Increased Levels of Macrophage Migration Inhibitory Factor in Sera of Patients with Escherichia coli O157:H7-Induced Enterocolitis

Verotoxin-producing Escherichia coli O157:H7 is a pathogen that causes severe hemorrhagic enterocolitis and sometimes leads to serious conditions such as hemolytic-uremic syndrome. Various inflammatory parameters have been assessed for their efficacy in predicting the severity of E. coli O157:H7-induced colitis (9, 10), but no serological biomarker has been identified which can precisely determine the presence and severity of this colitis. Macrophage migration inhibitory factor (MIF) is a unique cytokine that has been shown to play a role in several inflammatory diseases (4, 7). Here, we report increased levels of MIF in sera of patients with verotoxin-producing E. coli O157:H7-induced enterocolitis.

Serum samples were obtained from 24 patients with E. coli O157:H7-induced enterocolitis, 30 sex- and age-matched patients with bacterial enterocolitis induced by pathogens other than O157:H7 (Salmonella, Vibrio, and non-verotoxigenic E. coli) as disease controls, and 55 healthy individuals as normal controls. Serum MIF concentrations were measured with an enzyme-linked immunosorbent assay specific for MIF (IDLISA; Sapporo Immunodiagnostic Laboratory, Sapporo, Japan) as described previously (7). E. coli O157:H7 was diagnosed by stool culture, which is the gold standard diagnostic method for this pathogen.

As shown in Fig. 1, the serum MIF levels in patients with E. coli O157:H7-induced enterocolitis (23.67 ± 2.36 ng/ml; P < 0.001 versus normal controls and disease controls) were sixfold higher than those in normal controls and threefold higher than those in disease controls (3.99 ± 0.60 ng/ml, 0.875, 0.950, and 0.840, respectively). Furthermore, serum MIF levels provided a level of differentiation of subjects with E. coli O157:H7-induced enterocolitis that was comparable to that afforded by detection of E. coli O157:H7 from stool culture (cutoff value, sensitivity, specificity, and positive predictive value by serum MIF: 12.5 ng/ml, 0.875, 0.950, and 0.840, respectively). On the other hand, leukocyte counts and levels of C-reactive protein in peripheral blood samples were increased in patients with E. coli O157:H7-induced enterocolitis and disease controls compared with normal controls, but there was no significant difference between patients with E. coli O157:H7-induced enterocolitis and disease controls (data not shown).

We previously demonstrated that MIF is a pivotal cytokine in the pathogenesis of inflammatory bowel disease and other types of colitis (8). In addition, recent studies have shown that MIF is immediately released in large quantities from the pituitary and other tissues in response to lipopolysaccharide (LPS) (1, 3). In particular, increased MIF levels in serum have been reported in patients with endotoxemias such as sepsis (4, 6). In mice, targeted disruption of MIF diminishes the immune response to LPS (2). Thus, MIF is considered to be an important molecule in the development of endotoxemia and subsequent severe inflammation.

It has been hypothesized that LPS is involved in the pathophysiology of verotoxicogenic E. coli infection (5). In this study, we showed that serum MIF levels were markedly increased in patients with verotoxin-producing E. coli O157:H7-induced colitis compared with those in patients with nonspecific colitis and healthy individuals. These findings suggest that the acute-phase response of circulating MIF to the endotoxin induced by E. coli O157:H7 plays a role in the development of enterocolitis. Although further studies are needed to clarify the role of MIF in the pathophysiology of E. coli O157:H7-induced colitis, MIF may make a major contribution to the development of enterocolitis caused by verotoxicogenic E. coli O157:H7.

**REFERENCES**

1. Bernhagen, J., T. Calandra, R. A. Mitchell, S. B. Martin, K. J. Tracey, W. Voeiter, K. R. Manogue, A. Cerami, and R. Bucala. 1993. MIF is a pituitary-derived cytokine that potentiates lethal endotoxemia. Nature 365:756–759.

2. Bozza, M., A. R. Satoskar, G. Lin, B. Lu, A. A. Humbles, C. Gerard, and J. R. David. 1999. Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. J. Exp. Med. 189:341–346.

3. Bucala, R. 1994. Identification of MIF as a new pituitary hormone and macrophage cytokine and its role in endotoxic shock. Immunol. Lett. 43:23–26.

4. Calandra, T., B. Echtenacher, D. L. Roy, J. Pugin, C. N. Metz, L. Hultner, D. Hennmann, D. Mannel, R. Bucala, and M. F. Glauser. 2000. Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nat. Med. 6:164–170.

5. Karpman, D., H. Connell, M. Svensson, F. Scheutz, P. Alm, and C. Svensborg. 1997. The role of lipopolysaccharide and Shiga-like toxin in a mouse model of Escherichia coli O157:H7 infection. J. Infect. Dis. 175:611–620.

6. Lehmann, L. E., U. Novendor, S. Schroeder, T. Pietsch, T. V. Spiegel, C. Putensen, A. Hoefl, and F. Stuber. 2001. Plasma levels of macrophage migration inhibitory factor are elevated in patients with severe sepsis. Intensive Care Med. 27:1412–1415.

7. Mizue, Y., J. Nishihira, T. Miyazaki, S. Fujiwara, M. Chida, K. Nakamura, K. Kikuchi, and M. Mukai. 2000. Quantitation of macrophage migration inhibitory factor (MIF) using the one-step sandwich enzyme immunosorbent assay: elevated serum MIF concentrations in patients with autoimmune diseases and identification of MIF in erythrocytes. Int. J. Mol. Med. 5:397–403.

8. Ohtawara, T., J. Nishihira, H. Takeda, S. Hige, M. Kato, T. Sugiyama, T. Iwanaga, H. Nakamura, Y. Mizue, and M. Asaka. 2002. Amelioration of
dextran sulfate sodium-induced colitis by anti-macrophage migration inhibitory factor antibody in mice. Gastroenterology 123:256–270.

9. Proulx, F., C. Litalien, J. P. Turgeon, M. M. Mariscalco, and E. Seidman. 1998. Inflammatory mediators in hemorrhagic colitis and hemolytic uremic syndrome. Pediatr. Infect. Dis. J. 17:899–904.

10. Proux, F., E. Seidman, M. M. Mariscalco, K. Lee, and S. Carroll. 1999. Increased circulating levels of lipopolysaccharide binding protein in children with *Escherichia coli* O157:H7 hemorrhagic colitis and hemolytic uremic syndrome. Clin. Diagn. Lab. Immunol. 6:773. (Letter.)

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