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Spine

Inflammatory mediators of cerebrospinal fluid from patients with spinal cord injury☆

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Abstract

Background: The guidelines of MP treatment for acute SCI are still under debate. We examined the inflammatory mediators of CSF in patients with SCI and assessed the effect of MP treatment.
Methods: We studied 7 patients with acute SCI at the cervical level and examined the mediators of CSF in patients by cytokine antibody array, ELISA and gelatin zymography.
Results: We found that levels of IL-6, IL-8, monocyte chemoattractant protein-1, neutrophil-activating peptide 2, intracellular adhesion molecule-1, soluble Fas, tissue inhibitors of metalloproteinase 1, and matrix metalloproteinases-2 and -9 were upregulated in patients with complete SCI without MP treatment as compared to patients with MP treatments, incomplete SCI, or controls. Nerve growth factor was upregulated in patients with MP treatment.
Conclusions: We suggest that a neuroinflammatory CSF profile after complete SCI could be suppressed with MP treatment via downregulating the expression of various cytokines.

Keywords: Spinal cord injury; Cytokine; Methylprednisolone

1. Introduction

The pathophysiology of acute SCI includes an initial, mechanical injury and a series of secondary events such as ischemia-, calcium-, and sodium-mediated cell injury, excitotoxic cell death, inflammation, and apoptosis. Bracken et al [1] advocated MP as a neuroprotective agent in patients with SCI and reported favorable results in the Second and Third National Acute Spinal Cord Injury Studies. Although MP has been widely used for acute SCI, further appraisals are needed to substantiate its effect [6,10]. In this study, we examined the inflammatory mediators in CSF collected from patients with SCI patients who received spinal surgery and compared the changes in levels of cytokine in the CSF of patients who did and did not receive MP treatment.

Abbreviations: cSCI, complete SCI; CSF, cerebrospinal fluid; CT, control group; ELISA, enzyme-linked immunosorbent assay; ICAM-1, intracellular adhesion molecule-1; icSCI, incomplete SCI; IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; MMP-2, matrix metalloproteinases-2; MMP-9, matrix metalloproteinases-9; MP, methylprednisolone; NAP-2, neutrophil activating peptide 2; NGF, nerve growth factor; SCI, spinal cord injury; sFas, soluble Fas; TIMP-1, tissue inhibitors of metalloproteinases 1.

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2. Case description and methods

2.1. Subjects

We recruited 7 patients (mean age, 52.71 ± 16.4 years) with acute SCI at the cervical levels as shown in Table 1. We gave a bolus dose (30 mg/kg intravenously) of MP to patients with SCI who arrived within 8 hours of their injury, followed by an infusion of 5.4 mg/kg per hour over a 23-hour period. We did not, however, give MP to patients with SCI who arrived 8 hours after injuries. Table 1 lists the demographic and clinical data of patients including controls. One patient (no. 7) who had cSCI did not receive MP treatment (−MP). His CSF was taken 4 days after trauma. Another patient (no. 1) who had cSCI received MP treatment (+MP). Among 5 patients with icSCI, 2 (patients 2 and 3) were treated with MP, whereas the other 3 (patients 17-19) were not. Complete SCI is defined as complete loss of neurologic functions including motor, sensory, and sphincters below the injured level; otherwise, it is incomplete. We obtained CSF samples 1 to 4 days after SCI and frozen at −80°C until used. The 12 patients in the CT group were diagnosed as described in Table 1. Patients signed consent forms in accordance with the clinical study guidelines established at the Shin Kong Wu Ho-Su Memorial Hospital before CSF sampling. The follow-up period after SCI and treatment was more than half a year for each patient. There was no functional improvement for the patients with cSCI either with or without MP treatment. But the functional improvements for the icSCI groups were impressive. For data analysis, we grouped the patients into 5 groups: cSCI −MP, cSCI +MP, icSCI −MP, icSCI +MP, and CT.

