Expression of Galectins-1 and –3 Correlates with Defective Mononuclear Cell Apoptosis in Patients with Juvenile Idiopathic Arthritis

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ABSTRACT. ***Objective.*** Juvenile idiopathic arthritis (JIA) is characterized by hyperplasia of synovial cells and accumulation of mononuclear inflammatory infiltrates, which are locally maintained through a balance between cell proliferation and apoptosis. Although defective clearance of activated T cells in RA joints has been explained by alterations of the Fas-Fas ligand system, this has not been confirmed in synovial tissue of patients with JIA. We evaluated the relation between expression of galectin-1 (Gal-1) and galectin-3 (Gal-3) (ß-galactoside-binding proteins with pro- and anti-apoptotic properties, respectively) and the apoptosis and proliferation rates of infiltrative lymphocytes in synovial tissue of patients with JIA.

Methods. Using slide cytometry and *in situ* end labeling we observed dysregulated apoptosis of infiltrating mononuclear cells within the synovial tissue of patients with JIA.

Results. Patients with pauciarticular JIA showed minimal apoptosis, high Bcl-2 expression, and high or normal proliferation rates, while patients with polyarticular disease showed the lowest apoptotic indexes, accompanied by low Bcl-2 expression and low proliferation rates. We found that Gal-1 expression is downregulated and Gal-3 expression is upregulated in synovial tissue from patients with JIA.

Conclusion. In patients with polyarticular JIA, accumulation of inflammatory cells is mainly due to downregulated apoptosis, whereas in patients with pauciarticular disease the process results from increased proliferation. Defective mononuclear apoptosis in synovial inflammatory infiltrates from patients with JIA could be explained in part by decreased Gal-1 and increased Gal-3 expression. (J Rheumatol 2001;28:1914–22)

Key Indexing Terms:
APOPTOSIS Bcl-2 GALECTIN-1 GALECTIN-3
JUVENILE IDIOPATHIC ARTHRITIS SYNOVIAL PROLIFERATION

Juvenile idiopathic arthritis (JIA) encompasses a heterogeneous group of chronic inflammatory diseases of unknown etiology. Similar to rheumatoid arthritis (RA), JIA is characterized by hyperplasia of synovial lining cells, lymphocyte inflammatory infiltrates, overexpression of proinflammatory cytokines [e.g., interleukin 1ß (IL-1ß), tumor necrosis factor-α, interferon-γ], decreased expression of antiinflammatory cytokines (e.g., IL-4, IL-10, transforming growth factor-ß), and extensive destruction of articular cartilage in later stages of the disease[1-3].

Inflammatory infiltrates are locally maintained through a balance between cell proliferation and apoptosis. Both processes are under similar control mechanisms, involving both protooncogenes and tumor-suppressor genes[4,5]. The importance of dysregulated apoptosis in the etiology of autoimmune diseases has been highlighted by observation of autoimmune disorders in MRL-*lpr*/*lpr* or C3H-*gld*/*gld* mouse strains that carry spontaneous mutations in Fas or Fas ligand genes[6], and in particular by the dominant Fas gene mutation associated with the Canale-Smith syndrome, a human autoimmune lymphoproliferative disorder[7].

It seems that defective clearance of activated T cells in RA joints might be due to a dysfunction of Fas ligand positive T cells or inhibitory effects on Fas/Fas ligand interactions by soluble Fas antigen and/or an inhibitory Fas signal

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transmission by deficient expression of the Fas ligand[8,9]. However, these findings could not be extended to the synovial fluid from patients with JIA[10], suggesting that, most likely, other "non-Fas" related factors could be involved in the regulation of proliferation and apoptosis of lymphocytes within inflamed joints in JIA.

Recently, galectin-1 (Gal-1)[11], a 14.5 kDa protein with ß-galactoside-binding specificity, has been shown to induce apoptosis of activated mature T cells[12-14] and immature thymocytes[15]. In contrast, galectin-3 (Gal-3), a 29 kDa member of the same family, has been shown to rescue cells from programmed cell death[16]. Thus, Gal-1 and Gal–3 may represent an additional family of proteins similar to the Bcl-2 family, where different members exhibit sequence similarity, yet have opposite effects on cell survival[11]. We recently provided experimental data supporting the concept of an *in vivo* therapeutic role for Gal-1 in a murine experimental model of arthritis by using gene and protein therapy strategies[17]. A single injection of genetically modified DBA/1 fibroblasts engineered to secrete Gal-1, at onset of the arthritic process, was sufficient to achieve a dramatic arrest in overall disease progression, as judged by clinical, histopathological, and immunological manifestations of arthritis. This effect was reproduced by continuous daily administration of recombinant Gal-1. Investigation of the molecular mechanisms implicated in this therapeutic effect revealed that T cells from mice treated with Gal-1 increased their susceptibility to antigen induced apoptosis[17]. In addition, we have shown that Gal-1 induced apoptosis is preceded by activation of the AP-1 transcription factor and mediated by a Bcl-2-dependent pathway[18].

