Inhibited Apoptosis of Synovial Fluid Lymphocytes in Children with Juvenile Idiopathic Arthritis Is Associated with Increased Expression of Myeloid Cell Leukemia 1 and XIAP Proteins

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ABSTRACT. ***Objective.*** Inhibited apoptosis of lymphocytes present in synovial fluid (SFL) and persistently infiltrating synovial tissue may be crucial in the pathogenesis of rheumatoid arthritis (RA). Similarly, this may be the case in juvenile idiopathic arthritis (JIA). Little is known about lymphocyte apoptosis in this disease. Recently, we reported significantly enhanced apoptosis of peripheral blood lymphocytes (JIA-PBL) compared to synovial fluid (JIA-SFL) or healthy lymphocytes, with downregulation of p53 in JIA-SFL. In this study we assessed other possible molecular mechanisms of this phenomenon.

Methods. PBL from 31 children with JIA and 26 healthy children were examined. SFL obtained from 18 patients was also studied. Apoptosis was assessed by TdT-mediated dUTP-biotin nick-end labeling (TUNEL) method. Expression of several apoptosis-regulating proteins was analyzed, including myeloid cell leukemia 1 (Mcl-1), cross-linked inhibitor of apoptosis (XIAP), FLICE-inhibitory protein (FLIP), or Bcl-xL inhibitors and proapoptotic p53, Bcl-w, Bak, and Bid.

Results. We found significant overexpression of Mcl-1 and XIAP in JIA-SFL ($p < 0.001$ and $p < 0.02$, respectively). Expression of Mcl-1 and XIAP in SFL correlated inversely with the apoptotic index ($p < 0.002$ and $p < 0.01$, respectively). FLIP expression was also distinctly higher in SFL than in JIA-PBL; however, the difference was not statistically significant ($p = 0.061$). No statistically significant differences were found in the expression of other proteins between SFL and PBL.

Conclusion. This is the first study showing that upregulation of anti-apoptotic Mcl-1 and XIAP proteins, along with downregulation of p53 protein, is correlated with inhibition of JIA-SFL apoptosis. (J Rheumatol 2006;33:1684–90)

Key Indexing Terms:
JUVENILE IDIOPATHIC ARTHRITIS APOPTOSIS
PROTEINS SYNOVIAL FLUID LYMPHOCYTE

Defective regulation of apoptosis in cells present in synovial tissue, synovial fluid (SF), and circulating immune response cells may contribute to the pathogenesis of rheumatoid diseases. Inhibited apoptosis of residual stromal cells, synoviocytes, in patients with rheumatoid arthritis (RA) has been reported[1-3]. Inhibited apoptosis of T cells, correlated with impaired Fas signaling pathway, was also found in RA synovial tissue[4,5]. Moreover, deficient ligand expression was found on SF lymphocytes (SFL)[6]. Those abnormalities can be responsible for ineffective clearance of activated T cells in RA joints and persistent infiltration of rheumatoid synovium. Additionally, RA synoviocytes express elevated levels of tumor necrosis factor-α (TNF-α), inducing nuclear factor-κB (NF-κB) prosurvival signaling pathway[7].

Several other abnormalities in apoptosis-regulating protein expression in RA synovial tissue have been reported. Namely, coexpression of antiapoptotic Bcl-xL and, surprisingly, proapoptotic Bax proteins in synovial cells correlated with lower incidence of their apoptosis[3]. Moreover, expression of p53 has been found in RA[8,9] and juvenile idiopathic arthritis (JIA) synoviocytes[10], but clinical implications of these findings remain unclear.

One of the apoptosis-regulating proteins that may influence inhibition of RA synoviocyte apoptosis is Bcl-2, highly expressed in synovial tissue from RA patients[1,11]. Bcl-2 expression in synovial fibroblast was found to be correlated with higher viability of mitochondrial membrane, protecting synoviocytes from apoptosis[11]. Most recently, overexpression of FLICE-inhibitory protein (FLIP) in synovial tissue has been postulated as a mechanism responsible for inhibited syn-

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oviocyte apoptosis in RA[12]. Synovial macrophages were also found to express high levels of FLIP in patients with early stages of RA[2]. Moreover, the inhibitor of apoptosis (IAP) family of proteins, cross-linked IAP (XIAP) and cellular inhibitor of apoptosis 2 (cIAP2), were found to be expressed in unstimulated synovial cells from RA synovial tissue[13]. XIAP is a TNF-α-inducible specific inhibitor of apoptosis of the RA synovial fibroblast cell line[14], whereas in *ex vivo* RA synovial cells the expression of both XIAP and cIAP2 was found to be downregulated by TNF-α[13].

