Fibroblast-Like Synoviocytes from Rheumatoid Arthritis Patients Express Functional IL-15 Receptor Complex: Endogenous IL-15 in Autocrine Fashion Enhances Cell Proliferation and Expression of Bcl-xL and Bcl-2

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Fibroblast-Like Synoviocytes from Rheumatoid Arthritis Patients Express Functional IL-15 Receptor Complex: Endogenous IL-15 in Autocrine Fashion Enhances Cell Proliferation and Expression of Bcl-xL and Bcl-2

Mariola Kurowska,* Weronika Rudnicka,* Ewa Kontry,* Iwona Janicka,* Magdalena Chorazy,* Jacek Kowalczewski,† Maria Ziółkowska,* Sylvie Ferrari-Lacraz,‡ Terry B. Strom,§ and Włodzimierz Maśliński*‡§

The hallmark of rheumatoid arthritis (RA) is leukocytic infiltration of the synovium and expansiveness of fibroblast-like synoviocytes (FLS). The abnormal proliferation of FLS and their resistance to apoptosis is mediated, at least in part, by present in RA joints proinflammatory cytokines and growth factors. Because IL-15 exerts properties of antiapoptotic and growth factors, and is produced by RA FLS, we hypothesized that IL-15 participates in RA FLS activation. To test this hypothesis, we first examined whether RA FLS express chains required for high affinity functional IL-15R. Indeed, RA FLS express IL-15Rα at mRNA and protein levels. Moreover, we confirmed the presence of IL-2Rβ and common γ-chains. Interestingly, TNF-α or IL-1β triggered significant elevation of IL-15Rα chain at mRNA and protein levels. Next, we investigated the effects of exogenous or endogenous IL-15 on Bcl-2 and Bcl-xL expression, FLS proliferation, and apoptosis. Exogenous IL-15 enhanced RA FLS proliferation and increased the level of mRNA-encoding Bcl-xL. To test the role of endogenous IL-15 in the activation of RA FLS, an IL-15 mutant/Fcγ2a protein exerting properties of specific antagonist to the IL-15Rα chain was used. We found that blocking IL-15 biological activities using this protein substantially reduced endogenous expression of Bcl-2 and Bcl-xL, and RA FLS proliferation that was reflected by increased apoptosis. Thus, we have demonstrated that a distinctive phenotype of RA FLS, i.e., persistent activation, proliferation, and resistance to apoptosis, is related to the autocrine activation of IL-15Rs by FLS-derived IL-15. The Journal of Immunology, 2002, 169: 1760–1767.

The hallmark of rheumatoid arthritis (RA) is leukocytic infiltration of the synovium (1). This inflammatory process stimulates proliferation of apoptosis-resistant fibroblast-like synoviocytes (FLS). As a direct consequence, pannus formation and joint destruction result (2–5). Intraarticular expression of proinflammatory cytokines, especially TNF-α and IL-1β, play a key role in the pathogenesis of RA (6). IL-15, first identified in 1992, is expressed within the disease process and is produced by RA FLS, we hypothesized that IL-15 participates in RA FLS activation. To test this hypothesis, we first examined whether RA FLS express chains required for high affinity functional IL-15R. Indeed, RA FLS express IL-15Rα at mRNA and protein levels. Moreover, we confirmed the presence of IL-2Rβ and common γ-chains. Interestingly, TNF-α or IL-1β triggered significant elevation of IL-15Rα chain at mRNA and protein levels. Next, we investigated the effects of exogenous or endogenous IL-15 on Bcl-2 and Bcl-xL expression, FLS proliferation, and apoptosis. Exogenous IL-15 enhanced RA FLS proliferation and increased the level of mRNA-encoding Bcl-xL. To test the role of endogenous IL-15 in the activation of RA FLS, an IL-15 mutant/Fcγ2a protein exerting properties of specific antagonist to the IL-15Rα chain was used. We found that blocking IL-15 biological activities using this protein substantially reduced endogenous expression of Bcl-2 and Bcl-xL, and RA FLS proliferation that was reflected by increased apoptosis. Thus, we have demonstrated that a distinctive phenotype of RA FLS, i.e., persistent activation, proliferation, and resistance to apoptosis, is related to the autocrine activation of IL-15Rs by FLS-derived IL-15.
RA (28). The role of IL-15 in these processes is supported by evidence showing that administration of soluble IL-15Rα prevents collagen-induced arthritis in mice and effectively reduces inflammation, synovial hyperplasia, and adjacent bone erosion (29).