We evaluated the potential association between Gal-1, Gal-3, and Bcl-2 expression and apoptosis and proliferation rates of infiltrative lymphocytes in synovial tissue from patients with JIA.

MATERIALS AND METHODS

Patients. For this study, we used synovial tissue specimens from 16 patients with different clinical subtypes of JIA: pauciarticular arthritis (n = 4), polyarticular arthritis (n = 8), and systemic onset disease (n = 4). All 16 patients with JIA were followed by physicians in the Division of Pediatric Rheumatology, Floating Hospital for Children at New England Medical Center. All patients met the American College of Rheumatology classification criteria for the diagnosis of JIA[19]. Detailed clinical information about study patients has been published[2]. In 5 patients, synovial biopsy was performed for early diagnosis in the course of the disease (mean duration of arthritis 0.4 yrs), while in the remaining 11, synovial biopsy or arthroplasty was done late in the disease (mean duration of arthritis 7.3 yrs). All JIA samples were formalin fixed and paraffin embedded. Because of the insufficient amounts of synovial tissue, galectin-1 and 3 expression was studied in 13 tissue samples (3 patients with pauciarticular disease, 8 with polyarticular disease, 2 with systemic onset JIA). For comparison, synovial tissue was also obtained from 11 patients with various orthopedic conditions including plica (n = 4), congenital dislocation/subluxation of the hips (n = 2), and recurrent patellar subluxation (n = 5). In those patients, arthroscopic synovial biopsy was done for diagnostic purposes and no particular histopathological diagnosis was made. Those patients did not fulfill clinical criteria for any chronic rheumatic disease, but low grade synovial inflammation was detected[2].

Immunohistochemical detection of Ki-67 antigen. Sections were mounted on positively charged microscope slides (Superfrost Plus; Fisher Scientific, Fair Lawn, NJ, USA) and baked at 60°C for 2 h. Slides were routinely dewaxed and rehydrated. The endogenous peroxidase activity was then quenched for 10 min with 0.5% H_2O_2 in methanol. A microwave antigen retrieval method was used (20 min in 10 mM citrate buffer, pH 6.0, at 600 W), followed by incubation with a 1:100 dilution of a polyclonal horse serum (Dako, Glostrup, Denmark) for 20 min and with monoclonal MIB-1 antibody (2 μg/ml) for 18 h at 4°C (Calbiochem, Cambridge, MA, USA). Sections were then serially incubated with 1:200 dilution of biotinylated anti-mouse antibody for 30 min (Dako) and a 1:100 dilution of peroxidase labeled avidin-biotin complex for 60 min (Dako). All incubations were performed in a moist chamber at room temperature unless otherwise specified. The reaction was developed under microscopic control, using 3,3' diaminobenzidine tetrahydrochloride with 0.3% hydrogen peroxide as the chromogen substrate (Sigma, St. Louis, MO, USA), and the sections were counterstained with hematoxylin. Both positive (reactive lymph node) and negative (omission of the primary antibody) controls were run simultaneously. The threshold of positivity was experimentally established using the positive control in each staining batch. Only those nuclei with staining features similar to those of their corresponding positive control were considered to be positive. Criteria for nuclear staining and positivity were experimentally optimized in the corresponding positive control.

Slide cytometric analysis of nuclear DNA content. Feulgen stained sections were used for DNA quantification[20] using the CAS model 200 and Quantitative DNA Analysis software® (Becton-Dickinson). At least 300 nuclei were measured in each case and the results were recorded separately. Only complete, non-overlapping, and focused nuclei were interactively selected, beginning in the most cellular area, until completion in consecutive microscope high power fields (×400). Several 5 μm sections were used for this analysis, according to published protocols[21,22]. External diploid controls (rat hepatocytes; Becton Dickinson) were included in each staining batch to normalize results (1 slide/staining holder). They were used for setting the diploid G0/G1 limits and for calculating the DNA index of each G0/G1 cell population[23]. The histograms of nuclear optical densities were used to evaluate the DNA index (as referred to their corresponding diploid controls), the proliferation rate (PR = S + G2 + M/G1 + S + G2 + M, expressed as percentage), and the ratio between the nuclear area and the DNA content of the cells in each cell cycle phase. The last variable was also referred to the corresponding values in the histologically normal urothelial cells to normalize the results. Both mean nuclear area and nuclear area/DNA index ratio of G0/G1 cells were recorded. The latter represents a morphometric variable of apoptosis when coupled with *in situ* end labeling[24]. At least 2000 lymphocytes from control synovial tissues were used to calculate the reference nuclear area and DNA index.