Table 1
Patient demographics and clinic characteristics

Patient	Sex	Age	Clinical history	CSF albumin (mg/L)	WBC	MP
1	M	73	C spinal injury with cSCI	81	2	Y
2	M	45	Traumatic herniated intervertebral disc of C with central cord syndrome and icSCI	952	97	Y
3	M	65	C5/6 fracture and epidural hematoma with icSCI	ND	0	Y
4	F	78	Hydrocephalus	88	0	N
5	M	54	Herniated intervertebral disc of L4/5	ND	6	N
6	M	29	Hydrocephalus	96	2	N
7	M	43	C5/6 fracture and dislocation with cSCI	1763	0	N
8	F	19	T1 intraspinal, arachnoid cyst	35	45	N
9	M	41	Hydrocephalus	44	0	N
10	F	71	Left cerebellopontine angle tumor and hydrocephalus	365	1	N
11	F	74	C-spinal epidural hematoma and tumor	467	29	N
12	F	38	C3-T1 syrinx	307	8	N
13	F	48	Normal pressure hydrocephalus	19	ND	N
14	M	67	Left hemifacial spasm	316	ND	N
15	F	54	C2 spinal meningioma	1039	18	N
16	F	78	Normal pressure hydrocephalus	330	1	N
17	M	64	C-spinal injury with icSCI	297	4	N
18	M	25	C7 fracture with icSCI	180	3	N
19	F	54	C-spinal injury with icSCI	93	1	N

ND indicates not done; WBC, white blood cell.

2.2. Human cytokine antibody array

Transignal human cytokine antibody array 3 MA6020 (Panomics, Redwood City, Calif) consisted of 42 different cytokine antibodies spotted in duplicate onto a membrane. Arrays were analyzed according to the manufacturer's instruction. The intensities of the signals were photographed using a Kodak digital camera (DC290) and quantitated by densitometry (Kodak ID Image Analysis software 3.5; Kodak, Rochester, NY). The relative intensity of array data from patients with cSCI vs with MP treatments and controls is compared spot by spot. Only the spots with greater than 2-fold difference in relative intensity are listed.

2.3. Quantification of cytokines in CSF by ELISA

DuoSet cytokine ELISA kits (R&D Systems, Minneapolis, Minn) were purchased and analyzed by commercial protocols. All data are expressed as mean of the duplicated wells ± SEM.

2.4. Matrix metalloproteinase analysis by gelatin zymography

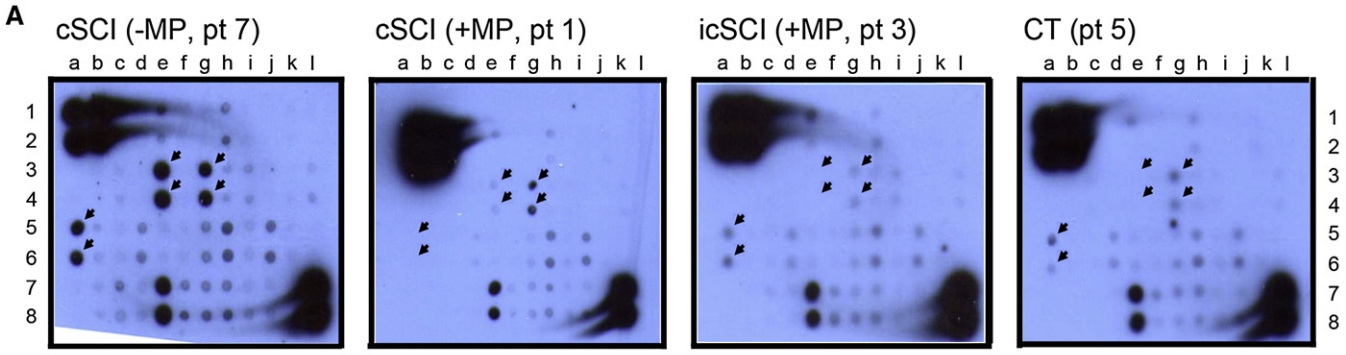
Matrix metalloproteinases were measured by gelatin zymography. Equal amount of total protein (5 μg) from each patient's CSF was separated on 10% sodium dodecyl sulfate–polyacrylamide gels containing gelatin substrate to analyze MMP-2 and MMP-9 production. Recombinant MMP-9 (5 ng) (Chemicon, Temecula, Calif) was used as the standard.

Semiquantitation of MMPs was conducted by scanning photographs of the gels with an imaging densitometer system (Kodak 1D Image Analysis software 3.5). For data analysis, patients with white blood cell counts greater than 5 per microliter were excluded.

