Increased Soluble Cytoplasmic Bcl-2 Protein Serum Levels and Expression and Decreased Fas Expression in Lymphocytes and Monocytes in Juvenile Dermatomyositis

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**ABSTRACT.** Objective. To evaluate soluble Fas antigen (sFas), sFas ligand (sFasL), soluble tumor necrosis factor-related apoptosis-inducing ligand, and soluble cytoplasmic Bcl-2 protein (sBcl-2) serum levels, Fas and Bcl-2 expressions in T and B lymphocytes and monocytes and relations with erythrocyte sedimentation rate, C-reactive protein (CRP), Childhood Myositis Assessment Scale, and manual muscle testing in juvenile dermatomyositis (JDM).

Methods. Serum levels were determined by ELISA and peripheral cell expressions by flow cytometry for patients with JDM or juvenile idiopathic arthritis (JIA), and healthy controls.

Results. Patients with JDM had increased sBcl-2, which correlated with CRP. Expression of Bcl-2 was increased and expression of Fas was decreased in CD3+, CD4+, and CD8+ T lymphocytes compared with JIA and/or healthy controls.

Conclusion. Patients with JDM presented a unique apoptosis-related proteins profile, which may contribute to disease development. (First Release August 1 2018; J Rheumatol 2018;45:1577–80; doi:10.3899/jrheum.171248)

Key Indexing Terms:
- APOPTOSIS
- JUVENILE DERMATOMYOSITIS
- SOLUBLE BCL-2
- FASL AND TRAIL
- LYMPHOCYTES
- MONOCYTES

Juvenile dermatomyositis (JDM) pathogenesis involves cell-mediated and humoral immune responses and is distinct from that of polymyositis and dermatomyositis1,2,3.

Fas, Fas ligand (FasL), and tumor necrosis factor–related apoptosis–inducing ligand (TRAIL) are apoptosis-promoting proteins. In turn, through a different pathway, cytoplasmic protein Bcl-2 protects cells from death. These protein expressions were demonstrated in muscle fibers and infiltrating inflammatory cells from patients with JDM4,5,6,7,8. Our group has observed altered soluble (s)Fas, sTRAIL, and sFasL levels, as well as expressions of Fas and Bcl-2 in lymphocytes and monocytes from patients with juvenile systemic lupus erythematosus (JSLE)9,10,11,12.

Because altered apoptosis-related protein serum levels13,14 and muscle fiber expressions4,5,6,7,8 have been observed, our study simultaneously evaluated sFas, sFasL, sTRAIL, and sBcl-2 serum levels; Fas and Bcl-2 expressions in peripheral T and B lymphocytes and monocytes, and their relationships with disease activity variables in patients with JDM.

**MATERIALS AND METHODS**

Our cross-sectional study randomly enrolled 34 patients fulfilling Bohan and Peter criteria for definite JDM15, 40 sex- and age-matched unrelated healthy individuals, and 33 juvenile idiopathic arthritis (JIA) disease controls.
(15 systemic, 11 polyarticular, and 7 oligoarticular)\textsuperscript{16}. Informed consent was obtained from parents and participants after approval by the CAPPesq ethical committee (No. 195.419). Subjects under suspicion of infection were excluded. Medical records were retrospectively evaluated regarding demographic, clinical, laboratory, and treatment data. Disease activity variables included erythrocyte sedimentation rate (ESR, by Westergren), C-reactive protein (CRP, by nephelometry), Childhood Myositis Assessment Scale (CMAS, range 0–28), and manual muscle testing (MMT, range 0–80)\textsuperscript{17,18}.

Soluble apoptosis-related protein serum levels were quantified using ELISA kits and following manufacturers’ instructions as detailed in our previous work\textsuperscript{9}. Duplicate undiluted or 100× diluted samples and standards were run in parallel for patients and controls. Optical density was measured in spectrophotometer at 450 nm and serum levels calculated using each standard curve and dilution factor.

Peripheral mononuclear cells were isolated and stained with monoclonal antibodies, using instruments and procedures detailed in previous studies\textsuperscript{10,11,12}. For flow cytometric analysis, lymphocytes were separated by gating of CD3 versus site scatter (SSC) and monocytes by gating CD14 versus SSC. Nonsialic cells were excluded from analysis by drawing gates not including events with low forward scatter (FSC) and high SSC light properties. Over study duration, instrument settings and procedures were identical. In each flow cytometry session, both patient and control samples were analyzed. Double-positive cells for Fas or Bcl-2 and CD3, CD4, CD8, CD19, or CD14 were identified by dot-plot histograms and the results are shown as percentage of double-positive cells in relation to the total number of lymphocytes or monocytes in the respective gate and as mean fluorescence intensity (MFI).