In situ end labeling of fragmented DNA. Since extensive DNA fragmentation is an important feature of apoptosis, visualization of DNA breaks has proved useful in the identification of apoptotic cells[25]. The extensive DNA fragmentation results in a high density of 5' protruding ends, which can be detected using the Klenow fragment of DNA polymerase I, with a mixture of labeled nucleotides[26,27]. Briefly, sections were routinely deparaffinized and hydrated. After incubation in 2× SSC buffer (80°C, 20 min) and protein digestion (500 μg/ml pronase in 10 mM Tris-HCl, pH 7.5, 10 mM EDTA, 0.5% SDS at room temperature for 25 min), sections were incubated with Klenow fragment of *E. coli* DNA polymerase I under appropriate conditions (20 units/ml in 50 mM Tris HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM dithiothreitol, 250 μg/ml BSA with 100 mM of each dNTP, maintaining a proportion of 11-digoxigenin-dUTP/dTTP of 0.35/0.65; 2 h at 37°C). Digoxigenin labeled DNA fragments were immunoenzymatically detected using an antidigoxigenin polyclonal Fab fragment labeled with alkaline phosphatase (1:100 dilution, Boehringer Mannheim, Germany). The enzymatic reaction was developed under microscopic control with nitroblue-

tetrazolium and X-phosphate. Sections were counterstained with diluted hematoxylin (25%), dehydrated, and mounted. Both positive (reactive lymph node) and negative (omitting DNA polymerase in the enzymatic reaction) controls were run simultaneously. The threshold of positivity was experimentally established using the positive control, and the *in situ* end labeling index was expressed as the percentage of positive nuclei referred to the total number of lymphocytes present in the same HPF as reported for mitosis fraction counting or *in situ* hybridization[27-29]. The whole lesion or at least 50 consecutive HPF were screened, beginning in the area with the most prominent infiltrate.

Immunohistochemistry for Gal-1 and Gal-3. Expression of Gal-1 and Gal-3 was studied in 13 patients with JIA and all control patients using immunogold complex preparations. Briefly, colloidal gold particles of 5 nm average diameter were prepared according to the method of Slot and Geuze combining sodium citrate and tannic acid as reducing agents[30]. Then particles were adsorbed to the IgG fraction purified from an antiserum raised against rabbit IgG (Sigma) (0.25 μg of protein was necessary to stabilize 1 μl of colloidal gold solution). Finally, the immunogold complex preparation was centrifuged at 60,000 ×g for 1 h before use, and the pellet was resuspended in PBS containing 1% (w/v) BSA (PBS-BSA) for 15 min and incubated with a 1:300 dilution of rabbit anti-human Gal-1 or anti-human Gal-3 antibody (kindly provided by Drs. J. Hirabayashi and K. Kasai) for 24 h at 4°C, as described[17,31]. Then sections were incubated with the optimal dilution of the 5 nm immunogold complexes for 1 h at room temperature. Finally, gold particles were visualized by the silver enhancement procedure (Sigma) and were observed in a Zeiss Photomicroscope III. To improve visualization, semi-thin sections were stained only with toluidine blue. Controls were processed as follows: tissue sections were incubated with anti-Gal-1 or anti-Gal-3 antibody preadsorbed with 10 μg of the specific antigen (recombinant human Gal-1 or Gal-3). A second control was performed by omitting the incubation with the first antibody.

Morphometric analysis of Gal-1 and Gal-3 expression. Synovial tissue from patients with JIA and control synovial tissues were recorded photographically with a Zeiss Photomicroscope III at ×200 magnification, scanned, analyzed morphometrically applying the THSCS Image Tool software, and scored semiquantitatively on a 5 grade scale. A value of 1 was conventionally assigned to Gal-1 and Gal-3 expression found in control synovial tissues.

Statistical analysis. Differences between groups were determined by analysis of variance and Tuckey's F test. P values < 0.05 were considered statistically significant.

RESULTS

Analysis of apoptotic and proliferative rates in JIA synovial tissue samples. Patients were first classified as early untreated patients (median age 7.6 yrs), or as long lasting chronic JIA (median age 7 yrs). In the first group (n = 4), 3 patients had pauciarticular disease and one patient had polyarticular arthritis. In all of them the median duration of the disease was 0.4 years. In the second group (n = 12 patients with chronic JIA), one patient had pauciarticular disease, 7 had polyarticular disease, and 4 had systemic onset disease. In this group the median duration of disease was 7.3 years. All patients with systemic JIA had a polyarticular disease course.

Low apoptotic indexes and low lymphocytic proliferation were general features of patients with long lasting chronic JIA. Patients with pauciarticular JIA (despite the duration of the disease) showed only minimal apoptosis, as detected by *in situ* end labeling and the highest Bcl-2 expression (3/4 patients). Those patients also had either normal or high lymphocyte proliferation, detected by a combination of slide cytometry and MIB-1 staining (Table 1).