On the other hand, synoviocytes may interact with immune response cells in SF, maintaining their survival and proliferative response. Stromal cell line from RA tissue has been shown to induce overexpression of Bcl-xL in activated lymphocytes present in RA inflammatory joints[15].

So far, these problems have been poorly explored in JIA. Recently, differences in the intensity of spontaneous lymphocyte apoptosis in peripheral blood (PBL) and in SF were reported in children with JIA[16]. Higher incidence of JIA-PBL was associated with elevated Bax/Bcl-2 ratio, mainly due to Bcl-2 downregulation. Further, a significantly decreased p53 expression has been found in JIA-SFL[17]. We assessed additional possible mechanisms involved in the regulation of PBL/SFL apoptosis on the level of apoptosis-regulating proteins. We measured the expression of proapoptotic Bcl-w, Bak, and Bid proteins as well as apoptosis inhibitors such as myeloid cell leukemia 1 (Mcl-1), Bcl-xL, FLIP, and XIAP. Additionally, to confirm our previous observation, the p53 protein was included in the current analysis.

MATERIALS AND METHODS

Patients. The study was performed in 31 children with JIA, including 20 girls and 11 boys, median age 12 years (range 3–18). Twenty-six age- and sex-matched healthy children served as controls. The diagnosis was established according to the Durban criteria[18]. Within the examined group, 10 children were untreated (newly diagnosed). In another 3 children only non-steroid anti-inflammatory drugs (NSAID) were used. Others were treated with methotrexate (10 children) or sulfasalazine (8 children). To avoid potential influence of that treatment on parameters analyzed, those children were enrolled in the study only if their clinical status allowed stopping drug administration for at least one week before blood sample collection.

The study was conducted in accord with the Helsinki Declaration and was approved by The Ethical Committee for Scientific Research at the Medical University of Lodz. All specimens were collected with the written consent of the parents.

Cell isolation. PB mononuclear cells (PBMC) were isolated from either heparinized blood (31 children with JIA and 26 healthy children) or heparinized SF (18 patients with JIA), obtained during exploratory or therapeutic joint puncture. A 1:1 (v/v) mixture of either PB or SF and Hanks' balanced salt solution, HBSS (Biomed, Lublin, Poland) was layered on top of Histopaque-1077 media (Sigma Diagnostic, St. Louis, MO, USA) in centrifuge tubes and centrifuged for 30 min at 200 g. The interphase region containing PBMC was collected and then washed twice, in HBSS and RPMI 1640 medium. Then, PB or SF PBMC were resuspended in 0.5 ml of phosphate buffered saline (PBS; Sigma Aldrich Chemie Gmbh, Steinheim, Germany) (1:1). Next, cells were fixed in 1% methanol-free paraformaldehyde (Polysciences, Inc., USA; 15 minutes, 0ºC) and in 70% ethanol (15 minutes, 0ºC). Afterwards, samples were stored at –20ºC, and then subjected to protein expression analysis.

Assessment of apoptosis. Apoptosis was assessed by DNA fragmentation detection according to TdT-mediated dUTP-biotin nick-end labeling (TUNEL) vs DNA content method, in the modification applying BrdUTP (APO-BRDU kit, Phoenix Flow Systems, San Diego, CA, USA)[19]. In this method, the 3'OH termini of the DNA strand breaks serve as primers and are labeled in this procedure with BrdU, when incubated with BrdUTP in a reaction catalyzed by exogenous terminal deoxynucleotidyl transferase (TdT). The incorporated BrdU is immunocytochemically detected by BrdU antibody conjugated to fluorescein isothiocyanate (FITC). Before the staining, cells were fixed (as above). After 60 min incubation with reagents, the cellular DNA was counterstained with 5 μg/ml of PI in the presence of 100 μg/ml RNase A (DNase-free) (Sigma Aldrich, Glostrup, Denmark) in PBS. Cell green (FITC) vs red (PI) fluorescence was measured during the next 15 min by dual-color flow cytometry.

The apoptotic index (AI) was calculated as a percentage of TUNEL-positive cells.

