

We investigated the serum macrophage migration inhibitory factor (MIF) levels of palmoplantar pustulosis patients, before and after the tonsillar provocation test. Higher serum MIF levels of palmoplantar pustulosis patients were decreased after the tonsillar provocation test ($n=29$). To confirm these phenomena, two patients with acute tonsillitis had their changes in body temperature, C-reactive protein (CRP) and serum MIF levels examined during the course of their illness. Surprisingly, increased MIF preceded fever and CRP elevation, and MIF subsequently decreased at the onset of fever and CRP elevation. Since MIF is an initiator of other proinflammatory cytokines, we suggest that the induction of MIF may precede other inflammatory conditions.

Key words: Migration inhibitory factor, Focal infection, Tumor necrosis factor alpha, Tonsillitis

Induction of macrophage migration inhibitory factor precedes the onset of acute tonsillitis

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Macrophage migration inhibitory factor (MIF) is an important cytokine in the innate immune system and plays an important role in the control of such inflammatory responses.¹ Once released, MIF induces the expression of proinflammatory mediators by macrophages and activates T cells, thereby promoting inflammatory and immune responses.² Elevated concentrations of MIF have been detected in sera from patients with a variety of inflammatory disorders such as atopic dermatitis.³

We investigated serum levels of MIF in patients with palmoplantar pustulosis (PPP) and compared them before and after the tonsillar provocation test (TPT) in each patient. PPP is a common chronic skin disease characterized by recurrent sterile pustules and usually also by erythematous, scaly skin on the palms and soles. It has been reported that PPP is

closely related to focal tonsil infections, and that tonsillectomy is often effective in curing this condition.⁴ The TPT is a well-established laboratory procedure to evaluate whether the tonsils participate in the etiology of inflammatory skin diseases such as PPP and psoriasis.⁴ Twenty-nine patients with PPP (10 males and 19 females; aged 19–78 years, mean age 46.3 ± 1.3 years) and 135 healthy volunteers (47 males and 88 females; aged 22–73 years, mean age 47.6 ± 0.82 years) were enrolled in the present study. None of the patients had been treated with systemic immunosuppressants before the TPT. Controls did not have any acute or chronic illness at the time of the study. All participants gave informed consent. The TPT was performed by indirect ultramicrowave irradiation (Micro thermy ME-320; OG Giken, Okayama, Japan) as described previously.⁴ Blood samples were collected before and 1 h after TPT. Serum MIF was measured by enzyme-linked immu-

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nosorbent assay (ELISA) as previously described.³ Briefly, the anti-human MIF antibody dissolved in 50 µl of phosphate-buffered saline was added to each well of a 96-well microtiter plate. After incubation for 1 h at room temperature, the plate was washed and 50 µl of biotin-conjugated anti-human MIF antibody was added. Following incubation, the plate was again washed. Then avidin-conjugated horseradish peroxidase was added to individual wells, which were further incubated for 1 h at room temperature. After washing again, 50 µl of substrate solution containing 200 µg of *o*-phenylenediamine and 10 ml of 30% hydrogen peroxide in 10 ml of citrate-phosphate buffer (pH 5.0) were added. Then the reaction was terminated with 50 µl of 1 N sulfuric acid. The absorbance at 492 nm was measured with an ELISA plate reader (Model 3550; Bio-Rad, Tokyo, Japan). For this assay we used recombinant human MIF to obtain the standard curve, in which good linearity was demonstrated between MIF concentrations (1–200 ng/ml) and absorbency. C-reactive protein (CRP) was measured using a Hitachi modular system 7350 (normal range, 0–0.39 mg/dl).

Table 1 summarizes the data. The mean serum MIF level before the TPT in the PPP patients was 11.32 ± 2.0 ng/ml ($n=29$), which was significantly higher than that of control subjects (4.78 ± 0.32 ng/ml, $n=135$; $p=0.0027$). In contrast, the mean serum MIF level in the PPP patients was significantly decreased after the TPT (8.3 ± 1.1 ng/ml)(before TPT versus after TPT, $p=0.016$). Elevation of CRP was detected after the TPT in each patient (data not shown).