In patients with polyarticular JIA, accumulation of inflammatory cells was mainly due to downregulated apoptosis (5/8), whereas in pauciarticular patients, the process resulted from increased proliferation (2/4). In the remaining patients, the inflammatory cells were possibly recruited from peripheral blood because they did not proliferate actively or downregulate apoptosis *in situ* (synovial tissue). Patients with polyarticular JIA had the lowest apoptotic indexes, as detected by *in situ* end labeling and slide cytometry. We were able to detect the presence of apoptotic cells by *in situ* end labeling in 50% of patients (4/8) with pauciarticular disease, while patients with polyarticular disease had either low (63%) or normal apoptosis (37%), as found by DNA histograms and compared to control samples (Figure 1).

In addition, decreased Bcl-2 expression (3/8 patients) was found in those patients compared to patients with pauciarticular disease (3/4 patients). No patient had high lymphocyte proliferation, as detected by slide cytometry, and MIB-1 expression was rare in patients with polyarticular disease (Figure 2).

Patients with systemic JIA differed in some aspects from pauciarticular patients. Normal or low apoptotic indexes (in 75% and 25% of patients, respectively) and detectable apoptosis (in 50% of the patients) were characteristics of those patients. Similarly to patients with polyarticular JIA, very low Bcl-2 expression and low lymphocyte proliferation were found in patients with systemic onset JIA.

Expression of Gal-1 and Gal-3 in inflamed synovial tissue from patients with JIA. To explore whether apoptotic and proliferation indexes correlated with the expression of Gal-1 and Gal-3, we analyzed synovial tissue samples from 14 patients in addition to 11 controls. In most patients, Gal-1 expression was found to be mainly localized at the level of the mononuclear infiltrate. However, it was also present (although to a lesser extent) in synovial cells (Figures 3A,

Table 1. Results of proliferation studies, apoptosis, and Bcl-2 expression in all 27 study patients.

	Low Normal Proliferation		High Proliferation	
	Low Apoptosis	Normal Apoptosis	Low Apoptosis	Normal Apoptosis
Oligo JRA, n = 4*	0 (0)	2 (50)	0 (0)	2 (50)
Poly JRA, n = 8	5 (63)	3 (37)	0 (0)	0 (0)
Systemic JRA, n = 4	1 (25)	3 (75)	0 (0)	0 (0)

* All patients with oligoarticular JRA were untreated prior to the synovial biopsy. The limits for 95% confidence intervals in control synovial samples were as follows: *in situ* end labeling 19.74–24.38; Bcl-2 1.2–4.33; proliferation rate (slide cytometry) 13.39–17.95; MIB-1 index 10.54–15.88.