3. Results

The cytokine profiles analyzed by the antibody array are listed in Fig. 1. Fig. 1C-E shows the spots with greater than 2-fold difference in relative intensity between patients. The present array analysis indicated selective increases in the levels of chemokines (IL-8, MCP-1) and proinflammatory cytokines (IL-6) in patients with cSCI and downregulated after MP treatment. This suggests that cSCI initiates an acute inflammatory reaction in the brain.

ELISA was used to verify the data from the array. Fig. 2 shows cytokines MCP-1, NAP-2, IL-8, IL-6, ICAM-1 (A); sFas and NGF (B); TIMP and MMP (C)



B

	a	b	c	d	e	f	g	h	i	j	k	l
1	Pos	Pos	Neg	Neg	ENA-78	GCSF	GM-CSF	GRO	GRO-α	I-309	IL-1α	IL-1b
2	Pos	Pos	Neg	Neg	ENA-78	GCSF	GM-CSF	GRO	GRO-α	I-309	IL-1α	IL-1b
3	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12	IL-13	IL-15	IFN-γ
4	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12	IL-13	IL-15	IFN-γ
5	MCP-1	MCP-2	MCP-3	MCSF	MDC	MIG	MIP-1δ	RANTES	SCF	SDF-1	TARC	TGF-β1
6	MCP-1	MCP-2	MCP-3	MCSF	MDC	MIG	MIP-1δ	RANTES	SCF	SDF-1	TARC	TGF-β1
7	TNF-α	TNF-β	EGF	IGF-I	Ang	OSM	Tpo	VEGF	PDGF β	Leptin	Neg	Pos
8	TNF-α	TNF-β	EGF	IGF-I	Ang	OSM	Tpo	VEGF	PDGF β	Leptin	Neg	Pos

