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Prominent Increase of Macrophage Migration Inhibitory Factor in the Sera of Patients with Uveitis

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Purpose. To investigate pathogenesis underlying endogenous uveitis, macrophage migration inhibitory factor (MIF) was quantified in sera of patients.

Methods. Sera were obtained from the 55 patients with uveitis (24 with Behçet's disease; 9 with Vogt-Koyanagi-Harada's [VKH] disease; 22 with sarcoidosis) and 58 healthy control subjects. MIF levels were determined by a human MIF enzyme-linked immunosorbent assay.

Results. The mean MIF levels in the sera of the patients with Behçet's disease, VKH disease, and sarcoidosis and of healthy control subjects were 60.4 ± 9.0 (mean ± SE) ng/ml, 16.5 ± 2.9 ng/ml, 27.1 ± 5.6 ng/ml, and 5.4 ± 0.04 ng/ml, respectively. The average levels of MIF in the sera of uveitis patients were significantly higher ($P < 0.0001$) than those of healthy control subjects. The high levels of MIF were especially noted in patients with Behçet's disease at the ocular exacerbation stage and patients with sarcoidosis at the severe uveitis stage.

Conclusions. Significant increase of MIF in sera was characteristic of uveitis, and MIF may be a useful laboratory parameter to use to comprehend the clinical course of uveitis. (*Invest Ophthalmol Vis Sci*. 1999;40:247-250)

Macrophage migration inhibitory factor (MIF) was reported as the first lymphokine produced by activated T cells in guinea pigs.[1] The MIF was determined by an inhibitory capacity of macrophage migration. Although it was suggested that MIF production was associated with the generation of cell-mediated immune responses, the biological role of MIF in vivo has been unclear for a long time. Recent studies have established that MIF is a critical cytokine in the delayed type hypersensitivity response, an important mediator of endotoxic shock, and a counter regulator of glucocorticoid action. Bacher et al.[2] showed that anti-MIF antibodies inhibited T-cell proliferation and interleukin-2 (IL-2) production in vitro and suggested that MIF played an important regulatory role in the activation of T cells induced by mitogenic or antigenic stimuli. Moreover, it was reported that anti-MIF antibodies inhibited immunologically induced crescentic anti-glomerular basement membrane glomerulonephritis in rats and collagen type II-induced arthritis in mice.[3,4] In human diseases, it was demonstrated that the serum MIF levels of the patients with atopic dermatitis were significantly higher than those of the healthy control subjects.[5]

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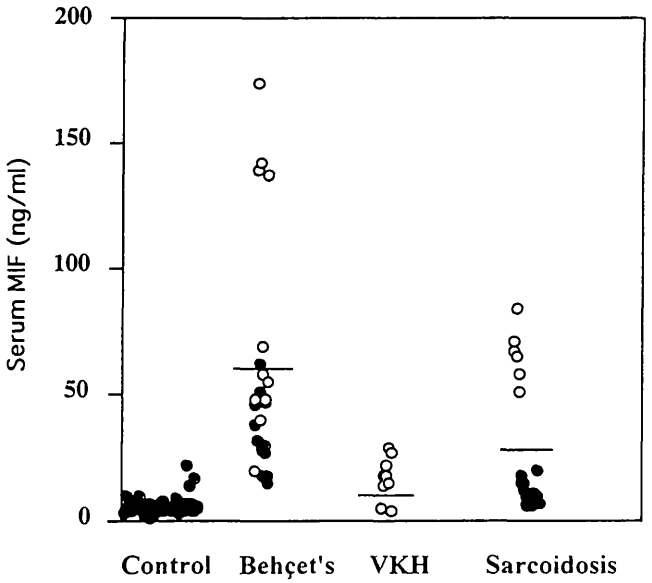

FIGURE 1. Serum macrophage migration inhibitory factor (MIF) levels in the patients with Behçet's disease, Vogt-Koyanagi-Harada's disease (VKH), sarcoidosis, and healthy control. *Open circles* represent the patients with active uveitis: at the exacerbation stage in Behçet's diseases and at the severe uveitis stage in VKH and sarcoidosis. *Closed circles* indicate the patients without active uveitis: at the convalescent stage in Behçet's disease and at the mild uveitis stage in sarcoidosis. The *horizontal lines* represent the mean concentration of MIF in each group.