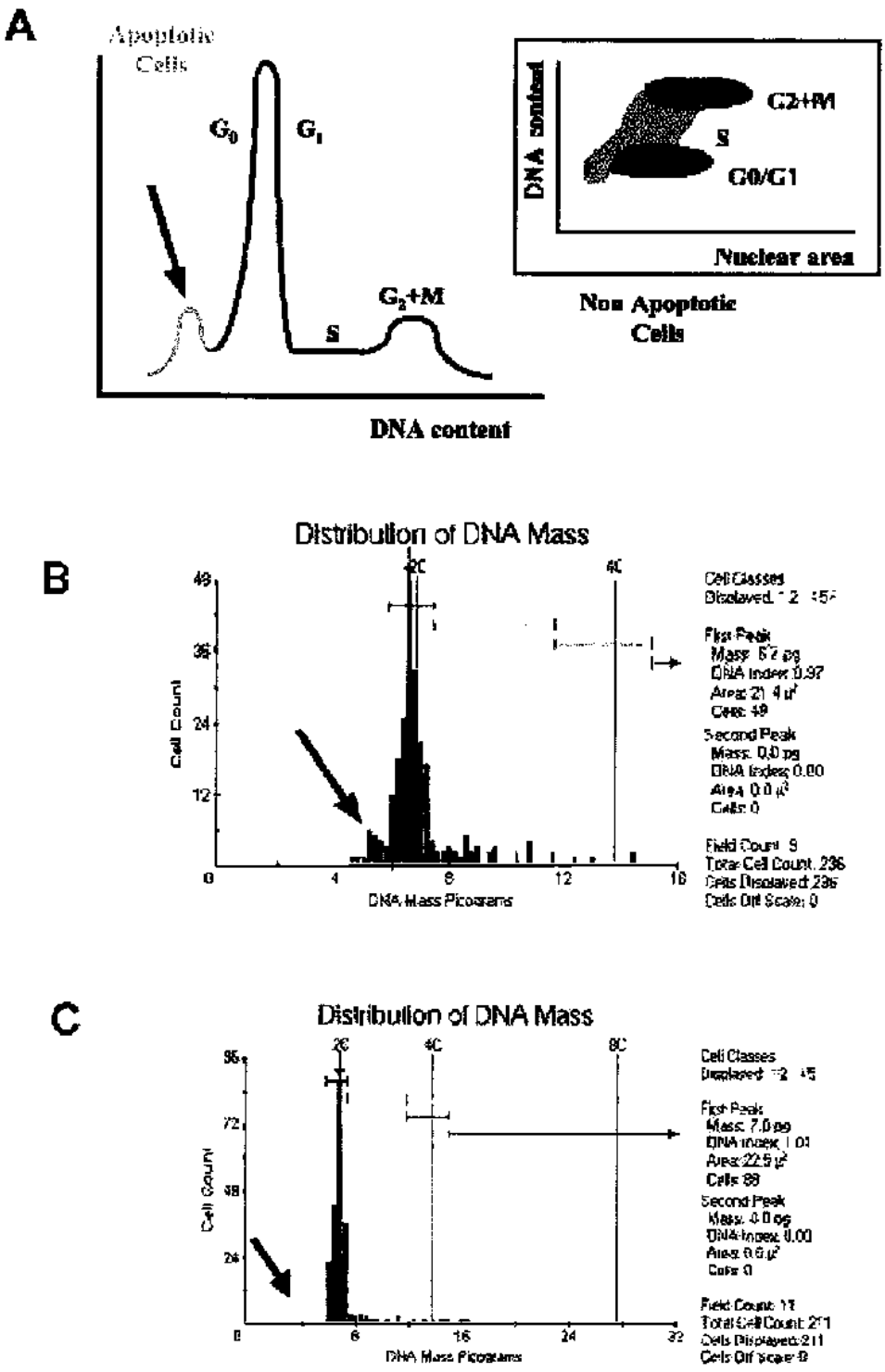

Figure 1. A. Schematic of DNA histogram analysis by slide cytometry after Feulgen's staining. The arrow indicates the fraction of apoptotic cells. Cells in S, G_2, and M phases were considered to be in the proliferative stage. B. DNA histogram of normal control lymphocytes. C. DNA histogram of a representative patient with long standing polyarticular JIA. Compared with the normal histogram, this patient had no apoptotic lymphocytes, and the number of proliferating lymphocytes was very low (S + G_2 + M phases).

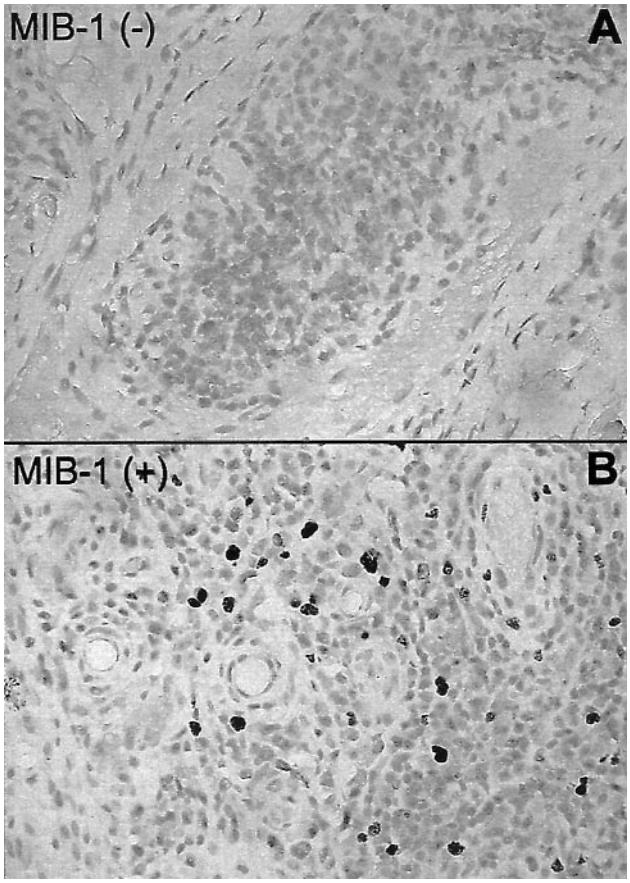

Figure 2. Immunohistochemical staining for MIB-1 (Ki-67) in the synovial tissue of a representative patient with long lasting polyarticular JIA (panel A, ABC peroxidase, original magnification ×400) and positive control patient with RA (panel B, ABC peroxidase, original magnification ×400). The absence of extensive MIB-1 staining was the most remarkable feature in the majority of patients with JIA.

3B). On the other hand, Gal-3 staining was found to be mainly restricted to fibroblast-like synovial cells (Figures 3C, 3D).

The comparative analyses of Gal-1 and Gal-3 expression with respect to their kinetic features (e.g., proliferation and apoptosis) are shown in Table 2.