FLS are believed to actively contribute to the pathogenesis of RA. By aggressive invasion into the cartilage and production of metalloproteinases, these cells directly contribute to the destruction of joint tissue. Moreover, FLS are sources of many factors important in the perpetuation of inflammation: IL-8, IL-6, GM-CSF, and IL-1α,2, c gastrin (7, 8). These factors, along with other mediators, stimulate the expansion of T cell subsets that contribute to the pathogenesis of RA. By aggressive invasion into the cartilage and production of matrix-degrading enzymes, FLS contribute to the destruction of joint tissue and play a role in the pathogenesis of RA.

The role of IL-15 in these processes is supported by evidence showing that administration of soluble IL-15Rα prevents collagen-induced arthritis in mice and effectively reduces inflammation, synovial hyperplasia, and adjacent bone erosion (29). FLS are believed to actively contribute to the pathogenesis of RA. By aggressive invasion into the cartilage and production of metalloproteinases, these cells directly contribute to the destruction of joint tissue. Moreover, FLS are sources of many factors important in the perpetuation of inflammation: IL-8, IL-6, GM-CSF, and IL-1α,2, c gastrin (7, 8). These factors, along with other mediators, stimulate the expansion of T cell subsets that contribute to the pathogenesis of RA. By aggressive invasion into the cartilage and production of matrix-degrading enzymes, FLS contribute to the destruction of joint tissue and play a role in the pathogenesis of RA.

\[ \text{IL-15} / \text{IL-15R} / \text{H9253} / \text{15DM/Fc} \]

supplemented with rIL-1

\[ \text{IL-15} / \text{IL-15R} / \text{H9252} \]

involved in perpetuation of inflammation: IL-8, IL-6, GM-CSF, and IL-1α,2, c gastrin (7, 8). These factors, along with other mediators, stimulate the expansion of T cell subsets that contribute to the pathogenesis of RA. By aggressive invasion into the cartilage and production of matrix-degrading enzymes, FLS contribute to the destruction of joint tissue and play a role in the pathogenesis of RA.

Materials and Methods

Patients

A group of 32 patients who fulfilled the American College of Rheumatology (Atlanta, GA) criteria for the diagnosis of RA and were undergoing knee synovectomy or joint replacement surgery as a normal part of clinical care were included in this study. Subjects were 26 females, 6 males, age 21–78 years, mean ± SEM 55 ± 2.4, and duration of disease was 1.5–40 years, mean ± SEM 14.4 ± 1.8.

Cells

Synovial fibroblast cell lines were prepared from synovial samples obtained from RA patients as described previously (35). FLS were used for experiments after three to seven passages.

Expression of mRNA-encoding IL-15, IL-15Rα, IL-2/IL-15Rβ, IL-2/IL-15Rγ, Bcl-2, Bcl-xL, and GAPDH