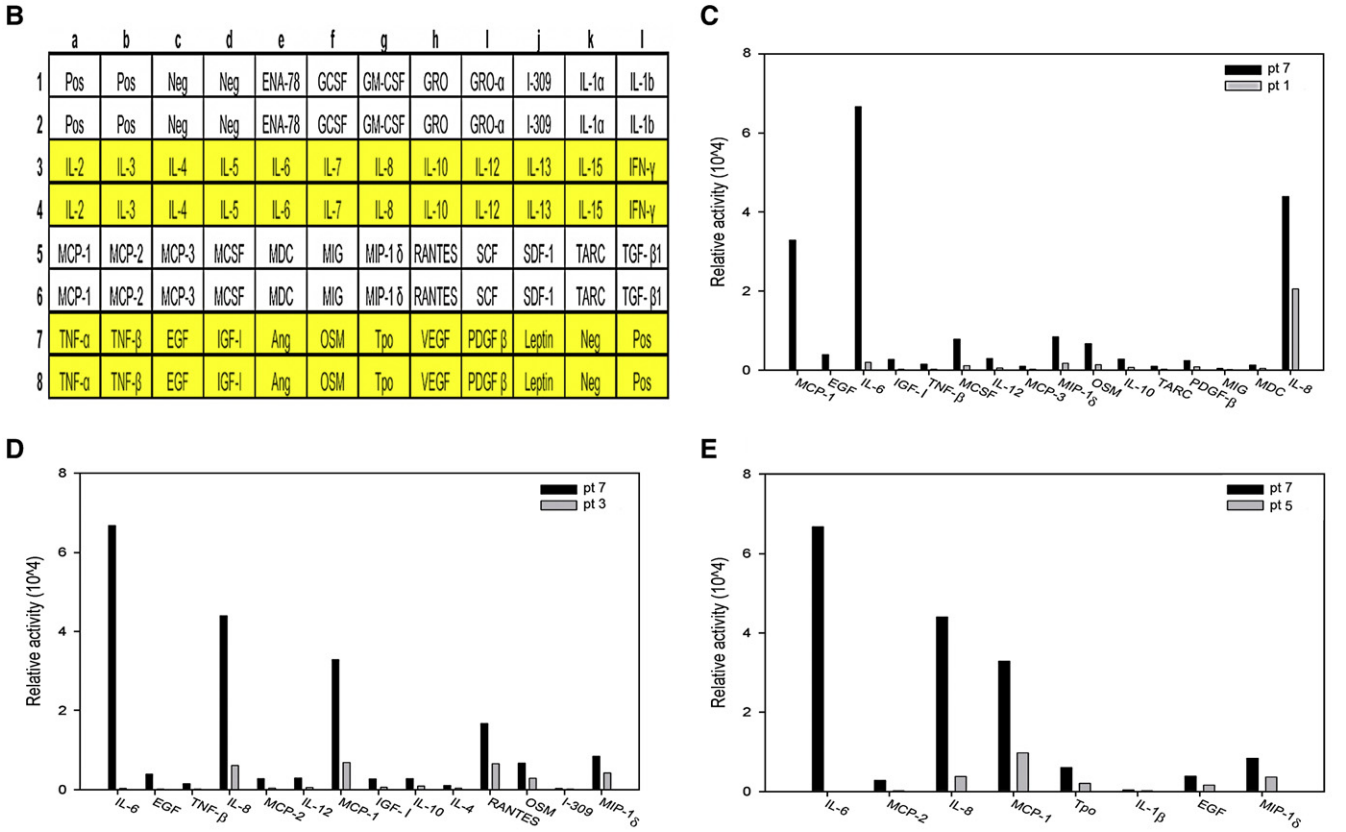


Fig. 1. Detection of cytokine expression profiles in 4 patients with SCI—cSCI −MP (patient 7), cSCI +MP (patient 1), icSCI +MP (patient 3), and CT (patient 5)—by antibody arrays is presented in panel A. Each dot represents immunoreactive staining against respective antibodies. Note the absence of staining at the negative control. Arrows indicate the positive signals of IL-6 (e, 3, 4), IL-8 (g, 3, 4), and MCP-1 (a, 5, 6) in patient 7 as compared to patients with MP treatment and control. A key to cytokine coordinates of panel A is shown in (B). Densitometric quantification of relative cytokine levels with greater than 2-fold difference between patients 7 and 1 (C), between patients 7 and 3 (D), and between patients 7 and 5 (E) are shown in diagrams.

expression in 5 groups of patients. Elevated CSF cytokines were measured in patients with cSCI as compared to patients with MP treatment and control. Gelatin zymography gel analysis showed that MMP-2 (72 kd) and MMP-9 (92 kd) activities were downregulated after MP treatment (Fig. 2C). Patients with MP treatment downregulate cytokines, sFas, TIMP, MMP-2, and MMP-9 activities, but upregulate NGF secretion. Therefore, these results suggest that inflammatory response mediated by increased cytokine expression may play an important role in the pathogenesis of acute SCI.

4. Discussion

This study aimed first to provide an overview of global changes in inflammatory mediator expression in CSF associated with SCI of humans. The findings show that inflammatory mediators in CSF markedly increased in patients with cSCI. These results agree with those noted in studies of rat models of SCI. Elevated levels of IL-8 have been seen in the CSF of patients with severe traumatic brain injury [8,14]. The CSF levels of IL-8, a pivotal cytokine in the pathology of brain injuries, have been correlated with

blood-brain barrier dysfunction [8]. Our results show that cytokine levels increased markedly (Fig. 2A), among which chemokines (MCP-1, NAP-2, IL-8) are produced by various cell populations to mediate trafficking of neutrophils and monocytes to inflammatory sites [13]. Elevated CSF IL-6 and ICAM-1 in patients with cSCI also agree with those in the literature [5,16].

Furthermore, the observed increase in sFas (Fig. 2B), involved in inflammatory activities [9,12], agrees with the reported upregulation at the lesion site after trauma and SCI. Neutralization of FasL further promotes regeneration and functional recovery after SCI [3]. Fas is involved in inflammatory activities [9,12]. Based on the findings of this study and in the literature, we suggest that upregulation of sFas expression may result from the inflammatory response at the lesion site. Nerve growth factor has been proved to promote sensory axon growth after injury [7]. Administration of NGF to SCI rats can protect the injured nerve tissues from apoptosis through stimulating bcl-2 and inhibiting bax protein expression [2]. Therefore, MP treatment increased NGF expression which may play a role in neuron protection through inhibition of apoptotic gene expression.