We then compared Gal-1 and Gal-3 expression in synovial tissue from untreated patients (who exhibited a shorter clinical duration of disease: 4 patients with pauciarticular and one with polyarticular disease) with those who were treated and who therefore had much longer duration of disease. No significant difference was found among treated and untreated patients, but we found a possible trend toward reduced expression of Gal-1 in treated patients compared to untreated patients (0.472 ± 0.320 vs 0.822 ± 0.263, respectively). No difference in Gal-3 expression was found between the treated and untreated groups (1.913 ± 1.224 vs 2.288 ± 1.014, respectively). Interestingly, significantly lower Gal-1 expression was found in synovial tissue samples from treated patients, compared to control patients ($p = 0.0032$), suggesting that a Gal-1 associated downregulation of apoptosis occurs in patients with long lasting JIA. The mean score values for Gal-1 and Gal-3 expression in JIA patients were 0.75 ± 0.5 and 2.05 ± 1.02, respectively. The individual expression values for both Gal-1 and Gal-3 compared to control synovial tissue values are shown in Figure 4.

In general, Gal-1 expression was found to be markedly reduced and Gal-3 upregulated in JIA synovial tissue samples compared to normal synovial tissue. The differences among groups were found to be statistically significant ($p = 0.05$).

Table 2. Comparative analysis of Gal-1 and Gal-3 expression with respect to their kinetic features. The normal range of proliferation and apoptosis was established according to the 95% CI in each case, i.e., 13.39–17.95% for proliferation and 19.74–24.38% for apoptosis. There were no significant differences in these values when they were compared to control patients.

	Gal-1 (Av ± SD)	Gal-3 (Av ± SD)
High proliferation	0.545 ± 0.431	1.885 ± 1.577
Low proliferation	0.616 ± 0.362	1.927 ± 1.087
Normal apoptosis	0.423 ± 0.289	1.730 ± 1.022
Low apoptosis	0.742 ± 0.354	2.068 ± 1.244

DISCUSSION

The rationale for this study arises from a growing body of evidence suggesting that defective apoptosis is one of the key processes leading to chronic inflammation and synovial proliferation within the arthritic joint. It is known that the synovial tissue microenvironment differs substantially with respect to apoptosis of synoviocytes or lymphocyte infiltrating cells[32-34]. Thus, we evaluated the apoptosis and proliferation of infiltrating mononuclear cells from patients with JIA.

Using different methods (slide cytometry and *in situ* end labeling) we observed reduced apoptosis of infiltrating lymphocytes within JIA synovial tissue. In addition, we found that only a small proportion of patients with JIA — those with pauciarticular disease — have high proliferative inflammatory infiltrates. Several reports support this finding. It has been shown that lymphoid aggregates and T cells within RA and JIA joints are relatively spared from undergoing apoptosis[32-34]. These cells seem to be protected from activation induced cell death by an abnormal synovial microenvironment[32]. Attempts at elucidating the molecular mechanisms involved in this enhanced protection from apoptosis revealed that deficient expression of Fas or Fas ligand was only partially responsible for insufficient apoptosis[8], suggesting that other mechanisms could play a role in this process. Alternatively, overexpression of Bax compared to Bcl-xL and Bcl-2 was found in synovial T cells from patients with ongoing apoptosis, but not in patients with few apoptotic cells[33]. An interesting study revealed an inverted