For the analysis of IL-15, IL-15Rα, IL-2/IL-15Rβ, and IL-2/IL-15Rγ mRNA expression, culture flasks (Nunc, Roskilde, Denmark) were seeded with 0.6–1.5 × 10⁶ synoviocytes/ml for 2–3 days. Then, fresh medium supplemented with rIL-1β (1 ng/ml), rTNF-α (10 ng/ml), rbFGF (1/5 ng/ml; all from R& D Systems, Abingdon, U.K.), or rIL-2 (250 U/ml; Genzyme Genetics, Framingham, MA) was added, and cells were incubated for an additional 4 h. To test whether exogenous IL-15 modulates the expression of mRNA-encoding Bcl-2 and Bcl-xL, cells were incubated with rIL-15 (25 ng/ml; R&D Systems) in medium containing a low concentration of FCS (0.5%). To determine the role of endogenous IL-15 on the expression of mRNA-encoding Bcl-2 and Bcl-xL, directly after seeding, cells were incubated for 24 h in medium containing 2% FCS supplemented with: 1) IL-15DM/Fcγ1a (murine Fcγ1a; 5–10 μg/ml; Ref. 36) or IL-15DM/Fcγ1b (human Fcγ1b), a high-affinity receptor site-specific agonist for IL-15Rα-chain protein (both from Cardion Pharmaceuticals, Boston, MA); 2) relevant isotype controls, murine IgG2a or human IgG1, respectively (both Abs from R&D Systems); 3) goat anti-human IL-15 neutralizing Ab (5 μg/ml; or 4) control total goat IgG (both Abs from R&D Systems). After 24 h, one-third of the culture medium was discarded and fresh medium with supplementary proteins were added for another 24 h. Total RNA extraction, cDNA template preparation, and the RT-PCR were done as previously described (37). Quantitative competitive RT-PCR was used to measure bcl-2, bcl-xL, and GAPDH mRNA expression. In this technique, cellular RNA-derived cDNA was coamplified with the internal control using the primers for both templates as previously reported (38). Products of competitive bcl-2, bcl-xL, and GAPDH PCRs, separated on 2% agarose gel, were densitometrically scanned using Kodak 1D Image analysis software (Eastman Kodak, Rochester, NY). For every sample, the ratio of tested to internal control product was calculated. Next, each sample was normalized to GAPDH level. Expression of IL-15, IL-15Rα isoforms, IL-2Rβ, and γc were measured in simple RT-PCR and analyzed as described above. The rate of amplification of the particular mRNA isoform was calculated by the ratio of tested to internal control product.

Table 1. Sequences of used primers and expected product sizes

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FIGURE 1. Expression of IL-15 in resting and TNF-α- or IL-1β-stimulated RA FLS. A and B, FLS from RA patients were incubated with TNF-α (10 ng/ml) or IL-1β (1 ng/ml) or medium alone (lane C) for 4 h followed by total RNA extraction. A, The RT-PCR was performed using a pair of primers recognizing sequences present in cDNA encoding both IL-15 isoforms: 528 bp-long cytosolic and 409 bp-long secretory/membrane-bound protein. B, The level of GAPDH mRNA was used as a control. Representative RT-PCR products of three independent experiments with similar result are shown. C and D, The membrane and cytosolic protein fractions were isolated from the RA FLS as described in Materials and Methods. Proteins (15 μg/lane) were separated on 15% SDS-PAGE. Representative Western blots for membranes (C) and cytosolic (D) protein of four (for TNF-α) and one (for IL-1β) with similar results are shown.
product was within exponential range. The fine details of this system are shown in Table I.