The 3 study groups were compared using the Kruskal-Wallis ANOVA test followed by the Mann-Whitney U test for comparisons of the 2 groups. After Bonferroni’s correction for multiple comparisons in posthoc analyses, only p values ≤ 0.01 were considered significant. Results are presented as median and interquartile range (IQR). Spearman’s rank test was used for correlations with disease activity variables.

**RESULTS**

Patients with JDM (22 girls) had median ages of 9.9 years (range 3.4–20.8), disease duration of 3.4 years (0.2–15.9), CMAS of 48 (28–52), MMT of 80 (33–80), ESR of 16.0 mm/h (2.0–55.0), and CRP of 0.7 mg/l (0.2–5.7). Twenty-two patients were taking oral prednisone, 15 methotrexate, 12 hydroxychloroquine, 4 cyclosporine, 1 cyclophosphamide, and 1 intravenous immunoglobulin. JIA disease controls (20 girls) had median age of 12.5 years (range 3.8–19.2), disease duration of 4.9 years (0.2–14.8), and ESR of 23.0 mm/h (2.0–56.0), and were taking antiinflammatory drugs, oral prednisone, and/or immunosuppressive therapy, according to clinical condition. Healthy controls (26 girls) had a median age of 10.9 years (range 2.1–20.3).

Soluble Bcl-2 levels were different among the 3 groups (p = 0.0004) and significantly increased in patients with JDM compared with healthy [median 29.3 ng/ml (IQR 15.1–39.9) vs 7.4 ng/ml (IQR 4.4–11.0), p = 0.0001] and JIA [6.4 ng/ml (IQR 3.2–30.5), p = 0.003, Figure 1A] controls. Moreover, sBcl-2 levels negatively correlated with CRP (r = –0.55; p = 0.01). sFas and sTRAIL levels were similar among groups (p = 0.1 and p = 0.9, respectively, Figure 2). Soluble FasL was detected in 1 patient with JDM (0.32 ng/ml), contrasting with 60% in healthy and 33% in JIA controls (Figure 2). No correlation was observed with CMAS, MMT, or ESR.

In addition, proportion of Bcl-2–expressing CD3+ T lymphocytes was different among the 3 groups (p = 0.01) and significantly higher in patients with JDM compared with healthy controls [median 60.7% (IQR 56.3–71.3) vs 52.1% (IQR 46.7–55.3), p = 0.007, Figure 1B]. As well, proportion of Bcl-2–expressing CD4+ T cells was different among groups (p = 0.003) and increased compared with healthy controls [median 36.3% (IQR 33.9–45.8) vs 27.8% (IQR 23.9–32.2), p = 0.0007] but not JIA controls [32.0% (IQR 26.3–36.5), p = 0.03, Figure 1C] considering Bonferroni’s correction. Moreover, expression levels of Bcl-2 in CD8+ T lymphocytes were different among groups (p = 0.007) and increased in patients with JDM compared with healthy controls [median MFI 32.4 (IQR 24.5–37.3) vs 23.1 (IQR 21.1–25.9), p = 0.004] and JIA controls [median MFI 24.4 (IQR 20.6–27.9), p = 0.006, Figure 1D]. Expressions of Bcl-2 in CD19+ B lymphocytes and CD14+ monocytes were similar in patients with JDM, healthy controls, and JIA controls. Surprisingly, expressions of Bcl-2 in T and B lymphocytes and monocytes did not correlate with ESR, CRP, CMAS, or MMT.

Otherwise, the proportion of Fas-expressing CD3+ T lymphocytes was different among the 3 groups (p = 0.03) and significantly decreased in patients with JDM compared with JIA controls [median 23.8% (IQR 20.6–56.5) vs 32.8% (IQR 28.3–36.4), p = 0.01]. Proportion of Fas-expressing CD4+ T lymphocytes was also decreased [median 14.1% (IQR 12.8–16.8) vs 17.2% (IQR 14.5–20.0), p = 0.01] compared with JIA controls. No difference was observed in proportion of Fas-expressing CD8+ T lymphocytes after Bonferroni’s correction [median 7.6% (IQR 5.5–14.6) vs JIA 13.6% (IQR 11.9–20.4), p = 0.03; Figure 2]. Expressions of Fas in B lymphocytes and monocytes were similar to those of healthy and JIA controls. No correlation was observed between Fas-expressing cells and disease activity variables.