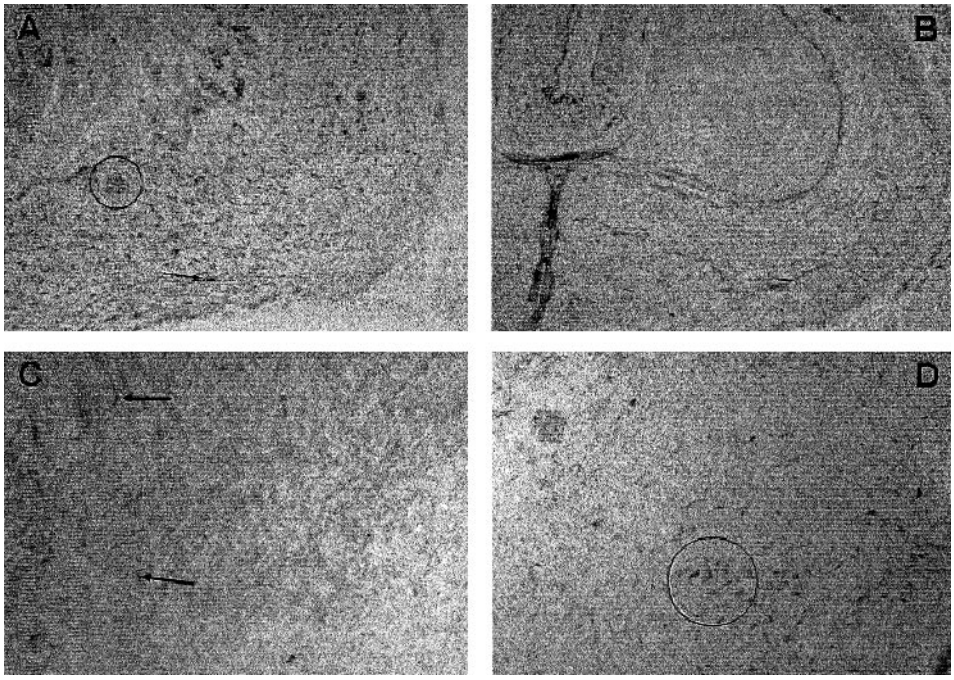

Figure 3. A. Immunogold staining for Gal-1 in a representative control patient. Extensive staining is mainly present around perivascular areas in mononuclear inflammatory cells (circled), and occasionally in fibroblast-like synovial cells (arrow; original magnification ×400). B. Immunogold staining for Gal-1 in a representative patient with long lasting polyarticular disease. The tissue sample is characterized by marked fibrosis. Low Gal-1 staining was detected. A negative control incubated with the anti-Gal-1 antibody preadsorbed with 10 μg/ml of recombinant human Gal-1 showed no staining (data not shown). C. Immunogold staining for Gal-3 in a representative control patient. Positive staining is mainly present in fibroblast-like synovial cells (arrows; original magnification ×400). D. Immunogold staining for Gal-3 in patients with long lasting systemic onset JIA and polyarticular disease. Extensive positive staining is present in fibroblast-like synovial cells (circled), and occasionally in mononuclear inflammatory cells (original magnification ×400).

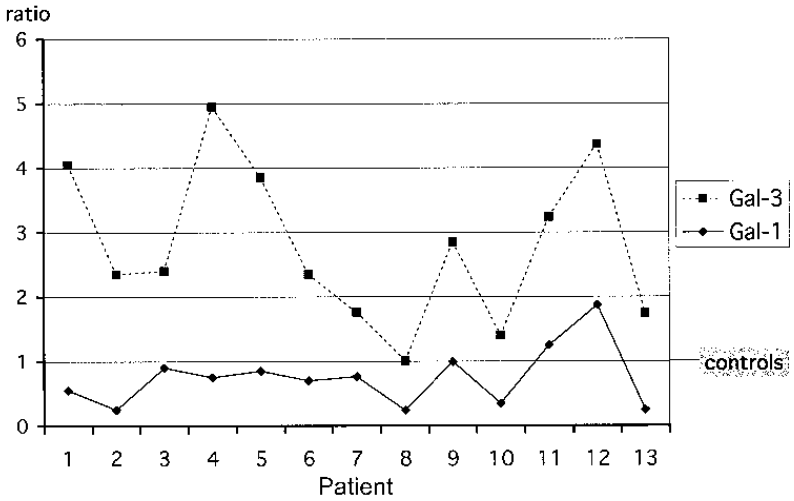

Figure 4. Morphometric analysis of Gal-1 and Gal-3 staining in individual patients with JIA (n = 13). A value of 1 was arbitrarily assigned to Gal-1 and -3 protein expression found in control synovial tissues, and the ratio of Gal-1 and 3 protein expression to the expression found in that tissue (y axis) compared to JIA synovial tissue is illustrated. The expression ratios varied among different patients. Only 2 patients who showed higher values for Gal-1 were affected by pauciarticular disease (Patients 11 and 12). Only Patient 7, who showed Gal-3 expression similar to control values, had long lasting systemic onset JIA and a polyarticular course (see text for details).

ratio of membrane bound Fas antigen (mFas) to soluble Fas antigen (sFas) in peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells from patients with early onset pauciarticular JIA compared to healthy individuals[10]. Since mFas/sFas ratios were similar in PBMC and mononuclear cells from synovial fluids, the authors concluded that the role of Fas/CD95 regulated apoptosis in early onset JIA was not locally limited to affected joints[10]. Similar findings were reported by Koczan, *et al*[34], where sFas in PBMC from patients with JIA could not be distinguished from that of children affected by bacterial or viral infections, although in PBMC from children with active JIA a predominance of the sFas mRNA was detected.

We found that patients with pauciarticular JIA showed normal apoptosis, high proliferation (as detected by *in situ* end labeling and slide cytometry), and high Bcl-2 expression. In contrast, patients with polyarticular disease (which closely resembles chronic RA) showed the lowest apoptotic indexes, together with low Bcl-2 expression, suggesting a different immunopathological mechanism in these patients.

These data are in agreement with recent findings suggesting that systemic onset JIA may be viewed as a different disease[35]. In principle, the susceptibility to apoptosis seems to be determined by relative levels and interactions between proteins of the Bcl-2 protooncogene family — Bcl-2, Bcl-xL, Bad, Bax, Bag, Bak and others — some of which, such as Bcl-2, protect from apoptosis, whereas others, such as Bax, promote apoptosis[36]. Bcl-2 was present in only 44% of JIA patients and its expression did not correlate with the longer duration of disease and lower apoptotic indexes.

