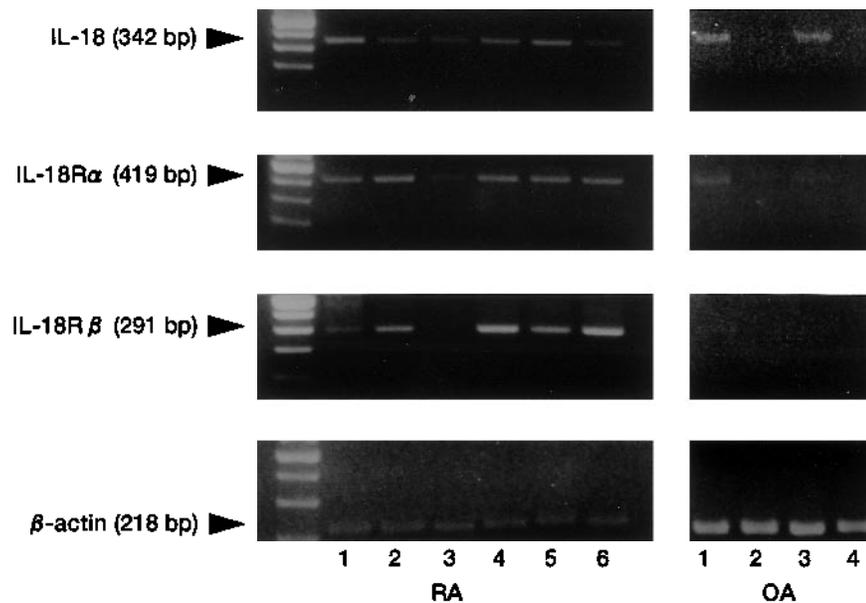
sublining region of synovia from patients with RA (Figures 1A, 1B). Double labeled immunohistochemical staining revealed that most IL-18 positive cells simultaneously expressed CD68 antigen (Figure 1C), but not CD3 antigen (Figure 1D). These findings indicate that macrophage-like synovial cells mainly produce IL-18 in RA ST. In ST of patients with OA, small numbers of IL-18-producing cells were found in the sublining region, but not in the lining layer (Figures 1E, 1F).

**Expression of IL-18 and IL-18R in synovial tissue.** Total RNA was extracted from ST of 18 RA and 4 OA patients, and the expression of IL-18, IL-18R $\alpha$ , IL-18R $\beta$ , and  $\beta$ -actin mRNA was determined by RT-PCR, as described in Materials and Methods (Figure 2). IL-18 mRNA was detected in 17 of 18 RA patients and 2 of 4 OA patients. Representative findings are shown. IL-18R $\alpha$  and 18R $\beta$  mRNA were detected in 6 and 5 of 6 RA patients, respectively. ST from OA patients did not express or very weakly expressed IL-18R $\alpha$  and IL-18R $\beta$  mRNA.  $\beta$ -actin mRNA was detected in all samples. Next, we examined the expression of IL-18 at the protein level in ST from 10 RA patients and 6 OA patients by Western blotting (Figure 3A). ST of all RA patients expressed both mature IL-18 (18 kDa) and pro-IL-18 (24 kDa). ST from all OA patients expressed pro-IL-18, but 4 of them did not express (Figure 3A, lanes 1 and 5) or very weakly expressed (lanes 2 and 6) mature IL-18. The ratio of mature IL-18 to pro-IL-18 was greater than 1.0 in 7 of 10 RA patients, while it was less than 1.0 in 5 of 6 OA patients. The ratio of mature IL-18 to pro-IL-18 of RA ST

