Synovial Membrane Expression of Matrix Metalloproteinases and Tissue Inhibitor 1 in Juvenile Idiopathic Arthritides

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ABSTRACT. ***Objective.*** Matrix metalloproteinases (MMP) are a large family of proteolytic enzymes involved in the remodeling of extracellular matrix during tissue resorption. We investigated synovial tissue expression of the main proteolytic enzymes (MMP-1, MMP-3, and MMP-13) and tissue inhibitor of metalloprotease 1 (TIMP-1) in juvenile idiopathic arthritides (JIA).

Methods. Expression of MMP-1, MMP-3, MMP-13, and TIMP-1 was studied by immunohistochemical analysis of synovial tissues, obtained at synoviectomy or arthroplasty from 9 patients with JIA, and was correlated with mononuclear cell infiltration into the lining layer.

Results. MMP-1 and MMP-3 were abundantly expressed in the lining layer, showing a high degree of correlation with macrophage infiltration (CD68+ cells). MMP-13 showed a lower degree of expression, with tissue distribution almost restricted to the sublining regions. TIMP-1 tissue distribution was similar to that observed for MMP-1 and -3, although with a definitely lower number of positive cells.

Conclusion. The expression of MMP-1 and MMP-3 in the synovium of patients with JIA was clearly correlated with the degree of inflammation. This indicates the possible role of MMP in the pathogenesis of synovitis in this group of pediatric idiopathic arthritides. Inadequate expression of tissue inhibitors may represent a crucial event for the development and perpetuation of tissue damage. (J Rheumatol 2002;29:1774–9)

Key Indexing Terms:
JUVENILE IDIOPATHIC ARTHRITIS MATRIX METALLOPROTEINASES
TISSUE INHIBITOR OF METALLOPROTEASE

In idiopathic inflammatory arthritides, proliferating synovial membranes play a pivotal role in cartilage and bone destruction[1,2]. In these disorders, macrophages and fibroblast-like cells are the major component of the lining layer of the synovial tissue, especially at the level of the cartilage-pannus junction. Both cell types produce proinflammatory cytokines, growth factors, chemokines, and degrading enzymes that combine to determine and perpetuate tissue damage[3,4].

Matrix metalloproteinases (MMP) are a large family of proteolytic enzymes produced by fibroblasts, macrophages, neutrophils, and chondrocytes upon stimulation with a number of growth factors, proinflammatory cytokines, and hormones[5]. The proteolytic activity of MMP is thought to represent a crucial component of both physiological (embryonic development, organ morphogenesis, angiogenesis) and pathological (chronic inflammatory diseases, tumors) conditions[6]. The main function attributed to MMP is the remodeling of extracellular matrix during tissue resorption[7].

In adult rheumatoid arthritis (RA), a clear overexpression of a number of MMP has been reported at the level of rheumatoid synovial tissue[8-16]. Some MMP (MMP-1, MMP-3, MMP-8, and MMP-13) are mainly produced by synovial macrophages and fibroblast-like cells of the lining layer, where they carry out proteolytic digestion of the extracellular matrix of cartilage and bone as well as synovial tissue remodeling.

Four different natural inhibitors of MMP are known (namely, tissue inhibitor of metalloprotease, TIMP-1 to 4), which interact with activated MMP in the inflamed tissue, forming a 1:1 stoichiometric complex[5]. TIMP-1 is the natural MMP inhibitor most abundantly expressed in the lining cells of adult rheumatoid synovium[12-14,17,18].

We investigated synovial tissue expression of the main proteolytic enzymes (MMP-1, MMP-3, and MMP-13) and of TIMP-1 in juvenile idiopathic arthritis (JIA), which, at

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least in its oligoarticular and systemic subtypes, can be considered characteristic of the pediatric age.