Cell proliferation assay

Proliferation of FLS was assessed by incorporation of tritiated thymidine. For the assay, 96-well flat-bottom culture plates (Nunc) were seeded with 5 × 10^3 FLS in 0.2 ml of culture medium containing 0.5% FCS for 72 h. Then, the culture medium was removed and replaced for 24 h with fresh medium alone or medium supplemented with 25 ng/ml IL-15 (R&D Systems). To determine the contribution of endogenous IL-15 in the FLS proliferation, cells were incubated with murine IgG2a (0.5 μg/ml) in medium alone or medium supplemented with 2% FCS for 24 h. Then, medium was replaced with fresh, supplemented medium containing 5% FCS at 37 °C. The effects of cytokines on the expression of mRNA-encoding IL-15R isoforms in FLS by RT-PCR analysis, the analysis of IL-15R expression in FLS and the role of endogenous IL-15 on the expression of Bcl-2, Bcl-xL proteins, and in apoptosis, FLS were cultured with IL-15DM/Fc2a (50 ng), or isotype-matched negative control Ab (R&D Systems). Next, cells were stained with PE-conjugated goat anti-mouse IgG (5 μg/sample; DAKO, Glostrup, Denmark). To confirm binding specificity of IL-15DM/Fc2a to IL-15rα, cells were incubated with recombinant human IL-15 (150 ng/sample) for 15 min before their incubation with IL-15rα antagonist. For intracellular Bcl-2 and Bcl-xL staining, cell aliquots were permeabilized using Cytotox/Cytoperm kit (BD Biosciences, Mountain View, CA) according to the manufacturer’s protocol. Pepermized FLS were incubated with specific mAb, FITC-labeled anti-Bcl-2 Ab (5 μg/sample; DAKO), or Ab against Bcl-xL (0.5 μg/sample; BD Transduction Laboratories, Lexington, KY) or relevant control Ab (DAKO). For Bcl-xL, a secondary PE-conjugated goat anti-mouse IgG (5 μg/sample; DAKO) was used. For apoptotic cell death analysis, FLS were stained with FITC-conjugated annexin V and propidium iodide according to manufacturer’s instruction (Roche Diagnostic Systems, Mannheim, Germany). All analyses were done using FACSCalibur and CellQuest software (BD Biosciences).

Statistical analysis

Data are expressed as mean ± SEM. Where appropriate, the different groups were tested for statistical significance using paired two-tailed Student’s t tests. Values of p < 0.05 were considered to be statistically significant.

Results

Regulation of IL-15 expression by proinflammatory cytokines in RA FLS

RA synoviocytes, especially when stimulated with TNF-α or IL-1β, secrete IL-15 (34). However, it is not known whether FLS express only one or both known IL-15 isoforms: secretory/membrane and cytokin/cellular (9), and whether IL-15 protein is present on the FLS surface. To determine the expression of mRNA-encoding IL-15 isoforms in FLS by RT-PCR analysis, the

![FIGURE 2. The effects of cytokines on the expression of mRNA-encoding IL-15rα isoforms. Analysis of IL-15rα mRNA expression in FLS; with exon 3 (A) or lacking exon 3 (B). FLS from RA patients were stimulated with TNF-α (10 ng/ml; n = 3) or IL-1β (1 ng/ml; n = 5), or bFGF (1/5 ng/ml; n = 2) or IL-2 (250 U; n = 2) for 4 h. Total RNA was isolated, and RT-PCRs for IL-15rα and GAPDH were performed. Data were densitometrically scanned. For every sample, the expression of IL-15rα was normalized based on GAPDH level. Values are the mean (percentage of nonstimulated control) ± SEM; *, p < 0.05; **, p < 0.02 vs control (paired two-tailed Student’s t test). C, The representative results of RT-PCR products (selected from A and B) are shown. Lane C, Resting control cells.](http://www.jimmunol.org/)
set of primers recognizing specific sequences present in both IL-15 isoforms was used. Resting RA FLS express both IL-15 isoforms (3/3 RA FLS lines). Interestingly, the expression of mRNA-encoding secretory/membrane-bound IL-15 increased after a 4-h stimulation with TNF-α or IL-1β (450 and 312%, respectively; p < 0.05) (Fig. 1A). In contrast, FLS stimulation with bFGF, a known synoviocyte growth factor (30), exerted no effect on IL-15 isoforms mRNA expression (data not shown).