Expression of apoptosis-regulatory proteins. Expression of apoptosis-regulating proteins was analyzed in the Ctrl-PBL, JIA-PBL, and JIA-SFL, such as Mcl-1, Bcl-xL XIAP, or FLIP inhibitors and pro-apoptotic Bcl-w, Bak, and Bid. Since the number of cells that can be obtained from a SF sample is relatively low, the flow cytometry technique was chosen as most suitable for this purpose. The measurements were made in fixed cells, and permeabilized with 0.1% polysorbate 20 (Tween-20) in PBS (Amersham Biosciences Inc., Freiburg, Germany). Additionally, mouse anti-human monoclonal antibodies (Mab) anti-p53 (clone DO-7; Dako, Glostrup, Denmark) were used in concentrations 1:30 and 1:15, respectively; time of incubation was 30 min, at room temperature, in the dark. Anti-Mcl-1 (Calbiochem-Novabiochem Corp., San Diego, CA, USA), anti-Bak and anti-Bid (both Abgent, San Diego, CA, USA), anti-Bcl-w (Becton-Dickinson, San Jose, CA, USA), and anti-Bcl-xL (BD Pharmingen, CA, USA) primary rabbit anti-human antibodies were used in concentration 1:500 (60 min at room temperature). Mouse anti-human anti-XIAP Mab (Oncogene Research Products, San Diego, CA, USA) and rabbit anti-human anti-FLIP antibody (Calbiochem-Novabiochem) were used in the dilutions 1:100 and 1:1000, respectively (time of incubation 60 min at room temperature). Secondary swine anti-rabbit or goat anti-mouse antibodies were used at concentrations 1:20 and 1:50, respectively (30 min incubation, at room temperature, in the dark). Every antibody dilution was made in 1% PBS-BSA (bovine serum albumin). Additionally, to exclude unspecific intracellular staining control measurements were performed. Staining with irrelevant IgG followed by fluorochrome conjugated secondary antibodies was done for every protein studied. All staining procedures were adapted to flow cytometry after a series of preliminary studies, performed on healthy or leukemic PBL, comparing results of flow cytometry measurements with Western blot and fluorescence microscopy analysis.

Flow cytometry analysis. All fluorescence measurements were performed by flow cytometry (FACScan; Becton-Dickinson), using standard fluorescence filters: FL1 (green) and FL2 (orange) or FL3 (red), when necessary. Roughly 10,000 cells were measured per sample. Using forward versus side scatter discrimination method, the population of lymphocytes was identified from other PBMC for further analysis. Levels of protein expression were evaluated by mean fluorescence intensity (MFI) of the particular sample.

Additionally, the percentage of protein-positive cells was calculated in every case. However, in the statistical analysis MFI values were used, as a sensitive, accepted indicator of changes in protein expression[20].

Assessment of other laboratory measures. In parallel to protein expression studies, serum hemoglobin level, PB leukocyte and platelet counts, antinuclear antibodies (ANA), erythrocyte sedimentation ratio (ESR), rheumatoid factor (RF), and level of C-reactive protein (CRP) were assessed in patients with JIA using routine methods.

Statistics. The differences between values in more than 2 groups were evaluated with the Kruskal-Wallis ANOVA rank and median test. Then the Mann-Whitney test was performed for indirect comparisons. Wilcoxon test

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for paired samples was used for comparison of the results from PB and SF in particular patients. The correlation between features was evaluated using the Spearman rank coefficient p. In all tests p values less than 0.05 were considered statistically significant.

RESULTS

Clinical and laboratory characteristics of examined children with JIA are shown in Tables 1A and 1B. The mean time interval from first symptoms of JIA and the diagnosis was 1.4 ± 0.8 years. Twelve children had oligoarthritis, 15 polyarthritis, and 4 systemic type of JIA onset. Additionally, 3 stages of JIA activity were distinguished based on clinical and laboratory criteria: (1) low activity (limitation of joint movement, without pain or swelling, no extraarticular symptoms, ESR < 20 mm/h, CRP < 10 mg/l); (2) moderate activity (moderate intensity of arthritis, and/or slightly elevated temperature, ESR 20–60 mm/hour, CRP 1–3 ng/ml); and (3) high activity (morning stiffness, pain and/or swelling of joints, and/or hepatosplenomegaly, fever, rash, and raised values for laboratory tests ESR > 60 mm/hour, CRP > 3 ng/ml)[9]. Disease activity was considered low in 12, moderate in 10, and high in 9 examined children with JIA.