MATERIALS AND METHODS

Synovial samples obtained at synovectomy or arthroplasty from 9 patients with JIA diagnosed according to the Durban criteria[19] were studied (Table 1). Patients' ages at the time of the study ranged from 10 to 29 years (mean 18.8). Disease onset occurred for all patients before the age of 16 years and disease duration ranged from 6 to 20 years (Table 1). Three patients had had a systemic onset with polyarticular course, one patient had a polyarticular RF negative onset, and 5 patients had oligoarticular onset (3 extended and 2 persistent)[19]. Prolonged drug treatment with nonsteroidal antiinflammatory agents, systemic and local steroids, and second-line therapy was administered to all patients. Treatment at the time of the study is reported in Table 1. Steroid administration in the previous 6 months was considered a criterion of exclusion from the study.

Evaluation of overall disease activity and the degree of active inflammation at the level of the involved joint at the time of surgery (measured on a 0–10 cm visual analog scale) was performed by 2 expert pediatric rheumatologists (VG, FF) and recorded together with the number of active joints, number of joints with limited range of motion, and erythrocyte sedimentation rate (ESR)[20,21].

Synovial membrane samples from a 24-year-old woman with RF positive RA and from a 13-year-old girl who underwent meniscectomy due to traumatic injury were studied as positive and negative controls, respectively (Patients 10 and 11, Table 1). Eleven synovial membrane specimens of 5 to 12 mm maximum diameter (mean 8 mm) were studied. Immunohistochemical staining was performed according to standard technique. Briefly, all specimens were fixed in 10% formalin for 24 h and embedded in paraffin. Four millimeter paraffin sections were deparaffinized in xylene, and rehydrated in descending grade (100–70%) ethanol. Endogenous peroxidase activity was blocked by 10 min treatment with 3% hydrogen peroxide in distilled water. Pretreatment of tissue sections in a 750 W microwave was performed for staining with MMP-1 and MMP-13 monoclonal antibodies (Mab) to carry out heat induced epitope retrieval. For MMP-3 and TIMP-1 Mab staining, enzymatic digestion was performed with pepsin (final concentration 2.5 mg/ml; Bio Genex, San Ramon, CA, USA).

Immunohistochemical reactions were performed in an automated immunostainer (Optimax, Bio Genex). Sections were incubated 30 min at room temperature with the following Mab: anti-CD68, clone kp1 (diluted 1:50; Dako, Glostrup, Denmark); anti-MMP-1, clone 41-1E5 (diluted 1:1000; Chemicon International, Temecula, CA, USA); anti MMP-3, clone 55-2A4 (diluted 1:1000; Chemicon); anti-MMP-13, clone 181-15A12 (diluted 1:400; Chemicon); and anti-TIMP-1, clone 147-6D11 (diluted 1:1000; Chemicon). All the anti-MMP Mab reacted with the precursor and with the biologically active form of the respective MMP. Sections were subsequently reacted for 30 min at room temperature with an anti-mouse Ig antibody conjugated to peroxidase labeled dextran polymer (EnVision, Dako). Reactions in the absence of primary antibody and with irrelevant antibodies of the same isotypes (anticytomegalovirus, clones DDG9 and CCH2, Dako) were carried out as negative controls. The antigen-antibody immunoreaction was visualized by diaminobenzidine substrate (DAB, Dako)[22]. Slides were counterstained with Mayer's hematoxylin.

Slides were evaluated blindly on 2 different occasions by 2 expert pathologists (CG, AM). In each sample all synovial areas were evaluated at the level of the lining and sublining layers. However, based on previous observations showing a prevalent expression of MMP and TIMP in the lining layer[3-6], a semiquantitative evaluation was performed at the latter site, according to the following criteria: macrophage (type A synoviocyte) infiltration was evaluated as number of CD68 (kp1) positive cells/high power field (hpf/40×) and classified as absent (–, < 3 positive cells/hpf), weakly positive (+, 3 to 10/hpf), moderately positive (++, 10 to 30/hpf), strongly positive (+++, 30–50/hpf), or intensively positive (++++, > 50/hpf)[13]. Expression of MMP and TIMP-1 was evaluated according to the same criteria. Type B synoviocyte infiltration was calculated as number of CD68 (kp1) negative fibroblast-like cells/hpf[13]. A mean of 6 hpf (range 4–12) were evaluated for each sample. The assignment of each sample to one of the above categories was based upon the predominant pattern observed. Intra- and interobserver variability was less than 5%.