Previous studies show that upregulated MMP expression in rats with SCI [4,11] has been correlated with blood-brain barrier dysfunction, inflammation, and tissue injury. MMP-9 null mice showed better functional outcomes after SCI as compared with wild-type controls [11]. In addition, MP was

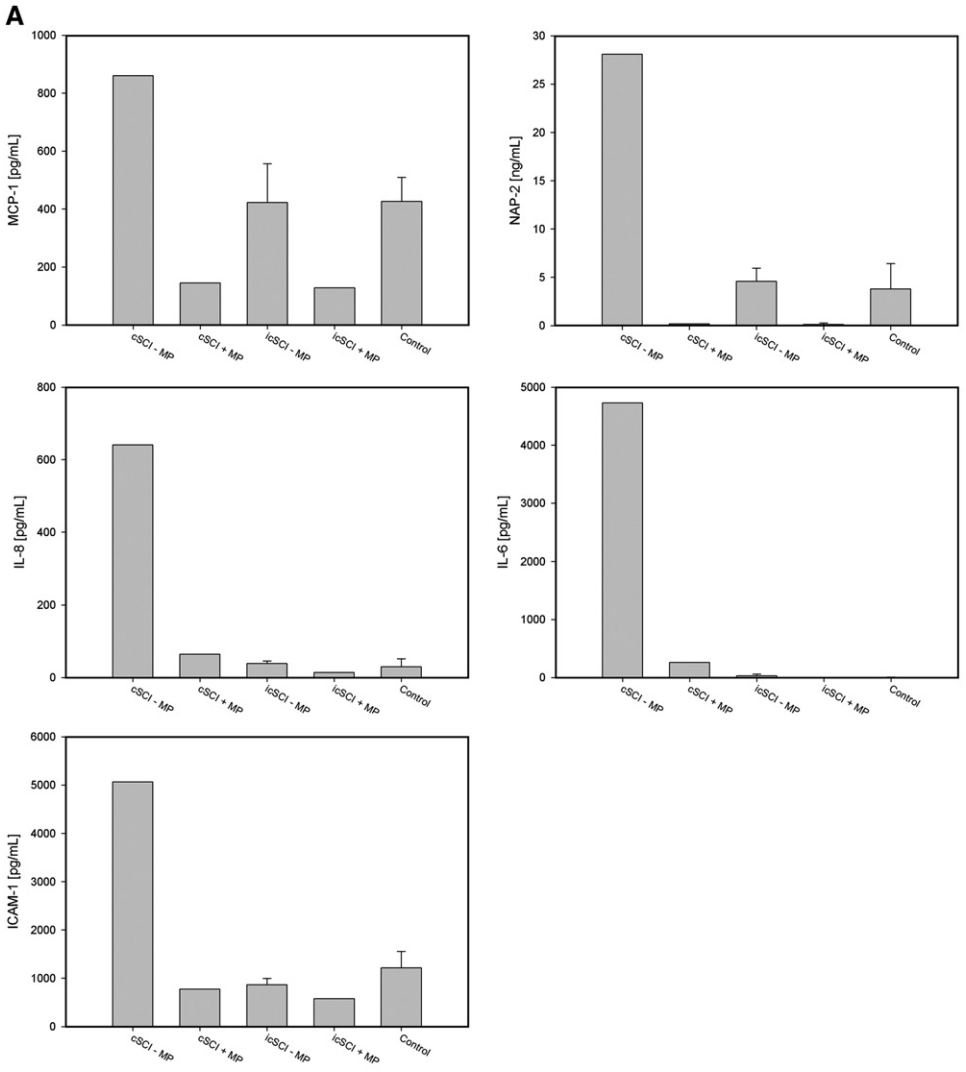


Fig. 2. Comparisons of individual inflammatory mediator level in 5 groups—cSCI −MP, cSCI +MP, icSCI −MP, icSCI +MP, and CT—are shown in diagrams. The levels of mediators were measured by ELISA. The levels of MCP-1, NAP-2, IL-8, IL-6, ICAM-1 (A), and sFas (B) in patients with cSCI-MP increased markedly, whereas the level of NGF increased after MP treatment for patients with cSCI (B). In panel C, MMP-2 and MMP-9 activities for each patient were analyzed by gelatin zymography. For each zymography gel, the first lane denoted recombinant MMP-9; other lanes denoted the patient's number as indicated in Table 1. TIMP concentration and densitometric quantification of MMP-2 and MMP-9 intensities are shown in diagrams (C). MMP-2 and MMP-9 activities in CSF were higher in patients with cSCI-MP than in those with MP treatment, and TIMP concentration was high in patients with cSCI−MP.

