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ORIGINAL ARTICLE

Pilot Study: Elevated Circulating Levels of the Proinflammatory Cytokine Macrophage Migration Inhibitory Factor in Patients With Chronic Spinal Cord Injury

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Abstract

Objective: To test the hypothesis that the proinflammatory cytokine macrophage migration inhibitory factor (MIF) is elevated in the circulation of patients with chronic spinal cord injury (SCI) relative to uninjured subjects, and secondarily to identify additional immune mediators that are elevated in subjects with chronic SCI.

Design: Prospective, observational pilot study.

Setting: Outpatient clinic of a department of physical medicine and rehabilitation and research institute in an academic medical center.

Participants: Individuals with chronic (>1y from initial injury) SCI (n=22) and age- and sex-matched uninjured subjects (n=19).

Interventions: Not applicable.

Main Outcome Measures: Plasma levels of MIF, as determined by a commercially available multiplex suspension immunoassay. The relationship between MIF levels and clinical/demographic variables was also examined. As a secondary outcome, we evaluated other cytokines, chemokines, and growth factors.

Results: Plasma MIF levels were significantly higher in subjects with chronic SCI than in control subjects ($P<.001$). Elevated MIF levels were not correlated significantly with any one clinical or demographic characteristic. Subjects with SCI also exhibited significantly higher plasma levels of monokine induced by interferon-gamma/chemokine C-X-C motif ligand 9 ($P<.03$), macrophage colony stimulating factor ($P<.035$), interleukin-3 ($P<.044$), and stem cell growth factor beta (SCGF-β) ($P<.016$). Among subjects with SCI, the levels of SCGF-β increased with the time from initial injury.

Conclusions: These data confirm the hypothesis that MIF is elevated in subjects with chronic SCI and identify additional novel immune mediators that are also elevated in these subjects. This study suggests the importance of examining the potential functional roles of MIF and other immune factors in subjects with chronic SCI.

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The estimated number of Americans living with traumatic spinal cord injury (SCI) ranges from 200,000 to over a million.[1,2] Even with advances in understanding the molecular responses to acute traumatic SCI, we remain without an effective therapy. The medical management of patients with chronic SCI therefore remains complex. One well-studied molecular cascade induced acutely by SCI is inflammation, which exacerbates neuronal loss and promotes secondary tissue damage.[3,4] The potential clinical importance of acute inflammation after SCI was recently

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highlighted by identification of a panel of cytokines elevated in subjects with acute SCI that correlated with and was predictive of functional outcomes.[5]

Less is known about the extent and impact of inflammation in the chronic phase of SCI. Inflammatory cells were detected in human spinal cord tissue for years after initial SCI.[6] A relatively small number of elevated inflammatory mediators were identified in the circulation of subjects with chronic SCI.[7-11] These include interleukin (IL)-6, IL-2 receptor (IL-2R), and intercellular adhesion molecule-1, with the highest levels found in SCI subjects with pressure ulcers.[9] Subjects with chronic SCI also had elevated levels of IL-2 and tumor necrosis factor alpha (TNF-α).[8,10] In addition to cytokines, autoantibodies have been observed in preclinical and clinical studies[8,10,12] of chronic SCI, and autoimmunity-promoting cytokines were recently shown to be elevated in subjects with chronic SCI.[11]

Inflammatory cytokines influence many complications of SCI, including pressure ulcers, urinary tract infections (UTIs), accelerated atherogenesis, bone loss, reduced lung capacity, spasticity, autonomic dysreflexia, and pain. Increasingly, inflammatory mediators are implicated in these processes in chronic SCI and in some studies[13,14] correlate with them. The objective of this study was therefore to identify candidate inflammatory mediators elevated in plasma of subjects with chronic SCI that may serve as new therapeutic targets for primary outcomes and/or secondary complications of SCI.