Correlations among the above variables was performed using nonparametric Spearman's rank test. Differences between the degrees of concomitant MMP and TIMP-1 expression were evaluated by the Wilcoxon rank test.

RESULTS

The results of the studies on the degree of synoviocyte infiltration are reported in Table 2. A slight predominance of type A (CD68+) versus type B synoviocytes (CD68–) was noted both in patients with JIA and in the RA control. Conversely, as expected, fibroblast-like cells were found to be predominant in posttraumatic synovial membrane (Table 2 and Figure 2, panel A)[1]. Thus, infiltration of type A synoviocytes (CD68+) was considered a sensitive marker of the degree of synovial inflammation[1,2].

The proportion of CD68+ cells was significantly correlated with the physician clinical assessment of the

Table 1. Clinical characterization of JIA and RA patients and control (Patient 11) at the time of study.

Patient	Sex	Disease Duration	JIA Subgroup or Other Conditions	Ongoing Treatment	Source	Global Disease Score	Single Joint Score	No. of Active Joints	ESR
1	M	7	Extended oligo	NSAID, MTX	Knee	2	0.8	6	16
2	F	20	Systemic	CS, MTX	Hip	6.8	0.9	0	62
3	F	11	Polyarticular	CS, AZA, NSAID	Knee	6.7	3.2	6	40
4	M	18	Systemic	NSAID	Hip	7.1	4.9	28	50
5	M	6	Persistent oligo	CSA, NSAID	Knee	4	6.8	1	14
6	M	13	Extended oligo	NSAID, MTX	Wrist	5.2	5.4	3	23
7	M	7	Extended oligo	CS, MTX	Knee	3.9	2.8	1	18
8	M	10	Persistent oligo	NSAID	Knee	1.8	2.9	1	14
9	F	7	Systemic	CS, NSAID, MTX	Knee	5	3.8	4	13
10	F	7	RA	CS, CSA, NSAID	Knee	6.9	6	33	6
11	F	—	Posttraumatic	—	Knee	—	—	—	—

NSAID: nonsteroidal antiinflammatory drugs, CS: corticosteroids, MTX: Methotrexate, CSA: Cyclosporine, AZA: Azathioprine, ESR: erythrocyte sedimentation rate, mm/h.

Table 2. Immunohistochemical analysis of type A (CD68+) and B (CD68–) synoviocyte infiltration, and MMP and TIMP-1 expression in respective synovial tissue.

Patient	Source	Synoviocytes		MMP-1	MMP-3	MMP-13	TIMP-1
		Type A	Type B				
1	Knee	1+	2+	1+	1+	1+	1+
2	Hip	2+	2+	2+	1+	1+	1+
3	Knee	3+	3+	3+	3+	–	2+
4	Hip	3+	2+	3+	4+	2+	2+
5	Knee	4+	3+	4+	3+	2+	2+
6	Wrist	4+	3+	3+	4+	2+	1+
7	Knee	2+	2+	2+	2+	1+	1+
8	Knee	2+	2+	2+	2+	2+	1+
9	Knee	3+	2+	3+	2+	2+	2+
10	Knee	4+	3+	4+	3+	2+	2+
11	Knee	1+	2+	2+	2+	–	2+

–, negative: < 3 positive cells/high power field (hpf); 1+, weakly positive: 3–10 cells/hpf; 2+, moderately positive: 10-30 cells/hpf; 3+, strongly positive: 30-50 cells/hpf; 4+, intensively positive: > 50 cells/hpf.