Because posttranscriptional regulation of IL-15 expression is more important (9), the expression of IL-15 protein in the cytosolic and membrane protein fractions isolated from cultured FLS was examined for IL-15 expression by Western blotting technique. As reported previously for other cells (9), in FLS, several bands representing different levels of IL-15 glycosylation were detected. Similar amounts of highly glycosylated IL-15 protein were present in both cytosolic and membrane fractions of nonstimulated RA FLS lines (Fig. 1, C and D). However, upon TNF-α stimulation, a significant increase in IL-15 expression was detected in the membrane (257%; p < 0.05), but not in cytosolic fraction (Fig. 1, C and D). In contrast, IL-1β did not affect the IL-15 level in either protein fraction. These data were further confirmed by flow cytometric analysis of surface-expressed IL-15 after extensive washing of receptor-bound IL-15 with low pH glycine buffer. Stimulation with TNF-α, but not with IL-1β, results in the increase of surface-expressed IL-15 (data not shown). Neither bFGF nor IL-2 enhanced surface-expressed IL-15 (data not shown). Thus, the TNF-α upregulated membrane-bound/secretory isoform of IL-15 is likely to dominate the pool of IL-15 produced by FLS.

RA FLS express all receptor chains required for IL-15-triggered signal transduction (IL-15Rα, IL-2/15Rβ, and IL-2/15Rγ)

To determine the expression of mRNA encoding for IL-15Rα, the set of primers recognizing extracellular domains of IL-15Rα isoforms was used (13). Using these primers, PCR yields two IL-15Rα chain-related products: 444 and 543 bp, lacking and containing exon 3, respectively. Each of the FLS samples isolated from nine RA patients constitutively expressed mRNA for both IL-15Rα chain isoforms (Fig. 2). Moreover, RA FLS expressed both IL-2Rβ and γ encoding mRNA (Fig. 3). Therefore, FLS express mRNA, encoding all three chains required for the formation of high affinity functional IL-15R complex. Surface-expressed IL-15Rα chain was further analyzed using previously described mutated IL-15 fused to the Fc fragment of murine IgG2a, IL-15DM/Fcγ2a (36), and flow cytometry. Roughly 50% of nonstimulated FLS express IL-15Rα (Fig. 4).

FIGURE 3. Expression of mRNA encoding IL-2Rβ and γ in RA FLS. The comparison of IL-2Rβ (A) or IL-2Rγ (B) mRNA expression in three RA FLS lines and PBMC from a healthy blood donor. Results are shown as RT-PCR products. Twenty microliters (FLS) and 5 μl (PBMC) of cDNA obtained from 1 μg total RNA were used as template. Lanes 1-3, FLS lines from three different RA patients.

FIGURE 4. Surface expression of IL-15Rα on resting and cytokines stimulated RA FLS. FLS were stimulated with cytokines as described in Fig. 2, but for 48 h. Cells were harvested by trypsin/EDTA treatment, incubated with IL-15DM/Fcγ2a or mouse IgG2a, and stained with PE-labeled goat anti-mouse IgG. IL-15Rα expression in nontreated (A and B) or TNF-α or IL-1β (A) or IL-2 or bFGF-stimulated cells (B). Representative histograms of three independent experiments with similar results are shown. C and D, Recombinant human IL-15 inhibits binding of IL-15DM/Fcγ2a to TNF-α-stimulated RA FLS. C, Cells were stained as described above. D, Cells were incubated with recombinant human IL-15 (150 ng) on ice for 15 min before staining with IL-15DM/Fcγ2a. E, The comparison of MFI. Data are expressed as the mean of MFI ± SEM. **, p < 0.008 and *, p < 0.03 vs spontaneous expression of IL-15Rα (paired two-tailed Student’s t test).