Our results are in agreement with those reported by Sioud, *et al*[33] of low Bcl-2 expression in T cells in JIA and RA patients, but are in disagreement with those found in clonally expanded CD4+CD28– T cells from RA patients[37]. These apparent discrepancies could be explained by the fact that T cells with high Bcl-2 expression predominate in early disease (that is, pauciarticular), while macrophages are the predominant infiltrating cells in long lasting disease[38-42] (Harjacek M, unpublished observations).

Interestingly, low Bcl-2 expression correlated well with low IL-2 mRNA expression in patients with JIA, and this value was lower than in control synovial tissue[2]. This result is noteworthy, since IL-2 is one of the main cytokines responsible for controling Bcl-2 protein expression[43]. As well, IL-4, an antiinflammatory Th2 derived cytokine that exerts a proliferative and anti-apoptotic effect on T and B cells, is associated with upregulation of Bcl-2/Bcl-xL[44,45]. In this regard, we (in the same patient population) and others have shown that low antiinflammatory cytokine mRNA expression (i.e., IL-4 and IL-10) in JIA is a hallmark of the disease[2,45]. The presence of IL-4 mRNA in only 6/16 patients with JIA is consistent with the results reported for IL-2[2], and suggests the causal relationship with low Bcl-2 expression in these patients.

Since dysregulated apoptosis in JIA could only be partially explained by defects in the Fas/Fas L system, we investigated a novel immunoregulatory mechanism in which the key molecule is a carbohydrate-binding protein. Galectins have recently emerged as immunomodulatory proteins that exert their functions by cross-linking oligosaccharide ligands on cell surface glycoconjugates. Galectin-1, a member of this family, has been implicated in critical functions such as immunomodulation[17,46], cell growth regulation[11,47,48], cell adhesion[13], and metastasis[49] by recognition of a mosaic of specific extracellular glycoproteins such as fibronectin and laminin and cell surface receptors such as CD45 and CD43. Experimental evidence is now emerging to support the potential use of galectins or their antagonists in treatment of autoimmune disorders, inflammation, and tumor spreading[11]. In this regard, we recently reported that genetic delivery of recombinant Gal-1 suppresses autoimmune and inflammatory responses via T cell apoptosis in collagen induced arthritis, an experimental model of RA[17]. Since Gal-1 administration suppressed the inflammatory disease through apoptosis of activated synovial T cells, we speculated that modulation of Gal-1 expression in synovial tissue could be an alternative mechanism to explain dysregulated apoptosis. Gal-1 has been also shown to induce *in vitro* apoptosis of activated T cells and immature thymocytes[12,14,15]. On the other hand, Gal-3, a 29 kDa member of the same family, has been shown to rescue cells from apoptosis[16]. We report that Gal-1 expression is downregulated in synovial tissue from patients with JIA in broad agreement with diminished susceptibility to programmed cell death. This result, together with upregulated Gal-3 expression, is intriguing in terms of the opposite effects reported for these 2 ß-galactoside-binding proteins. Moreover, we found that downregulation of Gal-1 expression in JIA synovial tissue is tightly correlated with longer duration of the disease.

Since Gal-1 has been shown to induce T cell apoptosis, whereas Gal-3 had the opposite effect, these 2 proteins may represent an additional family of proteins similar to the Bcl-2 family, where different members exhibit sequence similarities, yet have opposite effects on cell survival[11]. Galectin-1 has been shown to induce *in vitro* apoptosis of activated T cells by transducing signals through selectively glycosylated receptors such as CD43 and CD45, particularly the CD45RO splicing product[14,15,50,51]. It has been shown that Gal-1 induces a second signal that, together with T cell receptor signaling, sensitizes dividing T cells to undergo apoptosis[52]. In our experimental model, gene therapy using Gal-1 suppressed arthritis and induced a switch from a Th1 proinflammatory response toward a Th2 mediated immune response, thus correcting the cytokine imbalance. Thus one may hypothesize that high IL-1ß mRNA expression found in 13/16 patients[2] could be associated with low Gal-1 expression, because recombinant Gal-1, at least *in vitro*, effectively inhibits proinflammatory cytokine production by activated T cells[13,17].

Our data are in agreement with the model presented by Rabinovich, *et al*[13], in which Gal-1 is secreted at low physiological concentrations from immunocompetent or bystander cells after the completion or exacerbation of an inflammatory or immune response to inhibit T cell adhesion to extracellular matrix glycoproteins and reduce the amount of proinflammatory cytokines, but does not induce T cell apoptosis. If this regulatory mechanism is not sufficient to achieve homeostasis, enhanced secretion of Gal-1, together with prolonged stimulation and persistence in the extracellular milieu, would finally induce apoptosis of activated T cells.

We conclude that impaired lymphocyte apoptosis in patients with JIA could be explained, at least in part, by decreased Gal-1 expression and increased Gal-3 expression in synovial tissue. Further studies involving more patients and other forms of chronic arthritides are warranted to confirm the role of these carbohydrate-binding proteins in chronic synovial cell inflammation.

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