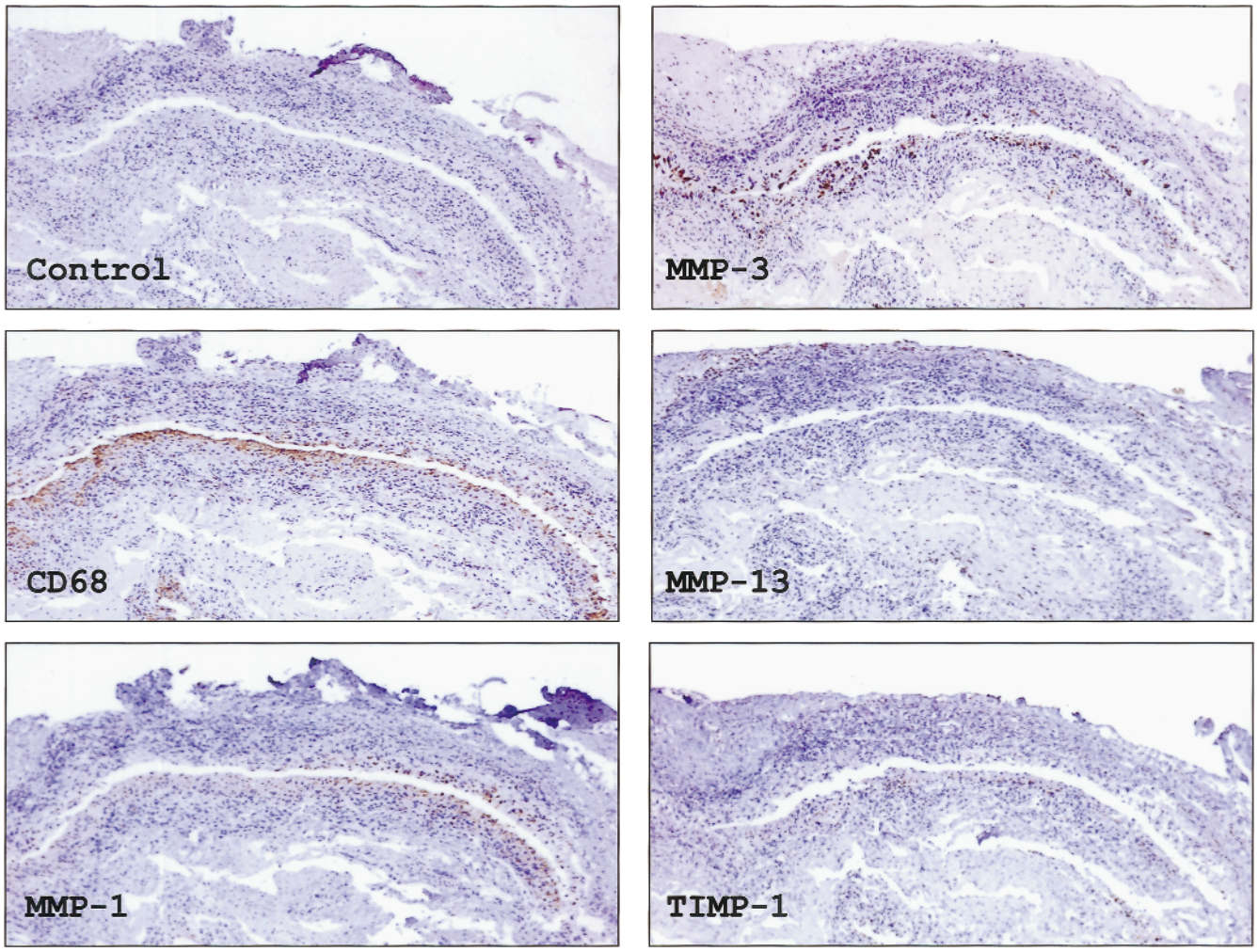

Figure 1. Immunohistochemical study of the synovial tissue of a 14-year-old boy with persistent oligoarticular JIA (Patient 5). Expression of CD68, MMP, and TIMP-1 within the same synovial area. The intraanalysis negative control was performed using irrelevant isotype matched primary antibodies (see Materials and Methods). The lining layer appears thickened and heavily infiltrated with type A (CD68+) synoviocytes. MMP-1 and -3 are clearly expressed in the lining layer with the same distribution of CD68+ cells. MMP-13 expression is restricted to the deepest portion of the lining layer. TIMP-1 distribution shows a lower number of positive cells in respect to MMP-1 and -3. Original magnification ×10.