Apoptosis of synovial fluid lymphocytes in JIA. The rate of lymphocyte apoptosis differed statistically depending on assessed compartments (p = 0.0001, Figure 1). AI in JIA-SFL (mean 0.31 ± 0.45%) was significantly lower than in PBL from both patients with JIA (2.51 ± 1.15%; p = 0.002) and controls (0.84 ± 0.53%; p = 0.01). There were no differences in lymphocyte AI between untreated and treated children with JIA.

Table 1A. Characteristics of patients with JIA.

Characteristic	No. of Patients/Value
Total number	31
Sex, girls, boys	20, 11
Age, median/range, yrs	12/3–18
Type of onset, no. of patients	
Systemic disease	4
Polyarthritis	15
Oligoarthritis	12
Disease activity, no. of patients	
Low	12
Moderate	10
High	9
Rheumatoid factor, no. of positive patients (%)	4 (12.9)
Antinuclear antibodies, no. of positive patients (%)	11 (35.5)

Table 1B. Laboratory results, by disease activity.

Result	Disease Activity		
	Low, n = 12	Moderate, n = 10	High, n = 9
Hemoglobin, mg/dl	12.9 ± 0.5	11.8 ± 0.2	10.0 ± 0.6
Leukocytes, g/l	5.5 ± 1.4	6.1 ± 2.6	7.3 ± 1.9
Platelets, g/l	247.8 ± 33.2	309.0 ± 73.5	529.0 ± 86.7
ESR, h	7.5 ± 3.0	32.5 ± 14.8	83.0 ± 36.9
CRP, ng/ml	0.1 ± 0.2	1.0 ± 0.8	6.1 ± 2.06

Expression of apoptosis-regulating proteins in JIA and healthy lymphocytes. Among the apoptosis-regulating proteins studied, only Mcl-1, XIAP, and p53 protein expression showed significant differences between children with JIA and controls or between PBL and SFL (Table 2).

Thus, Mcl-1 expression was significantly higher in JIA-SFL (MFI 139.7 ± 62.4) than in JIA-PBL (MFI 90.3 ± 37.9; p = 0.0015) and healthy control PBL (Ctrl-PBL) (MFI 102.7 ± 57.1; p = 0.045). Similarly, XIAP expression was the highest in SFL (MFI 104.0 ± 37.7), when compared to either JIA-PBL (MFI 77.6 ± 31.0; p = 0.011) or Ctrl-PBL (MFI 73.4 ± 41.6; p = 0.016) (Figure 2). Moreover, expression of both Mcl-1 and XIAP in children with JIA correlated inversely with the percentage of TUNEL-positive SFL (p = –0.69; p = 0.001 and p = –0.52; p = 0.027, respectively) (Figure 3).

Expression of p53 was significantly decreased in JIA-SFL (MFI 30.5 ± 14.8) in comparison to either Ctrl-PBL (MFI 49.9 ± 19.2; p = 0.039) or JIA-PBL (MFI 57.1 ± 22.1; p = 0.011) (Table 2, Figure 2).

FLIP expression also showed a distinct trend toward higher values in JIA-SFL; however, this relationship was slightly below statistical significance. Namely, MFI in JIA-SFL was 147.5 ± 27.5 versus 95.4 ± 35.4 in Ctrl-PBL (p = 0.053) and 101.7 ± 39.7 in JIA-PBL (p = 0.061) (Table 2).

Additional statistical analysis on paired samples (N = 18) showed a higher level of statistical significance of differences in the expression level of Mcl-1 and XIAP between JIA-PBL and JIA-SFL (p < 0.001 and p = 0.003, respectively). This also confirmed the significance of differences in p53 expression (p = 0.025) (Table 2).

No statistically significant differences were found in expression of other apoptosis-regulating proteins between SFL and PBL (Table 2). There were no differences in protein expression between children newly diagnosed with JIA and those already treated with NSAID, methotrexate, or sulfasalazine.

Protein expression and clinical and laboratory measures of JIA children. Expression of apoptosis-regulating proteins was correlated with several clinical and laboratory indicators of patients with JIA. Peripheral blood leukocyte count was found to be correlated with Mcl-1 (R = 0.38; p = 0.041) and Bcl-xL (R = 0.36; p = 0.048) protein expression. Interestingly, significantly higher expression of Bcl-w protein was found in boys than in girls with JIA (p = 0.043).

