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-x<sub>L</sub> and Bcl-2<sup>1</sup>

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The hallmarks of rheumatoid arthritis (RA) are 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-2β 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-x<sub>L</sub> expression, FLS proliferation, and apoptosis. Exogenous IL-15 enhanced RA FLS proliferation and increased the level of mRNA-encoding Bcl-x<sub>L</sub>. To test the role of endogenous IL-15 in the activation of RA FLS, an IL-15 mutant/Fc<sub>γ</sub> subunit was used. We found that blocking IL-15 biological activities using this protein substantially reduced endogenous expression of Bcl-2 and Bcl-x<sub>L</sub>, 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)<sup>1</sup> 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 inflammatory reaction in the joint containing a smaller (21 aa) signal sequence, is found within the cytoplasm or nucleus (9–11). The high-affinity IL-15R is heterotrimeric. The complex contains β and common γ-chain (γ<sub>γ</sub>) subunits that also serve as essential components of the IL-2R. The third receptor component is the IL-15-specific IL-15Rα chain. The shared IL-2/15 β and γ<sub>γ</sub> are responsible for transducing IL-15- and IL-2-triggered intracellular signals. The specificity for IL-15 versus IL-2 binding is provided by unique cytokine-specific, private α-chain receptor subunits. Monomeric IL-15Rα, but not IL-2Rα, chains provide a cytokine-specific high-affinity (K<sub>d</sub> = 10<sup>−11</sup> M) binding site. Expression of both IL-2 and IL-15Rα receptors is restricted to activated T cells, while IL-15- and IL-15Rα transcripts have a broader tissue distribution (12, 13).

McInnes et al. (14, 15) suggested that IL-15 may play a primary role in the development of RA. High levels of this cytokine are present in synovial fluid, synovial membrane, and serum isolated from RA patients. IL-15 may increase the number of cells involved in inflammatory reaction in the joint by directly: 1) stimulating migration of neutrophils and T lymphocytes into the joint (14, 16, 17), 2) protecting these cells from apoptosis (18, 19), and 3) triggering the proliferation of memory CD8<sup>+</sup> and CD4<sup>+</sup> naive CD8<sup>+</sup> T cells (20–22), and/or indirectly by inducing expression of proinflammatory cytokines TNF-α (15, 23), IL-1β (23), IL-17 (24), and IL-8 (25, 26), as well as inflammation-inhibiting free radicals (27). IL-15 may also participate in local bone destruction in

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<sup>4</sup>Abbreviations used in this paper: RA, rheumatoid arthritis; FLS, fibroblast-like synoviocytes; bFGF, basic fibroblast growth factor; γ<sub>γ</sub>, common γ-chain; MFI, mean fluorescence intensity.
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 involved in perpetuation of inflammation: IL-8, IL-6, GM-CSF, PGE2 (3), autocrine growth factor-basic fibroblast growth factor (bFGF; Ref. 30), and angiogenic factor-vascular endothelial growth factor (31). In addition, the phenotype of RA FLS is similar to transformed cells as the phenotype notable for anchorage independence, spontaneous expression of protooncogenes, c-myc, c-fos, mutated p53, and expression of antiapoptotic proteins Bcl-2, Bcl-xL, and sentrin-1 (2, 32, 33). Moreover, FLS are important sources of IL-15 in RA joints. Indeed, IL-15 expressed by RA FLS may be responsible for intraarticular T cell activation and expansion (34). The aim of this study was to test the hypothesis that IL-15 is critical to FLS activation and survival.

### 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 who 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 ± 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 × 10^6 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/Fcγ/2a (murine Fcγ/2a; 5–10 μg/ml; Ref. 36) or IL-15DM/Fcγ/1 (human Fcγ/1), a high-affinity receptor site-specific antagonist for IL-15-Rα 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 pair of 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. The 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 isoforms is shown.

### 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 results 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 membrane (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 × 10^5 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) or antagonist of IL-15Rα (IL-15DM/Fc2a) (0.5 μg/ml; Ref. 36), and FLS were cultured for an additional 72 h. [3H]Thymidine (2 μCi/ml; Amersham Pharmacia Biotech, Little Chalfont, U.K.) was added 18 h before the termination of the cell culture, and radioactivity of the samples were measured as described (35).

### Western blotting

To assess spontaneous, or TNF-α (10 ng/ml) or IL-1β (1 ng/ml) triggered expression of IL-15 protein, synoviocytes (1 × 10^5/ml) were cultured in medium containing 5% FCS at 37°C for 48 h. Expression of IL-15 was analyzed in cytosolic and membrane protein fractions according to the method previously described (39). Proteins were separated on 15% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA). Membranes were blocked with 5% nonfat milk and incubated with rabbit anti-human IL-15 Ab (2 μg/ml; PeproTech, London, U.K.) overnight at 4°C. The bands were visualized by application of peroxidase-conjugated goat anti-rabbit IgG (dilution, 1/2000; Sigma-Aldrich, St. Louis, MO) and ECL system (Amersham Pharmacia Biotech). 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 < 0.002 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.

![Figure 2](http://www.jimmunol.org/)

**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 < 0.002 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.

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 cytosolic/nucleus (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 < 0.002 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.
The comparison of IL-2Rβ and γc 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.

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

To determine the expression of mRNA encoding for IL-15Rα, the 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-1, 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 up-regulated membrane-bound/secretry isomorph of IL-15 is likely to dominate the pool of IL-15 produced by 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).

**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 isotype control, #, p < 0.03 vs spontaneous expression of IL-15Rα (paired two-tailed Student’s t test).
FIGURE 5. IL-15 stimulates FLS proliferation. A, Exogenous IL-15 stimulates FLS proliferation. FLS from six RA patients were cultured for 72 h in medium with low FCS (0.5%). Then, the culture medium was replaced for 24 h with fresh medium or fresh medium supplemented with IL-15 (25 ng/ml). [3H]Tdr was added simultaneously with IL-15. Data are expressed as mean (percentage of nonstimulated control) ± SEM; *, p < 0.02 compared with medium alone (paired two-tailed Student’s t test). B, IL-15Rα antagonist inhibits FLS proliferation. RA FLS were incubated with murine IgG2a (2 μg/ml) in medium with 2% FCS for 24 h, then medium was replaced with fresh, supplemented with mouse IgG2a (0.5; 2 μg/ml) or IL-15DM/Fcγ2a (0.5; 2 μg/ml). Cells were cultured for 72 h. [3H]Tdr was added 18 h before culture termination. All data are the average of triplicate samples, and the error bars represent the sample SEM. Similar results were obtained in two independent experiments. #, p < 0.03 vs nontreated cells (medium); *, p < 0.04 vs IgG2a (0.5 μg/ml) treated cells; **, p < 0.009 vs IgG2a (2 μg/ml) treated cells (paired two-tailed Student’s t test).

low FCS (0.5%). As shown in Fig. 5A, provision of IL-15 significantly enhanced proliferation of quiescent FLS (176% of unstimulated control).

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 stereo-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-xL gene expression in RA FLS (Fig. 6, B and C); however, in contrast to PBMC (data not shown), IL-15 did not elevate bcl-2 gene expression (Fig. 6, A and C). 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-15R 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. 10B) was observed in comparison with FLS cultured in the presence of isotype matching control IgG (11%; Fig. 10A). Similar data were obtained when IL-15R antagonists were 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...
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 increase 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, 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 γ. 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 Corrigall 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-15Rα, 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 distinct 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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References