The etiologic characteristics of uveitis vary among areas and races around the world because genetic and environmental factors are strongly linked to the occurrence of inflammation. In Japan, Behçet's disease, sarcoidosis, and Vogt-Koyanagi-Harada's (VKH) disease have the highest frequency. These are systemic inflammatory diseases in which immunologic disorders seem to be involved. To elucidate the pathologic role of MIF in uveitis, especially those associated with systemic disorders, we examined MIF levels in the sera of the patients with uveitis.

MATERIALS AND METHODS

Patients

Blood samples were obtained from 24 patients with Behçet's disease, 9 patients with VKH disease, and 22 patients with sarcoidosis. These diseases were diagnosed in the Uveitis Survey Clinic of Hokkaido University Hospital. The following diagnostic criteria were used: Behçet's disease, criteria as established by the Behçet's Disease Research Committee of Japan; sarcoidosis, criteria established by the Diffuse Pulmonary Disease Research Committee of Japan; and VKH disease, Sugiura's criteria.[6] Fifty-eight healthy subjects of similar age distribution served as controls. Blood samples were collected at the first medical examination in our clinic. To examine the relationship between MIF levels and disease activity, blood was drawn again from 4 patients with Behçet's disease at different stages. Corticosteroid was not administered to any of the patients before their blood samples were collected. Most patients with Behçet's disease were under colchicine or cyclosporin A therapy or both. All VKH disease patients were in the active phase when the blood samples were collected. These patients gave their informed consent for collection of blood samples. This study was performed according to the tenets of the Declaration of Helsinki.

Cytokine Determination

Venous blood samples were collected in sterile vacuum tubes and centrifuged for 30 minutes. The separated serum samples were separated into aliquots and stored at −70°C. MIF concentrations of these serum samples were quantified by enzyme-linked immunosorbent assay. In brief, the anti-human MIF antibody was added to each well of a 96-well microtiter plate and left for 1 hour at room temperature. All wells were filled with phosphate-buffered saline containing bovine serum albumin (1%) for blocking and left for 1 hour at room temperature. Samples were added in duplicate to individual wells and incubated for 1 hour at room temperature. After the plate was washed three times, 50 μl biotin-conjugated anti-human MIF antibody (IgG fraction) was added to each well. The polyclonal anti-human MIF antibody was generated by immunizing New Zealand white rabbits with purified recombinant human MIF. The IgG fractions (4 mg/ml) were prepared using Protein A Sepharose (Pharmacia, Uppsala, Sweden) according to the manufacturer's protocol. After incubation for 1 hour, streptavidin-horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Bio-Rad, Hercules, CA) was added to each well and incubated for 1 hour. Fifty microliters of substrate containing *o*-phenylenediamine (Wako, Osaka, Japan) and hydrogen peroxide (Wako) in citrate-phosphate buffer (pH 5.0) was added to each well. After incubation for 20 minutes, the reaction was stopped with sulfuric acid. The absorbance at 492 nm was measured using an enzyme-linked immunosorbent assay plate reader (model 3550; Bio-Rad). Data are expressed as the mean ± SE and median values. Statistical analysis was performed using Mann-Whitney *U* test and paired *t*-test. Calcula-

TABLE 1. Serum MIF Concentration of Patients with Sarcoidosis and Behçet's Disease

Disease	Stage	No. of Samples	Serum MIF (ng/ml) Mean ± SE	Median (ng/ml)
Sarcoidosis	Severe	7	63.3 ± 11.9*	57.2
	Mild	15	10.3 ± 4.4	8.3
Behçet's disease	Exacerbation	12	80.9 ± 15.4†	57.1
	Convalescent	12	39.9 ± 4.1	30.1
Control		58	5.37 ± 0.045	4.5

* $P < 0.0001$, compared with mild sarcoidosis; † $P < 0.005$, compared with convalescent Behçet's disease (Mann-Whitney *U* test).