There was no other statistically significant correlation of apoptosis-regulating protein expression in JIA-PBL or JIA-SFL and clinical indicators, such as age, sex, type of onset or activity of the disease, or with laboratory measures including PB leukocyte and platelet counts, hemoglobin level, presence of ANA or RF in serum, ESR, and CRP levels in patients with JIA.

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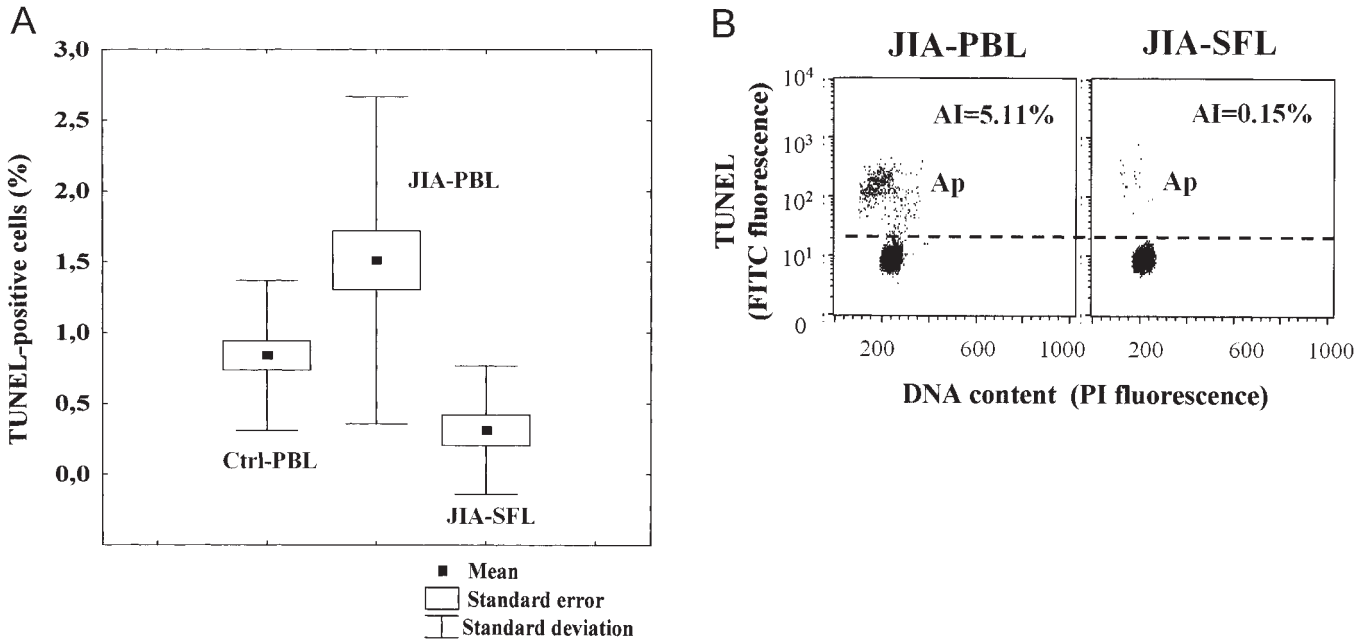

Figure 1. A. Apoptotic index of lymphocytes in synovial fluid from children with juvenile idiopathic arthritis (JIA-SFL) is significantly lower than in peripheral blood JIA lymphocytes (JIA-PBL) and in peripheral blood lymphocytes from healthy controls (Ctrl-PBL). p = 0.0001, Kruskal-Wallis test. B. Apoptosis detection by flow cytometry. The sample scatterplots show distribution of TUNEL-positive cells (green FITC fluorescence, Y axis) vs DNA content (red PI fluorescence, X axis). The percentage of TUNEL-positive cells (Ap) was defined as an apoptotic index (AI). The figure shows results obtained in samples from one representative patient. Relatively high spontaneous apoptosis of JIA-PBL was detected, whereas AI of JIA-SFL was very low. TUNEL: TdT-mediated dUTP-biotin nick-end labeling.

Table 2. Expression of apoptosis-regulating proteins in synovial fluid (SFL) and peripheral blood lymphocytes (PBL) from patients with juvenile idiopathic arthritis (JIA). Comparison to healthy control lymphocytes (Ctrl-PBL). Mean fluorescence intensity values ± SD are shown.