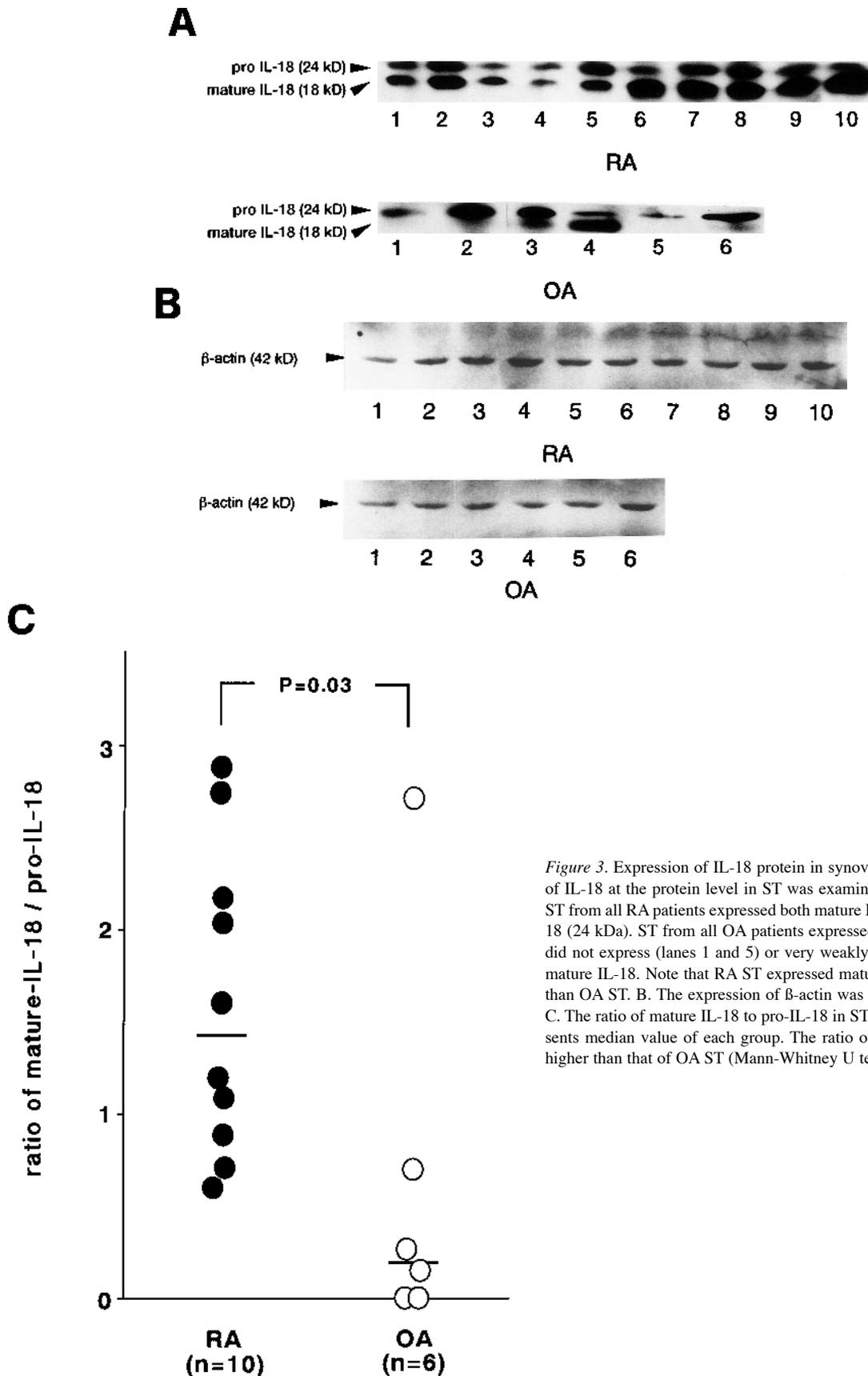
was significantly higher than that of OA ST (median and mean  $\pm$  standard deviation: 1.4 and 1.60  $\pm$  0.83 for RA; 0.22 and 0.65  $\pm$  1.06 for OA;  $p = 0.03$  by Mann-Whitney U test) (Figure 3C). The expression of  $\beta$ -actin was equivalent for all samples (Figure 3B). We also examined the spontaneous production of IL-18 from ST. Small pieces of ST (100 mg/ml) from 21 RA patients and 11 OA patients were cultured for 48 h without stimuli, and concentrations of IL-18 in the culture supernatants were measured by ELISA (Figure 4). IL-18 was detected in the supernatants from all patients. ST of some RA patients released large amounts of IL-18 in the culture supernatants, and there was a significant difference in the concentrations of IL-18 between RA and OA patients (median and mean  $\pm$  standard deviation: 2058.5 and 2086.4  $\pm$  874.5 pg/ml for RA; 1701.2 and 1803.6  $\pm$  610.9 pg/ml for OA;  $p < 0.05$  Mann-Whitney U test).