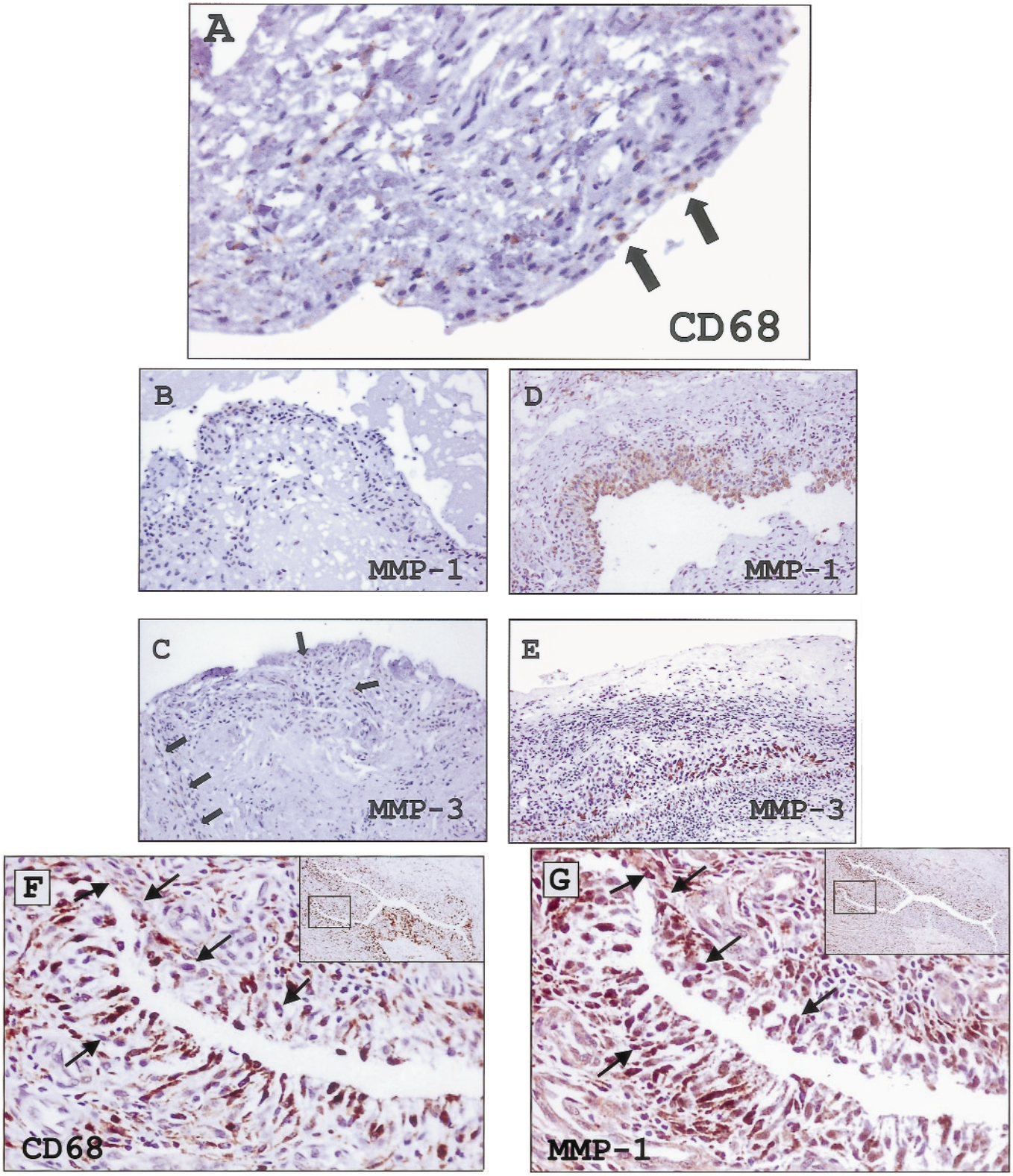

Figure 2. Panel A. Pattern of synoviocyte infiltration at the level of the lining layer in Patient 11 (posttraumatic meniscectomy): a few macrophage-like CD68+ cells (type A synoviocytes, arrows) are surrounded by fibroblast-like CD68– cells (type B synoviocytes) that represent the predominant cell type. Original magnification ×40. Panels B–E. Differential expression of MMP-1 and MMP-3 in JIA patients with different degree of synovial inflammation: B and C, low expression of MMP-1 and MMP-3, respectively, in Patient 2 (original magnification ×10; see Table 2); D and E, higher expression of the same MMP in Patient 5 (original magnification ×10; see Table 2). F and G, expression of CD68+ and MMP-1 in the same area at different magnifications (×10 and ×40, Patient 5); expression of MMP-1 by both CD68+ and CD68– synoviocytes (arrows) at high magnification (×40) is shown.

degree of inflammation of the involved joint ($r = 0.89$, $p = 0.001$), but not with the overall score of disease activity, number of active joints, and ESR.