TNF-α and IL-1β, but not bFGF or IL-2, enhance the expression of IL-15Rα in RA FLS

As shown in Fig. 2, IL-1β and TNF-α significantly enhanced the expression of mRNA encoding for both IL-15Rα isoforms in cultured FLS. In contrast, bFGF or IL-2 exerted no stimulatory effect on the IL-15Rα mRNA level. Moreover, TNF-α, and to a lesser extent IL-1β, enhance surface-expressed IL-15Rα. Both percentage of IL-15Rα positive (Fig. 4A) and IL-15Rα chain density (judged by changes of mean fluorescence intensity (MFI); Fig. 4E) were elevated. The specificity of IL-15Rα detection was confirmed by preincubation of FLS with 3-fold excess of recombinant human IL-15 for 15 min before staining with IL-15 DM/Fcγ2a. As expected, this procedure resulted in complete blockade of detection of surface-expressed IL-15Rα (Fig. 4, C and D).

IL-15 enhances RA FLS proliferation

To determine whether IL-15R complex expressed upon FLS is functional, cells were stimulated with IL-15 in medium containing

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Proliferation of cultured RA FLS depends in part on the autocrine IL-15/IL-15R activation

Nonstimulated FLS express IL-2Rβ and γc. Expression of both IL-15 and IL-15Rα is enhanced in the presence of TNF-α (Figs. 1, 2, and 4). Thus, it is possible that endogenously produced IL-15, in an autocrine fashion, stimulates IL-15Rs providing signals for proliferation and/or preventing cell apoptosis. This hypothesis was tested using IL-15DM/Fcγ2a (36). This protein, exerting properties of high affinity species-specific IL-15R antagonist, inhibited FLS proliferation in a dose-dependent manner, while isotype matching control IgG2a Ab did not (Fig. 5B). Therefore, our results suggest that FLS-derived IL-15 binds to and activates IL-15Rs expressed on these cells. An IL-15 autocrine pathway helps to drive RA FLS cell proliferation.

Endogenous IL-15 is responsible for enhanced expression of Bcl-xL and Bcl-2 in RA FLS

IL-15, a growth factor, also protects many cell types from apoptotic death (18, 19, 40). To determine whether IL-15 elicits antiapoptotic effects upon RA FLS, the effect of IL-15 on the expression of mRNA encoding antiapoptotic proteins Bcl-2 and Bcl-xL were examined. As expected, FLS express mRNA encoding for both Bcl-xL and Bcl-2 (Fig. 6). Exogenously added IL-15 enhanced bcl-2 gene expression in RA FLS (Fig. 6B and C), however, in contrast to PBMC (data not shown), IL-15 did not elevate bcl-2 gene expression (Fig. 6A). These results raised the possibility that levels of mRNA-encoding antiapoptotic proteins present in FLS depend on the autocrine IL-15 network. To test this hypothesis, RA FLS were incubated for 48 h in the presence of IL-15DM/Fcγ2a protein or control mouse IgG2a Ab. As illustrated in Fig. 7, provision of the IL-15Ra antagonist, in contrast to control IgG2a, significantly inhibited spontaneous expression of mRNA-encoding Bcl-xL and Bcl-2. Similar results were obtained in experiments, where goat anti-human IL-15 neutralizing Ab was used to block biological activities of endogenous IL-15 (Fig. 8). The expression of Bcl-xL and Bcl-2 proteins were confirmed using specific Abs and flow cytometric analysis. Indeed, FLS express both tested proteins (Fig. 9). As predicted from changes at mRNA levels, FLS cultured in the presence of IL-15Ra antagonist exert diminished levels of Bcl-2 and Bcl-xL (Fig. 9). Similar data were obtained when cells were cultured in the presence of neutralizing anti-IL-15 Abs (data not shown).
Endogenous IL-15 prevents apoptosis of RA FLS

In the next set of experiments, we tested whether autocrine IL-15-triggered elevation of antiapoptotic proteins is reflected by the resistance of FLS to apoptosis. Indeed, enhanced apoptosis (32%) of FLS cultured in the presence of neutralizing anti-IL-15 Abs (Fig. 10 B) was observed in comparison with FLS cultured in the presence of isotype matching control IgG (11%; Fig. 10 A). Similar data were obtained when IL-15R antagonist was used (data not shown). These results indicate that endogenously produced IL-15 contributes to the antiapoptotic status of RA FLS.