To confirm these phenomena, two patients with acute tonsillitis had their changes in body temperature, CRP and serum MIF levels examined during the course of their illness. Six-year-old and 9-year-old male patients were admitted to Hokkaido University hospital with acute tonsillitis. In both cases, increased serum MIF levels preceded both fever and CRP

Table 1. Serum MIF contents of PPP patients and MIF contents fell after the TPT

Patients	Number of patients	Serum MIF (ng/ml) ^a
Control	135	4.78 ± 0.32
PPP patients before the TPT	29	$11.32 \pm 2.0^*$
PPP patients after the TPT	29	$8.3 \pm 1.1^{**}$

^aThe Mann–Whitney U-test was used for analysis of the variance in serum MIF levels contents between several of the two groups. *Control versus patients before the TPT, $p=0.0027$, **PPP patients before the TPT versus after the TPT, $p=0.016$.

elevation, MIF levels were also reduced at the onset of fever and elevation of CRP (Fig. 1).

In vitro cytokine measurements of tonsil mononuclear cells stimulated with α -streptococcal antigens revealed significant tumor necrosis factor alpha (TNF- α) production in the culture supernatants from PPP patients but not controls.⁴ These findings suggested a close immunologic link between the tonsillitis and proinflammatory cytokines. It has been shown that the TPT induces an increased level of some inflammatory cytokines such as TNF- α , interleukin-1 and interleukin-6 in peripheral blood monocytes of patients with certain focal infectious skin diseases. We have observed two cases with acute tonsillitis, which showed a transient increase in serum MIF preceding an elevation of CRP and fever. In addition, MIF levels were decreased at the onset of fever and the elevation of CRP. Furthermore, significantly higher MIF contents were observed in the sera of patients with PPP than in normal subjects. Unexpectedly, the MIF serum level fell after the TPT. The fact that the serum MIF level significantly decreased at the onset of fever and elevation of CRP after the TPT seems to be a surprising, but interesting, result.

Monocytes and macrophages are the main sources of MIF, which is released after exposure to bacterial endotoxins and exotoxins such as lipopolysaccharide and streptococcal pyrogenic exotoxin A.² Thus, these effects may be mediated by a number of proinflam-

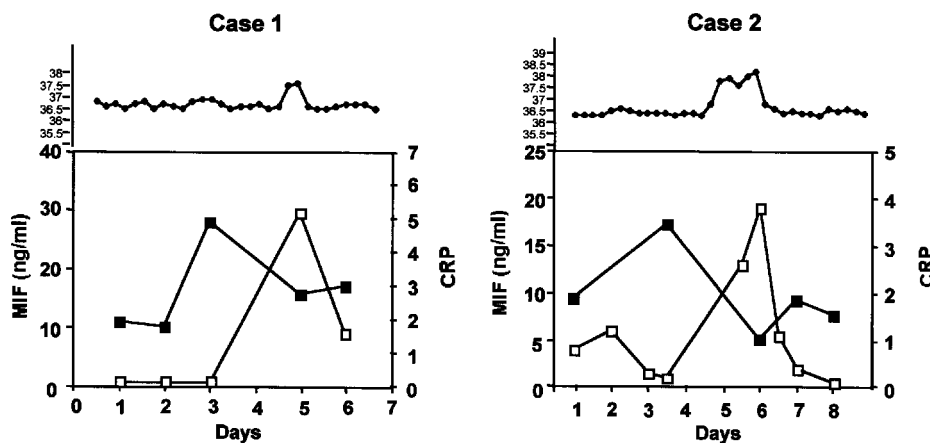


FIG. 1. Changes in body temperature, serum MIF, and CRP in patients with acute tonsillitis. The patients with acute tonsillitis (case 1, a 6-year-old male; and case 2, a 9-year-old male) were admitted to Hokkaido University hospital. Body temperature, CRP (□), and serum MIF (■) were examined during the course of admission.

matory cytokines, including TNF- α and IL-1.² Since MIF is an initiator of other proinflammatory cytokines such as TNF- α and IL-1, and regulates the induction of those cytokines,^{1,5} our findings suggest that the induction of MIF may precede that of other inflammatory conditions.

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