Incidence of Cytomegalovirus Reactivation in Patients with Inflammatory Connective Tissue Diseases Who Are Under Immunosuppressive Therapy

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ABSTRACT. ***Objective***. To evaluate the incidence and effect of cytomegalovirus (CMV) reactivation in patients with inflammatory connective tissue diseases (CTD) undergoing immunosuppressive therapy.

Methods. A total of 18 consecutive CMV seropositive patients undergoing immunosuppressive therapy for inflammatory CTD were enrolled. CMV reactivation was determined by detection of CMV-DNA in peripheral blood leukocytes (PBL) or plasma using quantitative real-time polymerase chain reaction.

Results. CMV reactivation was detected in PBL in 7 of 17 evaluable patients (41%), and in plasma in 5 of 17 patients (29%). Patients with detectable CMV-DNA in plasma were exclusively positive for CMV-DNA in PBL.

Conclusion. Patients with inflammatory CTD under immunosuppressive therapy are at high risk for CMV reactivation. The clinical significance of such an event and indications for antiviral therapy should be examined further. (J Rheumatol 2004;31:1349–51)

Key Indexing Terms:
CYTOMEGALOVIRUS REACTIVATION
INFLAMMATORY CONNECTIVE TISSUE DISEASE

Cytomegalovirus (CMV) is one of the opportunistic organisms causing serious infections in immunocompromised patients, including transplant recipients and patients with acquired immunodeficiency syndrome (AIDS). Patients with autoimmune diseases, especially inflammatory connective tissue diseases (CTD) or collagen diseases, could also be at increased risk for opportunistic infection by virtue of impaired immunity due to both the disease itself and the immunosuppressive therapy, including high dose corticosteroids and other immunosuppressive agents[1]. However, there have been a few case reports describing the development of CMV diseases in patients with inflammatory CTD such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA)[2-6]. Therefore, the incidence of CMV diseases in patients with inflammatory CTD has been suggested to be low. Two studies evaluated the association of CMV with inflammatory CTD[7,8]. Bendiksen, *et al* failed to detect active CMV reactivation in patients with SLE, whereas Rider, *et al* showed a high CMV seropositivity in patients with SLE. Thus, the results have been inconclusive, probably because these studies evaluated CMV status by serological examinations or detection of viruria. In this study, we prospectively evaluated the incidence of CMV reactivation in patients with inflammatory CTD who were receiving immunosuppressive therapy, using a recently developed sensitive, quantitative, real-time polymerase chain reaction (PCR).

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Submitted June 4, 2003; revision accepted January 28, 2004.

MATERIALS AND METHODS

Patients. CMV seropositive patients receiving immunosuppressive therapy for more than 4 weeks for refractory inflammatory CTD including SLE, polymyositis (PM), dermatomyositis (DM), systemic sclerosis, RA, rheumatoid vasculitis, cutaneous polyarteritis nodosa, microscopic polyangiitis (MPA), and their overlapping syndromes were evaluated[9-14]. Immunosuppressive therapy consisted of prednisolone (≥ 10 mg/day) alone or in combination with intravenously or orally administered cyclophosphamide, cyclosporin A, or azathioprine.

Definition of CMV reactivation and CMV disease. CMV reactivation was defined as detection of CMV-DNA in peripheral blood leukocytes (PBL) and/or in plasma with no clinical manifestations associated with CMV. CMV diseases were defined as clinical manifestations with a histological confirmation of CMV in affected tissues or organs.

Real-time PCR. PCR to detect CMV-DNA was performed as described[15].

DNA was extracted from PBL or plasma using QIAamp Blood Mini Kit (Qiagen GmbH, Hilden, Germany). The primers and probe were selected from the immediate early (IE) gene, as described[15]. The probe was dual-labeled with reporter dye and quenching dye. The PCR was performed using a TaqMan PCR kit (Applied Biosystems, Foster City, CA, USA). Nuclease degradation of the fluorogenic probe was detected as an increase in fluorescence intensity by the Model 7700 Sequence Detector (Applied Biosystems). As the standard, a plasmid containing the IE gene was constructed from pGEM-T vector (Promega, Madison, WI, USA). Quantification of CMV was carried out using the serially diluted standard, and the gene copy numbers were calculated by Sequence Detection System software v.1.6 (Applied Biosystems). The minimum detection levels were 20 copies per 1×10^6 cells for the PBL, and 100 copies per 1 ml for the plasma. Using this PCR technique with PBL and plasma, we detected no CMV-DNA in 16 CMV seropositive healthy volunteers.

RESULTS

Patient characteristics. Eighteen CMV seropositive patients were evaluable. Patient characteristics are shown in Table 1. Twelve of the patients were diagnosed as having SLE according to the revised classification criteria for SLE or its overlapping diseases[9]. Under the therapies described, the median numbers of lymphocytes and the median concentrations of serum immunoglobulin G (IgG) in these patients were 734/μl (range 70–2130) and 978 mg/dl (range 373–2260), respectively.