For JIA patients and the RA control, MMP-1 and MMP-3 displayed a variable expression, with a prevalent localization at the level of the lining layer (Figure 1 and Figure 2, panels B–E). A clear, albeit weak, expression was noted in posttraumatic synovial membrane as well (Table 2). In patients with JIA a clear correlation between the proportion of MMP-1 and MMP-3 positive cells and type A synoviocyte infiltration was found ($r = 0.95$, $p < 0.001$; $r = 0.83$, $p = 0.003$, respectively). Similarly, both MMP-1 and MMP-3 expression correlated with the physician clinical assessment of the degree of joint inflammation at the time of the study ($r = 0.83$, $p = 0.005$; $r = 0.88$, $p = 0.002$, respectively). Notably, at high magnification, MMP-1 and MMP-3 were found to be expressed by both CD68+ and CD68– synoviocytes (Figure 2, panels F and G).

MMP-13 expression was detected in 8/9 patients with JIA and the RA control, but not in posttraumatic synovial membrane. In JIA, the proportion of cells staining for MMP-13 was less than that of cells reactive with MMP-1 and MMP-3. Further, MMP-13 positive cells were detected almost exclusively in the deepest portion of the intimal layer (as shown in Figure 1). A significant correlation was noted with the clinical assessment of the degree of joint inflammation ($r = 0.76$, $p = 0.02$), but not with type A synoviocyte infiltration ($r = 0.5$, $p = 0.16$).

TIMP-1 tissue distribution was similar to that observed for MMP-1 and -3, in both JIA patients and controls, with a prevalent localization in the lining layer (Figure 1). However, the number of cells staining for TIMP-1 was definitely lower than that of MMP-1 ($p < 0.05$) and MMP-3 ($p < 0.05$) positive cells in the same samples. TIMP-1 expression showed no significant correlation with either CD68+ cells ($p = 0.09$) or physician clinical assessment of the degree of joint inflammation ($p = 0.1$).

DISCUSSION

The synovial membrane consists of 2 components: the intimal (or synovial lining) and subintimal layers. The intimal layer is highly cellular and, in normal conditions, is prevalently constituted by fibroblast-like (type B, almost 75%) and macrophage-like (type A, 25%) synoviocytes[1]. The subintimal layer is formed mostly by fibrous or adipose tissue and exerts essentially supportive functions. During chronic inflammation cells from the monocyte-macrophage lineage are recruited from circulation into the intimal lining layer, where they contribute to the development and perpetuation of tissue damage and synovial hypertrophy through the production of inflammatory cytokines, growth factors, and proteolytic enzymes[1,2].

MMP are a large family of zinc dependent endoproteases that degrade most extracellular matrix components. All MMP are synthesized as proenzymes and their expression is transcriptionally regulated by different growth factors and proinflammatory cytokines. Inactive pro-MMP are secreted and subsequently activated *in vivo* by tissue or plasma proteinases through proteolytic cleavage of the propeptide domain at the N-terminus of the molecule[5–7].

The crucial role of these proteolytic enzymes in the pathogenesis of cartilage and bone damage related to inflammatory synovitis has been shown in adult RA. Fibroblast collagenase (MMP-1) and stromelysin-1 (MMP-3) were the first MMP found to be overexpressed in RA synovitis[11-14]. Recently, collagenase 3 (MMP-13) mRNA has been detected consistently and specifically in the RA synovium[15,16,23].

To our knowledge, no information is available yet on the expression of MMP in synovial tissue of patients with JIA. Serum stromelysin-1 has been reported to be elevated in patients with JIA[24]. Recently, a clear overproduction of stromelysin-1 was found in synovial fluids of patients with JIA, in comparison with paired serum samples[25].

