Letters to the Editor

Rheumatology 2009;48:1460–1461
doi:10.1093/rheumatology/kep271
Advance Access publication 6 September 2009

MMP-12, a novel matrix metalloproteinase associated with giant cell arteritis

Sir, Identification of specific mediators of arterial damage in GCA is crucial to understand its pathogenesis, and may have important clinical implications both for diagnosis and treatment. Thirty-two non-consecutive patients who underwent a temporal artery biopsy for evaluation of GCA were included in this study. Nineteen of the 32 biopsies had histological evidence of GCA. Detailed pathological findings were recorded on every biopsy and assessed using semi-quantitative scales [1]. Patients' demographic and clinical information was collected. All subjects gave written informed consent. The study was approved by our institutional review board (Wills Eye Institute).

In brief, total RNA was isolated from the temporal artery biopsies. The concentration and quality of RNA was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Wilmington, DE, USA). DNA microarray analysis using Affymetrix U133 Plus 2.0 human genome arrays was performed in two temporal artery specimens with histologically proven GCA and two that were negative for GCA. We focused on identifying which of the 23 human MMPs were up-regulated in GCA. To confirm the expression levels of those genes up-regulated in the microarray analysis, relative quantification by real-time PCR was performed in all the 32 temporal artery biopsies, and gene expression between arteries with histological evidence of GCA and controls were compared. Single-strand cDNA was synthesized from total RNA. Real-time PCR SYBR Green assays with pre-designed primers for *MMP-2, -9, -12* and GAPDH (SABioscience Corp., Frederick, USA) were run in a Stratagene Mx3000P thermal cycler. All the assays were run in triplicate by an investigator blinded to the pathological and clinical data.

Demographic and clinical features of the patients are shown in Table 1. Those who had negative biopsy results were diagnosed with conditions different from GCA based on their clinical manifestations, the negative biopsy, their lack of response to steroid therapy and their clinical evolution. Seven biopsy-negative patients fulfilled the ACR classification criteria for GCA. In the GCA-negative arteries, >80% of the internal elastic lamina (IEL) was preserved, whereas IEL degradation was common in temporal arteries with histological evidence of GCA. Mild intimal hyperplasia was observed in some of the GCA-negative temporal arteries, but all the GCA-positive arteries showed a high level of intimal hyperplasia and in seven (36.8%) of these, the lumen was virtually occluded.

A total of approximately 2000 genes showed a significant differential level of expression in our microarray analysis. Approximately half of these were up-regulated and half were down-regulated. Among the MMPs, only *MMP-12* (fold change = 163; $P = 0.003$) and *MMP-9* (fold change = 21; $P = 0.026$) showed statistically significant up-regulation in GCA arteries compared with controls by microarray analysis. *MMP-2* was also up-regulated in GCA arteries, but this was not statistically significant ($P = 0.075$). Interestingly, *MMP-12* was the fifth most up-regulated gene, when ordered by the level of expression (see Appendix 1 available as supplementary data at *Rheumatology* Online). *MMP-9* occupied the 65th position in that list. Up-regulation of *MMP-12* and *-9* was confirmed by real-time PCR. The median fold change (interquartile range) for *MMP-12* was 67.7 (400.2) in GCA-positive biopsies *vs* 2.1 (3) in GCA-negative biopsies ($P = 0.003$). The median fold change for *MMP-9* was 20.7 (57.7) in GCA-positive *vs* 0.06 (0.5) in GCA-negative biopsies. *MMP-2* was also relatively up-regulated in GCA arteries [2.19 (4.15)] compared with GCA-negative biopsies [0.89 (1.14)], but this difference was not statistically significant ($P = 0.07$). Levels of expression of both *MMP-9* and *-12* correlated with typical GCA pathological findings (see Appendix 2 available as supplementary data at *Rheumatology* Online).

Our study is the first to use a broad-spectrum approach to define which of the 23 human MMPs are associated with histologically proven GCA. We are the first to have identified the overexpression of *MMP-12* gene transcripts in GCA lesions.

MMP-12 appears to participate in atherosclerosis and aneurysm growth by degradation of the elastic layers and basement membrane [2, 3]. We postulate that this enzyme may also contribute to elastic lamina degradation in GCA. The association of *MMP-9* with histologically confirmed GCA has been reported previously [1, 4–8]. *MMP-9* likely facilitates both IEL rupture and intimal hyperplasia [1]. Although the association of *MMP-2* with GCA is still controversial, most studies have found that *MMP-2* is ubiquitously expressed in temporal arteries both with and without vasculitis [1, 4, 6, 7, 9].