Discussion

We have now tested the hypothesis that IL-15 participates in the activation of RA FLS. First, we confirmed recent a report by Harada et al. (34) that RA FLS spontaneously produce IL-15, and that IL-15 expression is further enhanced by TNF-α and IL-1β. Next, we expanded their findings by analyzing the expression of the two known IL-15 isoforms (9, 11) in RA FLS. In resting RA FLS, both secretory/membrane-bound and cytosolic/nucleus IL-15 isoforms are present at mRNA and protein levels (Fig. 1). It was most interesting to note that TNF-α and IL-1β treatment significantly elevated mRNA levels encoding the secretory/membrane-bound isoform (Fig. 1, A and B). In keeping with this observation, we determined at the protein level that TNF-α stimulation raised

![FIGURE 7.](http://www.jimmunol.org/)

**FIGURE 7.** IL-15R antagonist inhibits bcl-2 and bcl-2 mRNA expression in RA FLS. RA FLS were cultured in medium alone or supplemented with IL-15DM/Fcγ2a (10 μg/ml) or mouse IgG2a (10 μg/ml) for 48 h. The evaluation of mRNA expression for bcl-2, bcl-xL, and GAPDH was performed as described in Fig. 6. Data for bcl-2 (A) and bcl-xL (C) are expressed as the mean (percentage of IgG2a-treated control) ± SEM of four experiments. ***, p < 0.0002; *, p < 0.04; ns, not statistically significant (paired two-tailed Student’s t test). B and D. The representative results of RT-PCR products (selected from A and C, respectively) are shown. The position and size of internal control and cDNA derived from cellular mRNA are marked.

![FIGURE 8.](http://www.jimmunol.org/)

**FIGURE 8.** IL-15 neutralizing Ab inhibits bcl-xL and bcl-2 mRNA expression in RA FLS. RA FLS were cultured in medium alone or supplemented with goat anti-human IL-15 (5 μg/ml) or goat IgG (5 μg/ml) for 48 h. Total RNA was isolated and competitive RT-PCRs for bcl-2, bcl-xL, and GAPDH, in the presence of internal controls, were performed. The RT-PCR products from one representative experiment of two with similar results are shown.

![FIGURE 9.](http://www.jimmunol.org/)

**FIGURE 9.** IL-15R antagonist decreases Bcl-2 and Bcl-xL protein expression in RA FLS. FLS were cultured in the presence of IL-15DM/Fcγ1 (10 μg/ml) or human IgG1 (10 μg/ml) for 48 h. Cells were harvested by trypsin/EDTA treatment, fixed, permeabilized, and stained with anti-Bcl-2 or anti-Bcl-xL Ab or appropriate isotype control followed by flow cytometric analysis. One representative experiment of three for Bcl-2 (A) and two for Bcl-xL (B) with similar results are shown.
the expression of highly glycosylated membrane bound, but not cytosolic, IL-15 isoform (Fig. 1, C and D). This was further confirmed by the flow cytometric analysis. However, despite the increased level of mRNA encoding membrane-bound IL-15 upon IL-1β stimulation, there was no rise in membrane-bound IL-15 protein. This apparent discrepancy between levels of IL-1β-triggered IL-15 at mRNA (rise) and protein (no effect) levels led us to the hypothesis that IL-1β, more efficiently than TNF-α, triggers activation of a specific protease that cleaves surface expressed IL-15 protein. Our preliminary results support this suggestion (M. Kurowska, W. Rudnicka, M. Ziolkowska, and W. Maśliński, manuscript in preparation). Taken together, we provide the evidence that RA FLS express both cytosolic and membrane-bound/secretory forms of IL-15 protein, and that stimulation of FLS by proinflammatory cytokines (TNF-α or IL-1β) enhance expression of only the latter isoform at mRNA level. In addition, our data correspond well with those of Harada et al. (34), who reported that both TNF-α and IL-1β increase IL-15 production at the protein level. Based on previous findings showing that IL-15 expressed on the surface of monocytes (41), keratinocytes (42), and dermal fibroblast (43) is biologically active, it is quite possible that cell surface IL-15 expressed upon RA FLS also exerts biological activity.