In our study, immunohistochemical investigation of JIA synovium showed clear expression of MMP-1 and MMP-3 in all the specimens examined[11-14], especially at the level of the lining layer[13,14]. Moreover, a clear correlation between such expression and type A synoviocyte infiltration was observed[1].

Thus, in our experience, the presence of a low number of CD68 and MMP positive cells in some patients appears to be related to the low degree of synovium inflammation at the time of the study. MMP expression in posttraumatic specimens has been reported[15] and was attributed to constitutive production of these MMP (or their proenzymes) by resident fibroblast-like synoviocytes, which represent the prevalent cell population at the level of the normal lining layer[15]. It is thus conceivable that recruitment of activated macrophages stimulates overexpression and activation of MMP proenzymes by macrophages themselves and by resident fibroblast-like synoviocytes (Figure 2, panels F and G)[26].

Recently, a comparative mRNA analysis of all human MMP in RA and traumatic synovial membrane showed that MMP-13 expression was restricted to patients with RA[15]. In our study MMP-13 was detected in 8/9 specimens, mainly from the deepest intimal layer. This observation has been reported in adult patients with RA and seems to be related to a predominant production of MMP-13 by fibroblast-like synoviocytes of the subintimal layers[23].

Another interesting point comes from the possible inadequate regulatory role of natural tissue inhibitors of MMP in the development of JIA associated synovitis. The biological activity of all MMP is inhibited *in vivo* by 4 specific endogenous tissue inhibitors. The TIMP control connective tissue breakdown both by blocking the action of the activated MMP and by preventing their activation[5].

In our study, immunohistochemical analysis showed

expression of TIMP-1 in all the specimens examined. However, the proportion of TIMP-1 positive cells was generally lower than that of MMP-1 and MMP-3 positive cells. This feature is consistent with an inbalance between MMP and TIMP protein concentrations at the level of synovial fluid, as described in patients with RA[27,28] and JIA[25].

We show that overexpression of MMP-1 and MMP-3 is clearly related with synoviocyte infiltration in the affected joints of patients with JIA. These findings point to the pathogenic role of matrix metalloproteinases in this group of pediatric idiopathic arthritides. Inadequate expression of their tissue inhibitors may represent a crucial event for the development and perpetuation of tissue damage.