Detection of CMV reactivation by real-time PCR. Except in 2 cases (Cases 2 and 3), PCR to detect CMV-DNA was performed on both PBL and plasma. Cases 2 and 3 were examined by PBL or by plasma. CMV-DNA was detected in PBL in 7 (41%) and in plasma in 5 (29%) of the 17 evaluable patients by real-time PCR (Table 1). All patients with detectable CMV-DNA in plasma had detectable CMV-DNA in PBL. The copy numbers of CMV-DNA in patients with detectable CMV-DNA ranged from 20 to 2.7×10^4 per 1×10^6 of PBL and from 1×10^2 to 3.2×10^3 per 1 ml of plasma (Table 1). In contrast, CMV-DNA was not detected in PBL in any of 12 patients with inflammatory CTD (4 with SLE, 7 with PM and/or DM, one with MPA) before immunosuppressive therapy was started.

Occurrence of CMV disease. During the study period, one patient (Case 1) with the highest copy numbers of CMV-DNA in both PBL and plasma developed disseminated CMV disease, including CMV pneumonitis and subsequent thrombotic thrombocytopenic purpura, which, despite ganciclovir administration, was fatal.

DISCUSSION

Despite the introduction of effective preemptive or prophylactic therapy against CMV infection or disease, CMV remains one of the common causes of opportunistic infections in immunocompromised patients such as transplant recipients or those with AIDS[16-18]. Although it has been shown that infectious disease is one of the major causes of life-threatening complications in patients with inflammatory CTD, CMV disease in these patients has been considered rare[1]. There have been only a few case reports describing the

Table 1. Patient characteristics and results of real-time PCR to detect CMV reactivation. Patients were treated with immunosuppressive therapy for more than 4 weeks. Real-time PCR using PBL and plasma could not detect CMV-DNA in healthy CMV-seropositive volunteers.

Patient	Sex	Age, yrs	Disease	Therapy	CMV Copy No. (/1×10^6 PBL)	CMV Copy No. (/ml plasma)
1	F	38	SLE	PSL + IV-CYC	2.7×10^4	3.2×10^3
2	M	55	DM	PSL + IV-CYC + CSA	3.4×10^3	—
3	F	71	MPA	PSL + IV-CYC	—	9.8×10^2
4	F	25	SLE	PSL + CSA	1.5×10^2	1.3×10^2
5	F	33	SLE	PSL + CSA + IV-CYC	20	$< 1.0 \times 10^2$
6	F	19	SLE	PSL	2.8×10^2	1.0×10^2
7	F	29	SLE + DM	PSL + IV-CYC + CSA	< 20	$< 1.0 \times 10^2$
8	F	46	SLE	PSL	< 20	$< 1.0 \times 10^2$
9	F	62	RV	PSL + AZT	25	$< 1.0 \times 10^2$
10	F	52	CPN	PSL + CYC + CSA	< 20	$< 1.0 \times 10^2$
11	F	69	RA + MPA	PSL + IV-CYC	29	1.0×10^2
12	F	38	SLE + SSc + PM	PSL	< 20	$< 1.0 \times 10^2$
13	F	19	SLE + SSc + PM + RA	PSL + IV-CYC	< 20	$< 1.0 \times 10^2$
14	F	30	SLE + PM	PSL + CSA	< 20	$< 1.0 \times 10^2$
15	F	36	SLE	PSL + CSA	< 20	$< 1.0 \times 10^2$
16	F	24	SLE	PSL	< 20	$< 1.0 \times 10^2$
17	F	33	SLE	PSL	< 20	$< 1.0 \times 10^2$
18	F	74	RV	PSL + AZT	< 20	$< 1.0 \times 10^2$

CMV: cytomegalovirus; PBL: peripheral blood leukocytes; SLE: systemic lupus erythematosus; DM: dermatomyositis; MPA: microscopic polyangiitis; RV: rheumatoid vasculitis; CPN: cutaneous polyarteritis nodosa; SSc: systemic sclerosis; RA: rheumatoid arthritis; PM: polymyositis; PSL: prednisolone; IV-CYC: intravenous cyclophosphamide; CSA: cyclosporin A; AZT: azathioprine.

development of CMV diseases in patients with inflammatory CTD[2-6]. Using a PCR technique, we observed a high incidence of CMV reactivation in patients with inflammatory CTD. Although the patient group was heterogenous, our results strongly suggest that patients with inflammatory CTD who are undergoing immunosuppressive therapy are at risk for CMV reactivation. The previously reported patients with CMV diseases associated with inflammatory CTD were undergoing intensive immunosuppressive therapy, except for a single case of disseminated CMV disease not associated with immunosuppressive therapy[19]. Thus we investigated the incidence of CMV reactivation in 12 patients with inflammatory CTD before initiating immunosuppressive therapy in order to clarify the effects of immune deficits associated with these diseases and immunosuppressive therapy on the susceptibility to CMV reactivation. CMV reactivation was detected in none of these patients. These findings strongly suggested that immunosuppressive therapy plays a crucial role in susceptibility to CMV reactivation in patients with inflammatory CTD.

In addition, CMV has been reported to affect the course of inflammatory CTD, namely via exacerbation or flare of the CTD[20,21]. Although some possibilities have been suggested, including cross-reactivity between CMV antigens and autologous antigens, the mechanism underlying the relationship between CMV and the activity of inflammatory CTD has not yet been fully elucidated. However, it is suggested that therapy against CMV reactivation in patients with inflammatory CTD may modify the activity of the CTD itself.

We conclude that patients with inflammatory connective tissue diseases who are undergoing immunosuppressive therapy are highly susceptible to cytomegalovirus reactivation. Further studies, including longitudinal analysis of viral load and longer followup to fully elucidate the incidence of clinically significant CMV diseases, should be conducted.

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