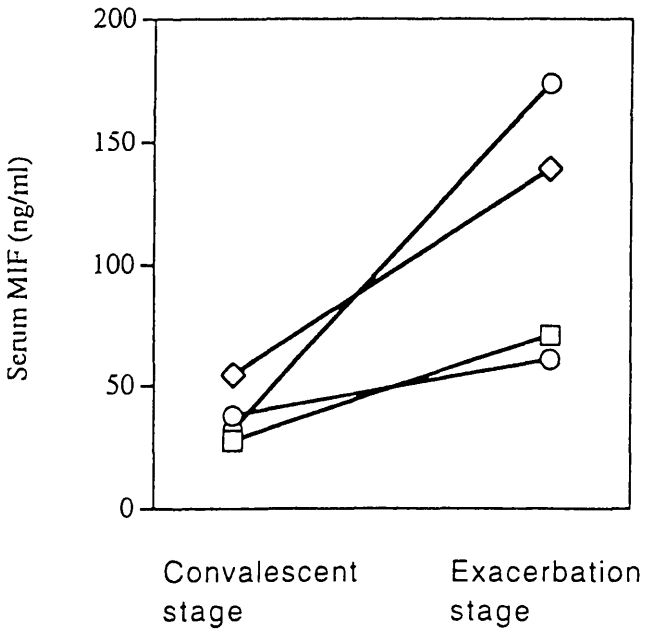

FIGURE 2. The serial observation of serum macrophage migration inhibitory factor (MIF) levels of 4 patients with Behçet's disease at the exacerbation stage and the convalescent stage of ocular disease. The serum MIF levels of these patients in the exacerbation stage are significantly higher than those in the convalescent stage.

tions were performed using the statistical software package Statview (Abacus Concepts, Berkeley, CA).

RESULTS

Serum MIF Levels in Patients with Uveitis

The average level of serum MIF from healthy control subjects was 5.4 ± 0.04 ng/ml. The mean serum MIF levels were 60.4 ± 9.0 ng/ml in Behçet's disease, 16.5 ± 2.9 ng/ml in VKH disease, and 27.1 ± 5.6 ng/ml in sarcoidosis (Fig. 1). The average value of each uveitis group was individually compared with that of the control group. The mean MIF levels in the sera of patients with these diseases examined were significantly higher than those of the healthy control subjects ($P < 0.0001$). When the mean MIF levels were compared among uveitis patients, the mean MIF level of Behçet's disease was significantly higher than those of the patients with VKH disease ($P < 0.0005$) and sarcoidosis ($P < 0.005$).

Correlation between Serum MIF Level and Stage of Ocular Disease in Sarcoidosis and Behçet's Disease

As shown in Figure 1, the patients with sarcoidosis were divided into two groups according to the MIF levels: 7 patients with quite high MIF levels (>50 ng/ml) and 15 patients with low MIF levels (<25 ng/ml). We found that all patients in the group with high MIF levels showed severe uveitis, and 15 patients in the group with low MIF levels were in chronic stage with mild uveitis. The mean MIF level in the former group (63.3 ± 11.9 ng/ml) was significantly higher than that of the latter group (10.3 ± 4.4. ng/ml; $P < 0.0001$; Table 1).

Patients with Behçet's disease have recurrent attacks that exacerbate the ocular diseases. We also analyzed the correlation between the serum MIF levels and the stage of ocular disease in patients with Behçet's disease. In Figure 1, open circles represent the patients in the exacerbation stage and closed circles represent those in the convalescent stage. The mean serum MIF level (80.9 ± 15.4 ng/ml) in the patients in the exacerbation stage was significantly higher than that in patients in the convalescent stage (39.9 ± 4.1 ng/ml; $P < 0.005$; Table 1).

Figure 2 shows the data of serial observations on four individual patients at various stages of Behçet's disease. The serum MIF levels of these patients in the exacerbation stage are significantly higher than those of patients in the convalescent stage ($P < 0.05$ by paired t-test).