**IL-18 and IL-12 synergistically stimulate IFN- $\gamma$  production by RA ST cells.** We next compared the effects of IL-12, IL-18, and IL-12 + IL-18 on IFN- $\gamma$  production by synovial tissue cells. IL-12 alone, but not IL-18 alone, could stimulate IFN- $\gamma$  production by RA synovial tissue cells (Figure 5A). In the presence of IL-12, however, IL-18 could synergistically stimulate IFN- $\gamma$  production by RA synovial tissue cells (Figure 5A). Neither IL-12, IL-18, nor IL-12 + IL-18 could stimulate IFN- $\gamma$  production by OA synovial tissue cells (Figure 5B). Experiments with synovial tissue cells from 2 other RA and 2 other OA patients showed similar findings.

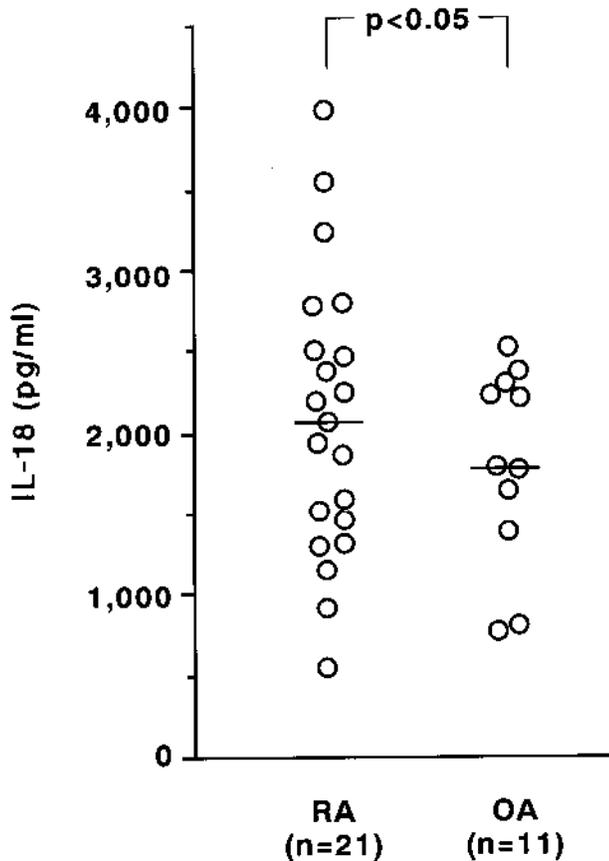
*IL-18 augments the ability of RA synovial T cells to produce*



**Figure 2.** Expression of IL-18,  $\alpha$  and  $\beta$  chains of IL-18 receptor (IL-18R $\alpha$  and IL-18R $\beta$ ) mRNA in synovial tissue (ST). Total RNA was extracted from ST, and IL-18, IL-18R $\alpha$ , IL-18R $\beta$ , and  $\beta$ -actin mRNA were detected by RT-PCR. ST from 17 of 18 RA and 2 of 4 OA patients constitutively expressed IL-18 mRNA. Six representative findings from 17 RA cases are shown. ST from 6 and 5 of 6 RA patients expressed IL-18R $\alpha$  and IL-18R $\beta$  mRNA. ST from OA patients did not express or very weakly expressed IL-18R $\alpha$  and IL-18R $\beta$  mRNA.