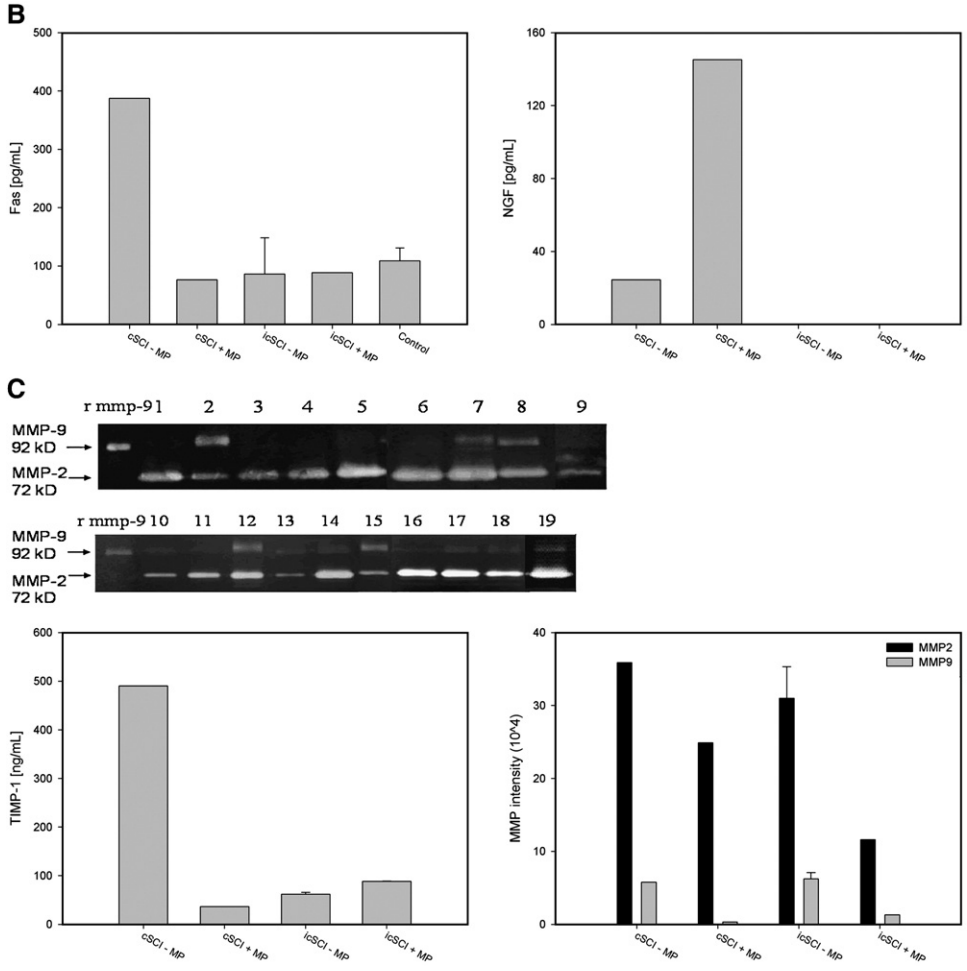


Fig. 2 (*continued*).

found to suppress MMP-9 expression after SCI [15]. The elevated concentrations of MMPs and TIMP-1 in CSF may be a regulatory mechanism involved in the extracellular matrix breakdown pathway.

In conclusion, we suggest that MP treatment may suppress the inflammatory processes by downregulating the expression of various cytokines, chemokines, adhesion molecules, sFas, MMPs, and TIMP molecules.

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Commentary

The authors present a very small patient series with significant and multiple patient variables. Despite these shortcomings, there may emerge a trend demonstrating MP's ability to affect cytokine mediators of inflammation. This study should be expanded and replicated at other centers if possible.

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