Proteins	Ctrl-PBL, n = 26 (1)	JIA-PBL, n = 31 (2)	JIA-SFL, n = 18 (3)	p Mann-Whitney	p Wilcoxon test* (2 vs 3; n = 18)
Bak	122.7 ± 32.7	124.4 ± 24.4	126.7 ± 56.7	NS	NS
Bid	86.7 ± 30.7	76.0 ± 29.1	73.5 ± 31.9	NS	NS
Bcl-w	99.9 ± 40.0	127.1 ± 29.1	111.9 ± 32.0	NS	NS
p53	49.9 ± 19.2	57.1 ± 22.1	30.5 ± 14.8	1 vs 3, p = 0.039 2 vs 3, p = 0.011	p = 0.025
Mcl-1	102.7 ± 57.1	80.3 ± 37.9	139.7 ± 62.4	1 vs 3, p = 0.045 2 vs 3, p = 0.0015	p = 0.0007
Bcl-xL	100.7 ± 41.6	136.0 ± 31.0	93.5 ± 37.7	NS	NS
XIAP	73.4 ± 41.6	77.6 ± 31.0	104.0 ± 37.7	1 vs 3, p = 0.016 2 vs 3, p = 0.011	p = 0.003
FLIP	95.4 ± 35.4	101.7 ± 39.7	147.5 ± 27.5	NS	NS

* Wilcoxon test for paired samples. NS: not significant.

DISCUSSION

Our results indicate that high expression of apoptosis inhibitors, Mcl-1 and XIAP proteins, in parallel with low expression of p53 protein, correlates with decreased rate of spontaneous apoptosis of JIA-SFL.

Inhibition of SFL apoptosis has been described in RA[4]. Deficient expression of Fas ligand on RA synovial lymphocytes was suggested to be responsible for ineffective clearance of activated cells in the RA joint[6]. However, some reports indicated overexpression of Fas and aberrant Fas signaling in SFL from patients with RA[4,5]. According to proteins responsible for regulation of the mitochondrial pathway of apoptosis, low Bcl-2 and high Bcl-xL expression have been shown in RA-SFL[4,21]. Moreover, SFL rapidly underwent apoptosis when cultured in vitro, suggesting that the blockage of its apoptosis is due to external regulation in

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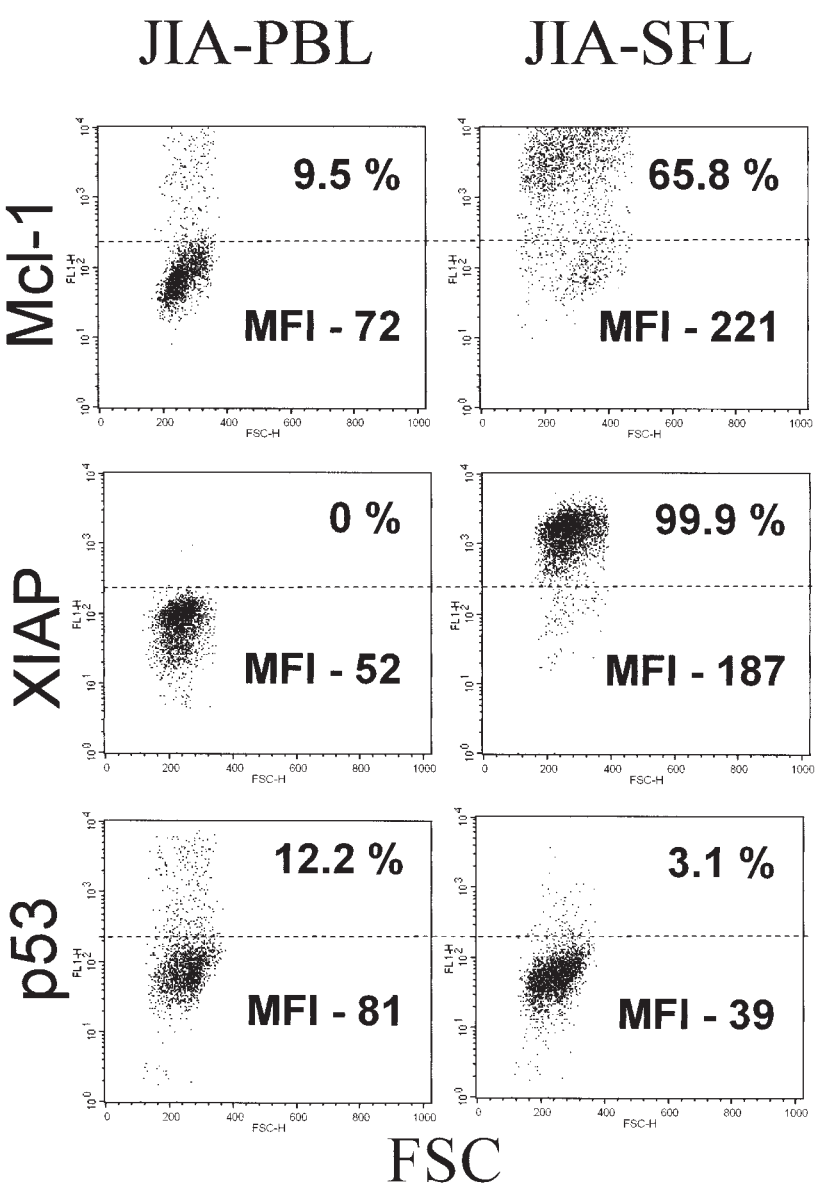