**Figure 3.** Expression of IL-18 protein in synovial tissue (ST). Expression of IL-18 at the protein level in ST was examined by Western blotting. A. ST from all RA patients expressed both mature IL-18 (18 kDa) and pro-IL-18 (24 kDa). ST from all OA patients expressed pro-IL-18, but 4 of them did not express (lanes 1 and 5) or very weakly expressed (lanes 2 and 6) mature IL-18. Note that RA ST expressed mature IL-18 more abundantly than OA ST. B. The expression of  $\beta$ -actin was equivalent for all samples. C. The ratio of mature IL-18 to pro-IL-18 in ST of RA and OA. Bar represents median value of each group. The ratio of RA ST was significantly higher than that of OA ST (Mann-Whitney U test).

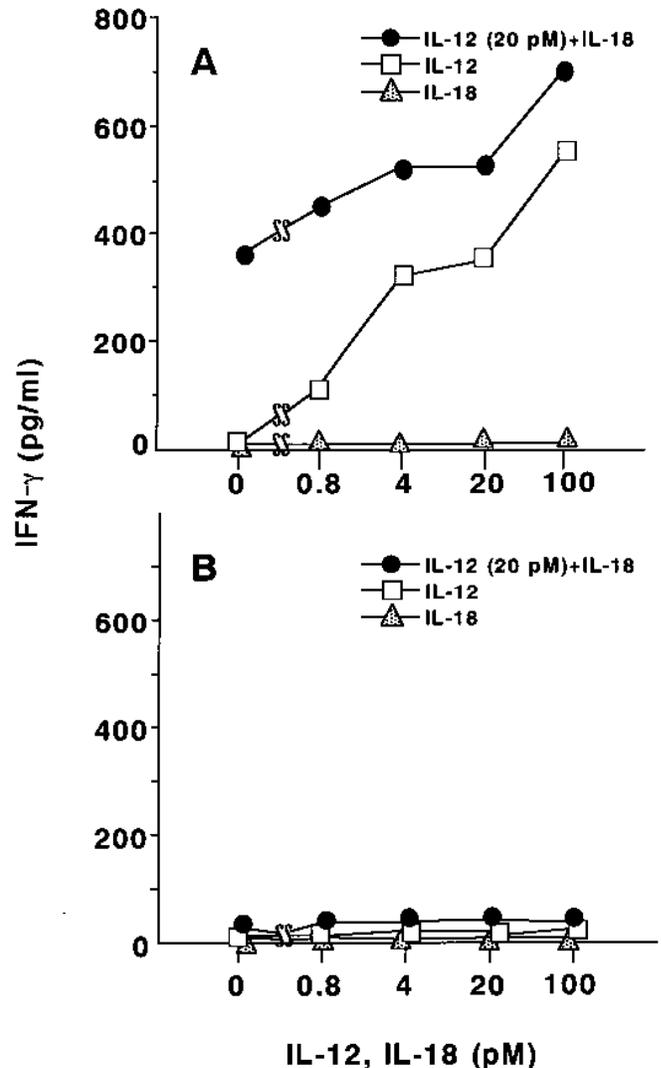


**Figure 4.** Production of IL-18 in synovial organ culture. Synovial tissues (ST) from both RA and OA patients were cultured 48 h without stimuli as described in Materials and Methods. IL-18 in the culture supernatants was measured by ELISA. Bar represents median value of each group. RA and OA ST released IL-18 into the culture supernatants, but some RA ST released large amounts of IL-18. There was a significant difference between RA and OA ST (Mann-Whitney U-test).