DISCUSSION

In the present study, we detected high levels of MIF in the sera of patients with uveitis. We examined Behçet's disease, sarcoidosis, and VKH disease because these diseases are representative of endogenous uveitis in Japan. These three kinds of diseases are systemic inflammatory diseases with uveitis and comprise almost 50% of uveitis patients in etiologic classification.[6] It has been reported that the endothelial cell is one of the sources of MIF production.[7] Retinal vasculitis is very severe in some uveitis patients, especially in those with Behçet's disease or sarcoidosis. In addition, the patients with Behçet's disease and sarcoidosis have systemic vasculitis, whereas systemic vasculitis is not seen in the patients with VKH disease. Average levels of serum MIF in Behçet's disease and sarcoidosis were higher than that of VKH disease. Thus, it seems that the source of serum MIF in uveitis patients, especially those with Behçet's disease or sarcoidosis, is endothelial cells of vessels. Severe and widespread vasculitis may induce structural destruction of the endothelial cells in patients with Behçet's disease or sarcoidosis. High levels of MIF were detected in the sera of patients with Behçet's disease and in sarcoidosis patients who had severe systemic inflammation. However, we found that patients with Behçet's disease in the ocular exacerbation stage showed higher levels of MIF than those in the convalescent stage. Moreover, sarcoidosis patients showed significantly higher levels of MIF at the severe uveitis stage than those at the mild uveitis stage. In these diseases, ocular and systemic inflammation do not always occur at the same time. The serum MIF level was associated with the severity and activity of ocular inflammation in the patients with Behçet's disease or sarcoidosis. Thus, MIF production in the eye appears to be related to the high serum MIF levels. To elucidate the role of MIF in the pathogenesis of uveitis, it is necessary to determine the major cell component, especially in the eye (i.e., endothelial cells and infiltrating macrophages and T cells in the region), that produces MIF.

When the MIF levels of uveitis patients were compared, the MIF level of patients with Behçet's disease was higher than those with sarcoidosis or VKH disease. Even in the convalescent stage, MIF levels were very high in Behçet's disease. This finding may indicate that the MIF plays a more important role in Behçet's disease than in other diseases. It has been established that cyclosporin A is effective for suppressing the attacks of the uveitis in the patients with Behçet's disease. Thus,

it is likely that T lymphocytes are involved in the induction of Behçet's disease. The activated T cells may release MIF in the early stage of Behçet's disease. It is thought that MIF is an inflammatory cytokine that induces the release of other cytokines. Indeed, tumor necrosis factor α, interferon-γ, IL-10, IL-12, and tumor necrosis factor receptor, which are involved in various inflammatory processes, were detected at high levels in Behçet's disease.[8-10] It seems to us that the elevation of the MIF level in the sera is involved in the induction of various inflammatory symptoms in Behçet's disease including uveitis.

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Corneal Transplantation in Antibody-Deficient Hosts

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Purpose. To examine the role of donor-specific antibodies, with or without complement, in rejection of orthotopic corneal transplants by using mice as recipients in which the genes for the heavy chain of immunoglobulin or the third complement component have been eliminated by homologous recombination.

Methods. BALB/c corneas were transplanted into eyes of B-cell-deficient (n = 17) or wild-type control C57BL/6 (n = 30) mice and into eyes of complement (C3)-deficient (n = 15) or wild-type control 129-C57BL/6 (n = 13) mice. After surgery all grafts were evaluated over 8 weeks in a masked manner by biomicroscopy for signs of rejection.

Results. The rates of corneal transplant rejection were similar among B-cell-deficient and C3-deficient mice compared with rejection rates in their respective wild-type control subjects. This similarity applied to the time course of rejection and to cumulative survival rates.

Conclusions. Neither donor-specific antibody nor the third component of complement play essential roles in acute rejection of orthotopic corneal allografts in mice. (*Invest Ophthalmol Vis Sci.* 1999;40:250-253)

Evidence that antibody and complement can contribute to allograft rejection has emerged from several recent studies of solid organ transplantation in laboratory animals. In rats immunosuppressed with cyclosporin-A or anti-CD4 monoclonal antibodies, the passive transfer of immune serum causes acute rejection of renal and cardiac allografts.[1] Similarly, repeated transfer of immune serum to severe combined immunodeficiency mice provoked chronic vascular lesions in cardiac allografts that were characterized by antibody and complement deposition and macrophage infiltration.[2] Finally, rats deficient in complement component C6 rejected cardiac allografts 2 weeks later than did wild-type rats.[3]

Despite this evidence in solid organ transplantation, the role for antibody and complement in the rejection of corneal allografts remains unclear. Cornea-specific and donor-specific antibodies have been detected in host serum after clinical[4] and experimental[5,6] corneal grafting, but it is not certain whether these antibodies contribute to graft rejection. Hutchinson et al.[5] have reported that although donor-specific serum alloantibodies are evoked by orthotopic corneal transplants, the antibodies are not detected until after the grafts have been rejected, and recently published data in Hedge et al.[7] indicate that passive transfer of donor-specific antibodies fails to cause

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