Figure 2. Expression levels of Mcl-1, XIAP, and p53 proteins in peripheral blood versus synovial fluid lymphocytes (PBL and SFL, respectively) as measured by flow cytometry. Representative scattergrams with the percentage and mean fluorescence intensity (MFI) as the measure of protein expression are shown. Mcl-1: myeloid cell leukemia 1. XIAP: cross-linked inhibitor of apoptosis.

the joint rather than to an intrinsic defect. In recent studies, interleukin 15 (IL-15) was shown to inhibit activation-induced apoptosis of RA-SFL and synoviocytes, via upregulation of Bcl-2 and Bcl-xL antiapoptotic protein expression in those cells[22]. Moreover, IL-15 expressed on JIA synovial vascular cells promotes their survival, which can lead to stabilization of new vascular structures formed in the synovium[23]. Abnormally high IL-15 levels were previously found in SF from children with JIA. Serum IL-15 concentrations exceeded by about 20 times those found in both JIA and healthy children[17].

In contrast to adult patients with RA, the data on lymphocyte apoptosis in children with JIA, either PBL or SFL, are scarce. Recently, we reported an increased incidence of spontaneous apoptosis in PBL from children with untreated JIA[17], which was correlated with downregulation of Bcl-2 in PBL[18]. No differences in either CD95 antigen expression on lymphocytes or soluble CD95 levels were found between children with JIA and healthy controls[17]. This was in accordance with another study showing that, in contrast to RA-SFL, PBL in patients with systemic or pauciarticular JIA did not show a defect in Fas-dependent apoptotic pathway[24].

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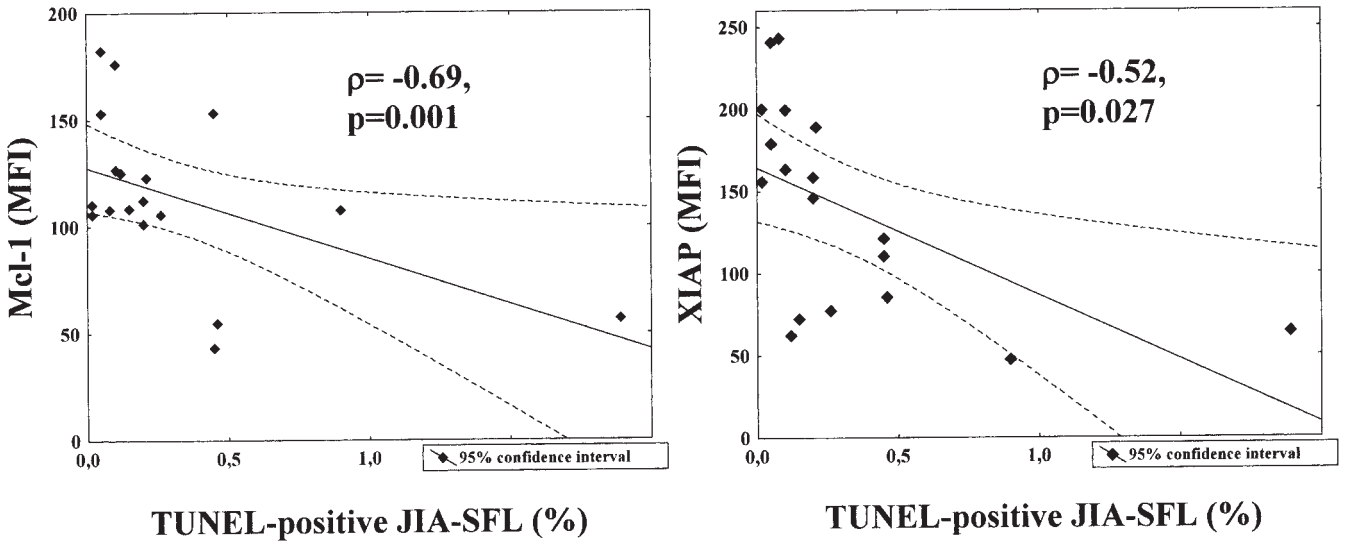