**IFN- $\gamma$ .** We further examined the effects of IL-18 on maintaining the IFN- $\gamma$  dominant T cell cytokine response in RA. Short term T cell lines were established from ST as described in Materials and Methods. When synovial T cells were expanded with anti-CD3 Mab + IL-2 + IL-12, they produced substantial amounts of IFN- $\gamma$  by PHA stimulation. Synovial T cells expanded with anti-CD3 Mab + IL-2 + IL-18 did not produce IFN- $\gamma$  by PHA stimulation. Synovial T cells expanded with anti-CD3 Mab + IL-2 + IL-12 + IL-18 produced significantly larger amounts of IFN- $\gamma$  than those expanded with anti-CD3 Mab + IL-2 + IL-12 (Figure 6). Thus, IL-18 showed synergistic effects with IL-12 in promoting the ability of synovial T cells to produce IFN- $\gamma$ . IL-4 was not detectable in any of the supernatants.

## DISCUSSION

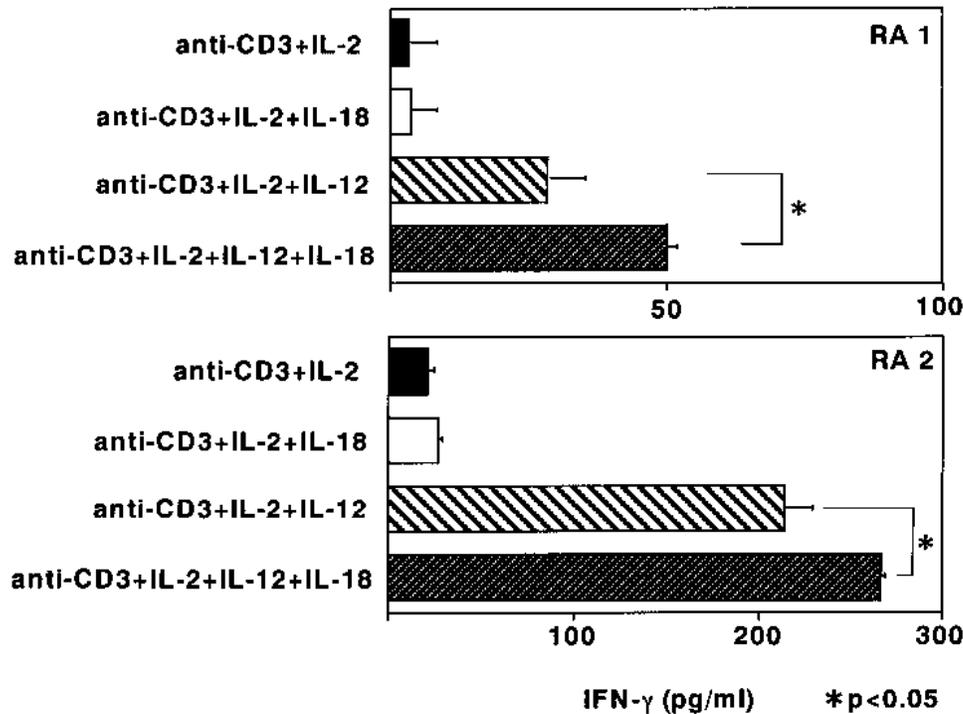
We examined the role of IL-18 in the immunopathogenesis of RA, focusing on its ability to induce IFN- $\gamma$  production of synovial T cells. Our findings reveal the expression of mature IL-18 in RA ST and its contribution to IFN- $\gamma$  produc-



**Figure 5.** Synergy between IL-12 and IL-18 in IFN- $\gamma$  production by synovial tissue cells. Synovial tissue cells from RA and OA patients were cultured with IL-12 alone, IL-18 alone, or IL-12 + IL-18 for 48 h. IFN- $\gamma$  in the supernatants was measured by ELISA. A. IL-12 alone, but not IL-18, could stimulate IFN- $\gamma$  production by RA synovial tissue cells. In the presence of IL-12, however, IL-18 could synergistically stimulate IFN- $\gamma$  production of RA synovial tissue cells. B. Neither IL-12, IL-18, nor IL-12 + IL-18 could stimulate IFN- $\gamma$  production of OA synovial tissue cells. Experiments with synovial tissue cells from 2 other RA and 2 other OA patients showed similar findings.

tion of synovial T cells. The response of synovial T cells to IL-18 was different between RA and OA, emphasizing the importance of IL-18 in synovial inflammation in RA.