Patients with JIA had soluble apoptosis-protein levels similar to those of healthy controls (Figure 1A and Figure 2), and considering Bonferroni’s correction, differences in proportion of Bcl-2–expressing and Fas-expressing cells were not significant (Figure 1B–E and Figure 2).

**DISCUSSION**

Studies regarding apoptosis-related proteins in patients with JDM are few; data are not conclusive and whether serum levels depict their expression in which cell types is not fully understood\textsuperscript{7,11,13,14,19}. Our study results parallel previous reports of Bcl-2 overexpression in muscle fibers\textsuperscript{4,6,7}. Thus we suggest that increased sBcl-2 levels reflect higher expression of Bcl-2 not only in myofibers but also in peripheral cells, because increased expression of Bcl-2 was shown in all peripheral T lymphocyte subsets. Previous findings and correlation with CRP support a hypothesis of an apoptosis role in...
disease development, which may lead to persistence of self-reactive lymphocyte clones and thus to autoimmunity, if Bcl-2 expressing cells remain viable 20. Otherwise, Bcl-2 overexpression can protect myofibers from death and may be part of a regeneration program 8. Therefore, further studies are needed to elucidate Bcl-2 protein function in JDM.

Increased Fas expression was observed in muscle fibers and infiltrating CD4+ and CD8+ T cells in polymyositis and dermatomyositis 4, 6, 8. In addition, Fas, TRAIL, and caspase-3 were identified in muscle biopsies in JDM 5. Decreased proportion of Fas-expressing peripheral CD4+ and CD8+ T lymphocytes reinforces the idea of a pathogenic role not

Figure 1. Soluble Bcl-2 levels and expressions of Bcl-2 in lymphocytes among the 3 groups in the study. JDM: juvenile dermatomyositis; JIA: juvenile idiopathic arthritis; MFI: mean fluorescence intensity.

Figure 2. Proportion of CD3+, CD4+, and CD8+ Fas+ T cells, and sFAS, sTRAIL, and sFasL levels in the 3 groups in the study. sFAS: soluble Fas antigen; sTRAIL: soluble tumor necrosis factor–related apoptosis–inducing ligand; sFasL: sFAS ligand; JDM: juvenile dermatomyositis; JIA: juvenile idiopathic arthritis.
only for Bcl-2 but also for Fas protein in JDM.

Interestingly, patients with JDM had expressions of Fas and Bcl-2 in B lymphocytes and monocytes similar to controls, contrasting with patients with JSLE, who presented decreased expressions of Fas and Bcl-2 and impaired apoptotic debris removal by monocytes, and increased expression of Fas in B lymphocytes.

Because steroids and immunosuppressive drugs are known to interfere with cell apoptosis and to both increase or decrease apoptosis-related protein expressions and serum levels, that sort of influence cannot be ruled out in our results.

Soluble Fas and sTRAIL levels were similar to healthy and JIA controls and did not correlate with ESR, CRP, CMAS, or MMT, though other authors have observed increased sFas levels in patients with JDM. Consistent with a previous study, sFasL was undetectable in a great number of patients with JDM. Hence, the sFas, sTRAIL, and sFasL roles in JDM pathogenesis and their use as biomarkers (as in patients with JSLE) still needs clarification.

Although studies of patients with rheumatoid arthritis and JIA showed increased sFas and sFasL levels, this inflammatory control group had cellular expressions and soluble levels of apoptosis-related proteins similar to healthy controls, reinforcing that the alterations observed herein are somewhat specific for patients with JDM.

Our study’s limitation was that FSC/SSC gating excluded debris but not all nonviable cells, and these may have contributed to the apoptotic signatures described. Otherwise, the study differentials were to solely evaluate patients with definite JDM, to analyze both apoptosis-inducing and inhibiting proteins not only in T and B lymphocytes but also in monocytes, and to simultaneously evaluate soluble forms, which gave the opportunity to draw a profile of apoptosis-related proteins in JDM. However, whether these results can be considered a primary pathogenic event or alternatively a secondary epiphenomenon remains to be elucidated.

Therefore, patients with JDM present an imbalance of apoptosis-related proteins, as previously shown for other autoimmune diseases, not only in muscle fibers but also in Bcl-2 and Fas peripheral lymphocyte expressions as well as serum levels, which may contribute to disease development.

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REFERENCES