REFERENCES

1. Bullogh PG. Joints. In: Sternberg SS, editor. Histology for pathologists. Philadelphia: Lippincott-Raven; 1997:107-26.
2. Gallagher PJ, Anderson JR. Histopathology. In: Maddison PJ, Isenberg DA, Woo P, Glass D, editors. Oxford textbook of rheumatology. Oxford: Oxford Medical Publications; 1998:755-98.
3. Firestein GS. Invasive fibroblast-like synoviocytes in rheumatoid arthritis. Passive responders or transformed aggressor. Arthritis Rheum 1996;39:1781-90.
4. Burmester GR, Stuhlmuller B, Keyszer G, Kinne RW. Mononuclear phagocytes and rheumatoid arthritis. Mastermind or workhorse in arthritis? Arthritis Rheum 1997;40:5-18.
5. Nagase H, Woessner F. Matrix metalloproteinases. J Biol Chem 1999;274:21491-4.
6. Sato H, Takino T, Okada Y, et al. A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 1994;370:61-5.
7. Bode W, Fernandez-Catalan C, Grams F, et al. Insights into MMP-TIMP interactions. Ann NY Acad Sci 1999;878:73-91.
8. Evanson JM, Jeffrey JJ, Krane SM. Human collagenase: identification and characterization of an enzyme from rheumatoid synovium in culture. Science 1967;158:499-502.
9. Harris ED Jr, Cohen GL, Krane SM. Synovial collagenase: its presence in culture from joint disease of diverse etiology. Arthritis Rheum 1969;12:92-102.
10. Okada Y, Nagase H, Harris ED Jr. A metalloproteinase from human rheumatoid synovial fibroblasts that digests connective tissue matrix components. Purification and characterization. J Biol Chem 1986;261:14245-55.
11. Okada Y, Takeuchi N, Tomita K, Nakanishi I, Nagase H. Immunolocalization of matrix metalloproteinase 3 (stromelysin) in rheumatoid synovioblasts (B cells): correlation with rheumatoid arthritis. Ann Rheum Dis 1989;48:645-53.
12. Docherty AJ, Murphy G. The tissue metalloproteinase family and the inhibitor TIMP: a study using cDNAs and recombinant proteins. Ann Rheum Dis 1990;49:469-79.
13. McCachren SS. Expression of metalloproteinases and metalloproteinase inhibitor in human arthritic synovium. Arthritis Rheum 1991;34:1085-93.
14. Firestein GS, Paine MM. Stromelysin and tissue inhibitor of metalloproteinases gene expression in rheumatoid arthritis synovium. Am J Pathol 1992;140:1309-14.
15. Konttinen YT, Ainola M, Valleala H, et al. Analysis of 16 different matrix metalloproteinases (MPP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. Ann Rheum Dis 1999;58:691-7.
16. Lindy O, Konttinen YT, Sorsa T, et al. Matrix metalloproteinase 13 (collagenase 3) in human rheumatoid synovium. Arthritis Rheum 1997;40:1391-9.
17. Osthues A, Knauper V, Oberhoff R, Reinke H, Tschesche H. Isolation and characterization of tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) from human rheumatoid synovial fluid. FEBS Lett 1992;296:16-20.
18. Hembry RM, Bagga MR, Reynolds JJ, Hamblen DL. Immunolocalisation studies on six matrix metalloproteinases and their inhibitors, TIMP-1 and TIMP-2, in synovia from patients with osteo- and rheumatoid arthritis. Ann Rheum Dis 1995;54:25-32.
19. Petty RE, Southwood TR, Baum J, et al. Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. J Rheumatol 1998;25:1991-4.
20. Giannini EH, Ruperto N, Ravelli A, Lovell DJ, Felson DT, Martini A. Preliminary definition of improvement in juvenile arthritis. Arthritis Rheum 1997;40:1202-9.
21. Ruperto N, Ravelli A, Migliavacca D, et al. Responsiveness of clinical measures in children with oligoarticular juvenile chronic arthritis. J Rheumatol 1999;26:1827-30.
22. Sabattini E, Bisgaard K, Ascani S, et al. The EnVision++ system: a new immunohistochemical method for diagnostics and research. Critical comparison with the APAAP, ChemMate, CSA, LABC, and SABC techniques. J Clin Pathol 1998;51:506-11.
23. Westhoff CS, Freudiger D, Petrow P, et al. Characterization of collagenase 3 (matrix metalloproteinase 13) messenger RNA expression in the synovial membrane and synovial fibroblasts of patients with rheumatoid arthritis. Arthritis Rheum 1999; 42:1517-27.
24. Matsuyama T. Tissue inhibitor of metalloproteinases-1 and matrix metalloproteinase-3 in Japanese healthy children and in Kawasaki disease and their clinical usefulness in juvenile rheumatoid arthritis. Pediatr Int 1999;41:239-45.
25. Gattorno M, Vignola S, Falcini F, et al. Serum and synovial fluid concentrations of matrix metalloproteinases 3 and its tissue inhibitor 1 in juvenile idiopathic arthritides J Rheumatol 2002;29:826-31.
26. Koch AE. Angiogenesis. Implication for rheumatoid arthritis. Arthritis Rheum 1998;41:951-62.
27. Martel-Pelletier J, McCollum R, Fujimoto N, Obata K, Cloutier JM, Pelletier JP. Excess of metalloproteases over tissue inhibitor of metalloprotease may contribute to cartilage degradation in osteoarthritis and rheumatoid arthritis. Lab Invest 1994;70:807-15.
28. Burger D, Rezzonico R, Li JM, et al. Imbalance between interstitial collagenase and tissue inhibitor of metalloproteinases 1 in synoviocytes and fibroblast upon direct contact with stimulated T lymphocytes: Involvement of membrane associated cytokines. Arthritis Rheum 1998;41:1748-59.