Here, we focused on the pleiotropic proinflammatory cytokine macrophage migration inhibitory factor (MIF), which is produced constitutively by many cell types.[15] Most relevant to SCI, MIF is produced by central and peripheral neurons, and microglia.[16-18] Despite this constitutive expression, recombinant MIF is toxic to spinal neurons in vitro.[19] At the cellular level, there are many potentially relevant functions of MIF, including induction of other cytokines (eg, TNF-α and IL-1), regulation of immune cell migration, and the seemingly unique ability among cytokines to override the anti-inflammatory effects of glucocorticoids.[20-28]

List of abbreviations:

AIS	**American Spinal Injury Association Impairment Scale**
CTACK	**cutaneous T cell–attracting chemokine**
GROα	**growth-related oncogene alpha**
HGF	**hepatocyte growth factor**
IFN	**interferon**
IL	**interleukin**
IL-2Ra	**interleukin-2 receptor antagonist**
IQR	**interquartile range**
LIF	**leukemia inhibitory factor**
MCP-3/CCL7	**monocyte chemoattractant protein 3/chemokine C-C motif ligand 7**
MCSF	**macrophage colony-stimulating factor**
MIF	**macrophage migration inhibitory factor**
MIG/CXCL9	**monokine induced by IFN-gamma/chemokine C-X-C motif ligand 9**
β-NGF	**beta-nerve growth factor**
SCF	**stem cell factor**
SCGF-β	**stem cell growth factor beta**
SCI	**spinal cord injury**
SDF1-α	**stromal-derived factor alpha**
TNF	**tumor necrosis factor**
TRAIL	**tumor necrosis factor-related apoptosis-inducing ligand**
UTI	**urinary tract infection**

While some immune cells do not migrate in the presence of MIF, human monocytes migrate toward higher levels of MIF, via its interaction with chemokine receptors.[29]

At the organ and systems levels, MIF is a critical, early, beneficial mediator in the response to infection.[30-34] However, elevated and prolonged levels of MIF are deleterious and correlate with negative clinical outcomes in acute conditions, including sepsis,[35] and in chronic conditions, such as rheumatoid arthritis,[36,37] lupus,[38,39] atherosclerosis,[40,41] and diabetes.[42-44] MIF has also been studied in the serum and/or cerebrospinal fluid of patients with a variety of neurologic disorders. For example, MIF levels are upregulated in patients with Alzheimer disease,[45,46] Parkinson's disease,[47] and multiple sclerosis.[48,49] In rodent models of SCI, MIF is elevated acutely and its absence improves functional outcome.[18,50]

Methods

Participants

This study was performed in accord with the ethical standards of, and was approved by, the local institutional review board. Written informed consent was obtained from all subjects before study enrollment. The study was publicized to potential subjects via letter from a study personnel physiatrist board certified in SCI medicine, on ClinicalTrials.gov, and by signs posted publicly. The following inclusion criteria were used for subjects with chronic SCI: (1) ≥18 years of age; (2) a history of SCI at any level; (3) an initial injury occurring at least 1 year before the study; and (4) classified with an American Spinal Injury Association Impairment Scale (AIS) grade of A to D. A physiatrist board certified in SCI medicine evaluated the subjects' injuries according to the

Table 1 Clinical and demographic characteristics of subjects with chronic SCI and uninjured control subjects

Characteristics	Uninjured	Chronic SCI	*P*
No. of subjects	19	22	
Sex (Male)	13 (68)	16 (72)	.78
Age (y)	41±2 (31–59)	48±3 (21–67)	.56
Mechanism of injury	NA		
MVC		12 (54)	
Fall		5 (23)	
Sport		2 (9)	
Violence		1 (4.5)	
Other		2 (9)	
AIS grade	NA		
A		14 (63.64)	
B		0 (0)	
C		3 (13.64)	
D		5 (22.73)	
Level	NA		
Cervical		15 (68.18)	
Thoracic		7 (31.81)	
Lumbar		0 (0)	
Years from injury	NA	12±1.5	

NOTE. Values are n (%), mean ± SEM (range), mean ± SEM, or as otherwise indicated. Percentages may not add up to 100% because of rounding.
Abbreviations: MVC, motor vehicle collision; NA, not applicable.