Next, we examined the expression of mRNA encoding all subunits of the IL-15R complex, i.e., IL-15Rα and IL-2/15 β and γc. Eight splicing variants of the human IL-15Rα have been identified (44). These include all combinations of exon 2 deletion, exon 3 deletion, and alternative use of exon 7 or 7*. Because all, except exon 2 deletion isoforms bind IL-15 with very high affinity (13, 44), we have chosen PCR primers located in exon 2 (sense) and exon 5 (antisense). Using these primers, amplification of mRNA encoding all IL-15Rα variants that contain exon 2, and thus, bind IL-15, can be detected. All tested RA FLS constitutively express mRNA encoding IL-15Rα isoforms that bind IL-15 (Fig. 2). In addition, we confirmed previous findings of Corrigan et al. (45) that RA FLS constitutively express an additional two subunits required for IL-15-triggered signal transduction, i.e., IL-2Rβ and γc (Fig. 3). Furthermore, we found that proinflammatory cytokines TNF-α and IL-1β, in contrast to bFGF and IL-2, significantly enhanced the expression of IL-15Rα isoforms at both mRNA and protein levels (Figs. 2 and 4).

The RA joint microenvironment contains high levels of IL-15 (14) and low levels of IL-2 (46). Moreover, the cell surface phenotype of RA FLS is notable for the presence of IL-15Rα (Fig. 4), but not IL-2Rα (45). Thus, it seems likely that IL-15, but not IL-2, participates in RA FLS activation. In fact, provision of exogenous IL-15 stimulates RA FLS proliferation (Fig. 5A) and enhances the expression of mRNA encoding the antiapoptotic protein Bcl-xL (Fig. 6, B and C). Because RA FLS secrete IL-15 and express both surface bound IL-15 and functional IL-15Rs, we tested the hypothesis that an IL-15-dependent autocrine loop contributes to the RA FLS phenotype. Indeed, we found that blockade of the IL-15 pathway using either an IL-15R antagonist or neutralizing anti-IL-15 Abs substantially reduces expression of Bcl-xL and Bcl-2 at mRNA and protein levels (Figs. 7–9). The latter finding, i.e., inhibition of Bcl-2 expression by anti-IL-15 treatment, indicates that also this antiapoptotic protein is regulated by IL-15. In contrast, exogenous IL-15 failed to increase Bcl-2 expression in RA FLS (Fig. 6, A and C). One possible explanation of these somehow opposite results is that endogenous RA FLS-derived IL-15 triggers maximal Bcl-2 expression, and the level of its expression cannot be further enhanced by addition of exogenous IL-15. The role of endogenous IL-15 in FLS activation was further confirmed in experiments where blockade of IL-15 pathway inhibited these cells proliferation (Fig. 5B) and increased their apoptosis (Fig. 10). The presence of a similar endogenous IL-15/IL-15Rα loop has been postulated as the mechanism of tumor propagation in multiple myeloma (47) and the pathogenesis of psoriasis (42). Recently, Sen and coworkers (48, 49) have postulated that Wnt and Frizzled families, which control cell fate determination during embryogenesis, may contribute to the autonomous RA FLS activation. Our findings are consistent with their observation that normal FLS transfected with wnt-5A expression vector acquired RA FLS-like, persistent activation status manifested by significant production of IL-15 (48, 49).

Taken together, our data provide the evidence that the distinctive phenotype of RA FLS, i.e., persistent activation, spontaneous proliferation, and resistance to apoptosis, is partially related to the autocrine activation of IL-15Rs by FLS-derived IL-15. These results further underline the important role of IL-15 in the pathogenesis of RA.

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