Immunohistochemical analysis revealed that synovial lining cells of RA ST, but not OA ST, expressed IL-18 protein. RA sublining cells also expressed IL-18 more abundantly than those of OA ST (Figure 1). Since some inflammatory stimuli such as lipopolysaccharides can induce IL-18 production, proinflammatory cytokines may also contribute to enhanced expression of IL-18 in RA ST. A



**Figure 6.** IL-18 augments the ability of synovial T cells from RA patients to produce IFN- $\gamma$ . Synovial tissue cells from 2 RA patients were cultured with anti-CD3 Mab in the presence of IL-2, IL-2 + IL-12, IL-2 + IL-18, or IL-2 + IL-12 + IL-18 for 72 h. Synovial tissue cells were washed and fresh medium containing the same cytokine, but not anti-CD3 Mab, was added. After culturing an additional 96 h, T cells were separated and stimulated with PHA for 24 h. IFN- $\gamma$  and IL-4 in the culture supernatants were measured by ELISA. Synovial T cells expanded with anti-CD3 Mab + IL-2 + IL-12 produced substantial amounts of IFN- $\gamma$  with PHA stimulation. Synovial T cells expanded with anti-CD3 Mab + IL-2 + IL-12 + IL-18 produced significantly larger amounts of IFN- $\gamma$  with PHA stimulation than those expanded with anti-CD3 Mab + IL-2 + IL-12.

recent study by Gracie, *et al*<sup>21</sup> reported the stimulatory effects of IL-1 $\beta$  and TNF- $\alpha$  on IL-18 production of synovial fibroblasts *in vitro*, which supports this possibility. Promoter of mouse IL-18 gene is TATA-less and the activity of the IL-18 promoter upstream of exon 2 acts constitutively in some cell lines<sup>26</sup>. In addition, the 3' untranslated region of the human IL-18 gene lacks the AUUUA destabilization sequence<sup>14,26</sup>. These features of IL-18 may explain the constitutive expression of IL-18 mRNA in normal human blood monocytes<sup>27</sup> and normal human chondrocytes<sup>28</sup>. The expression of IL-18 mRNA in ST from 17 of 18 patients with RA as well as 2 of 4 OA patients may also, in part, result from the constitutive activity of IL-18 gene promoter. It should be emphasized that IL-18 in RA ST was mainly the mature protein, while IL-18 produced by OA ST was mainly the precursor (Figures 3A, 3C). One OA patient (lane 4 in the lower panel of Figure 3A) expressed mature IL-18 more abundantly than pro-IL-18. Moderate infiltration of CD3+ lymphocytes and CD68+ macrophages to ST of this patient, detected by immunohistochemical analysis (data not shown), might be related to the relatively dominant expression of mature IL-18 in this particular case.