Table 1. Demographic and clinical variables of patients with histologically confirmed GCA and patients with negative temporal artery biopsy

	GCA patients, $n = 19$	Non-GCA patients, $n = 13$	*P*-value
Age, mean ± s.d., years	77.7 ± 7.8	79 ± 6.2	0.62
Female, *n* (%)	10 (52.6)	10 (76.9)	0.16
Number of patients on treatment before the biopsy, *n* (%)	8 (42.1)	2 (15.4)	0.11
Days of treatment, median (IQR)	0 (1)	0 (0)	0.15
ESR, mean ± s.d.	66.4 ± 38.6	52.3 ± 50.9	0.43
Headache, *n* (%)	9 (47.4)	5 (38.5)	0.62
Jaw claudication, *n* (%)	8 (42.1)	1 (7.7)	0.05
Scalp tenderness, *n* (%)	1 (5.3)	0 (0)	1
Temporal artery thickening, *n* (%)	6 (31.6)	1 (7.7)	0.19
Temporal artery tenderness, *n* (%)	2 (10.5)	2 (15.4)	1
Temporal artery decreased pulse, *n* (%)	2 (10.5)	1 (7.7)	1
Visual symptoms, *n* (%)	18 (94.7)	9 (69.2)	0.13
Fever, *n* (%)	0 (0)	1 (7.7)	0.41
PMR, *n* (%)	0 (0)	1 (7.7)	0.41

IQR = interquartile range.

Despite the limited number of biopsies included in the microarray study, the stringent statistical analysis used and the validation using real-time-PCR give us confidence in our findings. Although MMP mRNA may not correlate perfectly with protein concentration in tissues or enzymatic activity [10], our findings suggest that the potential role of *MMP-9* and *-12* in GCA pathogenesis deserves further study. Functional studies are needed to determine the specific contribution of these two enzymes in the development of arterial damage in GCA before any conjecture about their use as therapeutic target(s) can be made. Clinical studies will be required to determine whether serum levels of these MMPs are elevated in GCA patients. If so, our preliminary findings might have implications for diagnosis of GCA.

Rheumatology key message

- *MMP-12* and *-9* are the only MMPs that are overexpressed in GCA lesions.

Acknowledgements

The authors gratefully acknowledge Dr James A. Mahoney (Rheumatology, Johns Hopkins University School of Medicine) for consultation on molecular biology aspects of this study and technical assistance. P.S. is a Lowe Family Scholar at the Johns Hopkins Center for Innovative Medicine.

A.R.-P. was supported as a Post-Doctoral Fellow by the Vasculitis Clinical Research Consortium (U54 RRO 19497).

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

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Accepted 30 July 2009

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Rheumatology 2009;48:1461–1462
doi:10.1093/rheumatology/kep241
Advance Access publication 18 August 2009

Mesenteric and splenic cat-scratch disease during etanercept therapy

Sir, Cat-scratch disease (CSD) is a benign infectious disease caused by *Bartonella henselae*, and generally seen among children and young adults. Disseminated infection with extended granulomatous lymphadenopathy and visceral involvement is well known in immuno-deficient patients. We report a case of a systemic CSD with mesenteric lymphadenopathy and splenic involvement in a patient taking etanercept to treat PsA.

A 51-year-old man was admitted to hospital with an elevated fever for 1 week. He had a history of PsA treated with etanercept for 2 years. Two days after the last etanercept injection, he had noticed an isolated fever with night sweating.

Physical examination of the heart, lungs and abdomen was normal. In the left groin, a painful enlarged lymph node was palpable. There was no other detectable lymph node. We noticed few injuries on the legs without cellulitis that had been caused by a kitten.

Routine laboratory tests were within the normal range except ESR (103 mm/h) and CRP (88 mg/l). The following tests were negative: bacterial cultures of blood and urine, tuberculin skin test, serological tests for the HIV, Epstein–Barr virus, cytomegalovirus, syphilis, toxoplasma and bartonella.

Echography of the left groin revealed enlargement of lymph nodes and peripheral fat infiltration. Platelet anti-aggregation with clopidogrel was a contraindication for lymph node puncture.

Thoracoabdominal tomography showed pathological enlargement of mesenteric lymph nodes, peritoneal panniculitis and multiple hypodense round lesions in the spleen (Fig. 1). Bone biopsy revealed reactive plasmocytosis.

Infectious endocarditis was excluded by transesophageal echocardiography. CSD was suspected and we started a treatment with azithromycin (1 g/day for 5 days, but rapidly decreased to 0.5 g because of digestive adverse effects). The size of the lymph

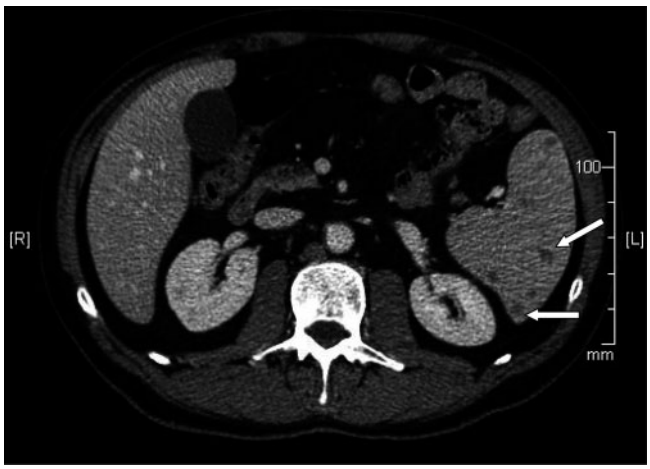

Fig. 1. Contrast-enhanced abdominal CT showing multiple small hypodense nodular lesions within the spleen.