Figure 3. Expression of Mcl-1 and XIAP proteins in synovial fluid lymphocytes (SFL) correlates inversely with apoptotic index (percentage of TUNEL-positive cells) of juvenile idiopathic arthritis lymphocytes (JIA-SFL). TUNEL: TdT-mediated dUTP-biotin nick-end labeling. Mcl-1: myeloid cell leukemia 1. XIAP: cross-linked inhibitor of apoptosis.

On the other hand, Knipp, *et al*[25] reported that the rate of SFL apoptosis was slightly increased when compared to PBL from children with JIA, with higher CD95 expression on SFL and soluble CD95 levels in SF, along with a substantial number of locally survived T cells. However, our study showed that the spontaneous apoptosis of JIA-SFL is distinctly inhibited compared to both JIA-PBL and ctrl-PBL. Previously we showed that JIA-SFL had significantly lower expression of p53 protein than JIA-PBL, which may be responsible for inhibition of apoptosis in these cells[18]. The downregulation of p53 protein in JIA-SFL has been confirmed also in our series. It is especially interesting in the light of evidence that expression of p53 protein is a characteristic feature of RA fibroblast-like synoviocytes[8]. Data available on p53 mutations in RA/JIA synoviocytes are contradictory[8,10].

The potential role of another antiapoptotic protein, FLIP, the inhibitor of caspase-8 (FLICE) pathway, in the pathogenesis of rheumatoid diseases was recently suggested. High FLIP levels were detected in RA synovium cells[2,12] and correlated with their inhibited apoptosis[2]. A distinct trend to higher expression of FLIP in JIA-SFL compared to JIA-PBL and Ctrl-PBL was also observed in our study, but the differences were not statistically significant. In the experimental RA model, both FLIP and Bcl-2 proteins were temporally and differentially expressed during development of adjuvant-induced arthritis[26], which could explain some differences in the reported data.

To date, there have been no other reports concerning the mechanisms regulating lymphocyte apoptosis in JIA. Our study showed that upregulation of Mcl-1 and XIAP correlated with lower proclivity of JIA-SFL to undergo apoptosis. However, since the average percentage of apoptotic JIA-SFL was very low, this finding should certainly be interpreted with caution. On the other hand these results correspond logically with the data showing that Mcl-1 and XIAP expression was significantly higher in JIA-SFL than in either JIA-PBL or ctrl-PBL, in which spontaneous apoptosis was significantly more pronounced. Until now, there were no reports on Mcl-1 or XIAP expression in either lymphocytes or synovial tissue cells from patients with RA and JIA. Mcl-1 protein is a member of the Bcl-2 family, inactivating proapoptotic Bak, thus exerting an antiapopototic effect[27]. XIAP, a member of the IAP family, plays a critical role as a major regulator of programmed cell death, above all inhibiting caspase-3, -7, and -9 activation[28]. Mcl-1[29] and XIAP[30,31] are considered to be important in either tumorigenesis or immune response by the anti-apoptotic effect and promotion of cell survival. However, it is likely that their overexpression can also influence SFL apoptosis during development of JIA.

There are reports suggesting the important role of IAP in other autoimmune diseases, including Sjögren's syndrome[32] and multiple sclerosis[33,34]. Based on those data and our current results, further studies on expression and regulation of the other family members, such as cIAP1 or cIAP2, are also warranted in JIA.

This is the first study showing that the upregulation of Mcl-1 and XIAP proteins, in parallel to downregulation of p53, is correlated with decreased proclivity of JIA-SFL to undergo apoptosis. This may result in maintaining autoimmunity and contributing to the development of joint destruction in JIA.

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