IL-18 precursor is cleaved into an active, mature mole-

cule at its authentic processing site, Asp<sup>35</sup>-Asn<sup>36</sup>, by an intracellular cystein protease, ICE/caspase-1<sup>15</sup> and released from the cell. ICE/caspase-1 activity has been detected in freshly isolated synovial tissue cells from patients with RA<sup>29</sup>, which may explain the higher processing activity for IL-18 by RA ST than by OA ST. The enhanced expression of mature IL-18 by RA ST is in agreement with the increased release of IL-18 by RA ST (Figures 3 and 4). It has recently been reported that IL-12 induced IFN- $\gamma$  production is dependent on ICE/caspase-1 processing of pro-IL-18 to its mature form<sup>30</sup>. Since IL-12 has been shown to induce IFN- $\gamma$  production of RA synovial tissue cells<sup>31</sup> (Figure 5), the ability of RA ST to produce mature IL-18 would be important for its production of IFN- $\gamma$ . Several investigators have reported IFN- $\gamma$  production of lymphocytes from RA ST or synovial fluid either by flow cytometry analysis or immunohistochemistry<sup>8-11</sup>. In both RA ST and synovial fluid, the numbers of IFN- $\gamma$ -producing T cells are significantly higher than those of IL-4-producing T cells<sup>11</sup>, indicating the shift toward the Th1 phenotype in the joints of RA patients. IFN- $\gamma$  in ST would act in paracrine manner to further induce Th1 dominance<sup>6</sup> as well as to stimulate synovial macrophages. We recently reported that IFN- $\gamma$  and

CD40 ligand synergistically increase TNF- $\alpha$  and IL-1 $\beta$  production of synovial tissue cells<sup>4</sup>. Thus, IFN- $\gamma$  would also be relevant for the immunopathogenesis of RA through the activation of synovial macrophages and the induction of other proinflammatory cytokines.

The response of RA synovial tissue cells to IL-18 (Figure 5) also differed from that of OA synovial tissue cells. We observed that IL-18 and IL-12 synergistically enhance IFN- $\gamma$  production by RA synovial tissue cells. It has been reported that IL-12 induced the expression of IL-18R $\alpha$  on T cells<sup>32,33</sup>, which may contribute to the synergy between IL-12 and IL-18 in RA synovial tissue cells. Although the expression of IL-18R $\alpha$  mRNA in RA synovial tissue cells has been observed by RT-PCR<sup>21</sup>, this is the first report describing the expression of IL-18R $\beta$  mRNA in RA ST and the expression of IL-18R $\alpha$  and IL-18R $\beta$  mRNA in OA ST. The expression of both chains of IL-18R is required for IL-18 signal transduction. ST from patients with OA did not express or very weakly expressed IL-18R $\alpha$  and IL-18R $\beta$  mRNA (Figure 2). These data would explain the lack of response to IL-12 + IL-18 of OA synovial tissue cells.

The importance of IL-18 for Th1 response through induction of IFN- $\gamma$  has been well documented<sup>13</sup>. We have shown that synovial T cells, expanded in the presence of anti-CD3 Mab, IL-2, IL-12, and IL-18, produced larger amounts of IFN- $\gamma$  than those expanded with anti-CD3 Mab, IL-2, and IL-12 (Figure 6). These findings, together with the preferential response of RA synovial tissue cells to IL-12 + IL-18, strongly suggest the contribution of IL-18 to IFN- $\gamma$  production of synovial T cells which would be relevant to the deviation of synovial T cells to the Th1 phenotype in RA.

Recently, it was reported that IL-18 induced TNF- $\alpha$  production by synovial tissue cells and that IL-18 could exacerbate arthritis in a murine collagen induced arthritis model<sup>21,22</sup>. Since IL-18 has been shown to stimulate the production of several cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-8<sup>18</sup>, and GM-CSF<sup>19</sup>, it may contribute to the immunopathogenesis of synovial inflammation in RA not only as an IFN- $\gamma$  inducer but also as a proinflammatory cytokine with a broad range of biological functions. The importance of IL-18 in arthritis will be clarified by destruction of the IL-18 gene in mouse models of arthritis.

We found that mature IL-18 is more abundantly expressed than pro-IL-18 in rheumatoid arthritis synovial tissue, and that it contributed to IFN- $\gamma$  production by synovial T cells in RA but not in OA. Further studies of the interaction of IL-18 with other cytokines and/or with surface molecules will be required to clarify its precise role in RA.

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