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ORIGINAL ARTICLE

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Clinical evaluation of patients with inflammatory connective tissue diseases complicated by cytomegalovirus antigenemia

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Abstract We evaluated the occurrence of cytomegalovirus (CMV) infection and the background characteristics in twenty-three hospitalized patients with inflammatory connective tissue diseases including systemic lupus erythematosus, polymyositis/dermatomyositis, rheumatoid vasculitis, microscopic polyangitis, and Takayasu's arteritis. Cytomegalovirus antigenemia was demonstrated in 10 of 23 evaluable patients. Five of ten patients with CMV antigenemia developed symptomatic CMV disease (all cases of fever, two cases of liver involvement, two cases of interstitial pneumonia, and one case of unknown organ involvement), whereas the remaining five patients were asymptomatic. Most of CMV antigenemia-positive patients had been administered intravenous steroid pulse, or in combination with immunosuppressive agents intravenously or orally because of refractory disease activity. Particularly, in patients who received intravenous methylprednisolone pulse in combination with additional intravenous cyclophosphamide pulse, the incidence of CMV antigenemia was markedly higher (four out of four). Four of ten CMV antigenemia-positive patients simultaneously showed detection of *Pneumocystis jiroveci* in induced sputum by PCR, increase in level of serum β-D-glucan and the finding of geographical ground-glass opacities on chest computed tomography. These findings suggested that patients with connective tissue diseases under intensive immunosuppressive therapies (intravenous steroid pulse in combination with additional intravenous cyclophosphamide pulse in particular) are highly susceptible to CMV infection and disease, and that patients complicated by CMV antigenemia are susceptible to combined opportunistic infection such as *Pneumocystis* pneumonia.

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Introduction

Survival in inflammatory connective tissue diseases (CTDs) represented by systemic lupus erythematosus (SLE) has predominantly improved by immunosuppressive therapy, including high-dose corticosteroids and the other immunosuppressive agents.[1] On the other hand, since about 1990 an increasing number of the case-reports have described the occurrence of serious opportunistic infection or disease in patients with CTDs.[2–11] These occurrences are considered by virtue of impaired immunity due to both the disease itself and the intensive immunosuppressive therapy.

Cytomegalovirus (CMV) infection is one of the most prevalent opportunistic infection in immunocompromised patients such as transplant recipients. Clinically, CMV infection is defined as detectable virus, as assessed by CMV antigenemia assay or quantitative polymerase chain reaction (PCR), and can be present in patients with symptoms or without symptoms.[12,13] Cytomegalovirus antigenemia is a useful marker for the early and rapid diagnosis of CMV infection and of the disease activity in immunodeficient patients.[14,15] The test is based on immunocytochemical detection of CMV immediate early antigens in blood leukocytes.[16] It is considered that the presence of CMV antigen-positive cells in circulating blood may reflect dissemination of reactivated CMV throughout the body, because of a highly correlation between CMV antigenemia and the symptoms of CMV disease.[16] We retrospectively evaluated the occurrence of CMV infection and the background characteristics of patients with CTDs under intensive immunosuppressive therapies.

Patients and methods

A total of 23 patients admitted to our department from October 2002 to May 2004, who had been receiving intensive immunosuppressive therapy for more than 4 weeks for refractory inflammatory CTD comprising 9 with SLE, 4 with polymyositis/dermatomyositis (PM/DM), 2 with systemic sclerosis, 2 with overlap syndrome, 2 with rheumatoid vasculitis, 2 with Takayasu's arteritis, 1 with microscopic polyangitis, and 1 with adult-onset Still's disease were evaluated. Immunosuppressive therapy consisted of oral prednisolone (PSL) (30–60 mg/day) alone, or intravenous methylprednisolone (mPSL) pulse (500 mg/day, for 3 days), or in combination with oral cyclosporine A (CyS) (150–300 mg/day), or additional intravenous cyclophosphamide (CYP) pulse (500 mg/day).

Cytomegalovirus antigenemia was examined by immunostaining assay using a monoclonal antibody (C10C11) against a CMV structural protein of the 65 kDa lower-matrix phosphoprotein (pp65).[14] The number of CMV antigen-positive polymorphonuclear neutrophils per 150 000 neutrophils in the peripheral blood was counted.

The presence of more than one CMV antigen-positive cell in circulating blood in two consecutive tests within a week was considered as CMV antigenemia, which is a marker for diagnosis of CMV infection. We diagnosed the patients with CMV antigenemia who developed clinical manifestations (fever, liver involvement, or interstitial pneumonia) as having symptomatic CMV disease.

Data are expressed as mean ± standard deviation. Intergroup comparisons were performed using Student's *t*-test. Statistical analysis was performed using the standard software package Statview version 4.5. $P < 0.05$ was considered to be significant.

Results

The CMV antigenemia test was performed for the diagnosis of CMV infection in blood samples from 23 patients with disease severity of CTDs, who had been receiving high-dose oral PSL alone, intravenous mPSL pulse, or in combination with oral CyS, or additional intravenous CYP pulse therapy. Table 1 summarizes the clinical characteristics of the background of patients at the time of performed antigenemia test. One CMV antigen-positive cell detected in three patients (cases 4, 10, and 18) was not considered as CMV antigenemia, because there was no detection of positive cell in two consecutive tests within a week. Consequently, 10 patients with CMV antigenemia and 13 patients without antigenemia were evaluated.

With regard to the background characteristics, patients who were treated with intravenous CYP pulse demonstrated a high incidence of CMV antigenemia. Particularly, in patients received intravenous mPSL pulse in combination with additional intravenous CYP pulse, the incidence of CMV antigenemia was markedly higher (four out of four). The median dose of oral PSL in patients with CTDs was 43mg daily (range 30–60) at the time of diagnosis of CMV antigenemia, and most of these patients were under tapering of PSL (Table 1). In addition, in patients with CTDs who were receiving the maintenance dose (5–28 mg daily) of oral PSL alone, CMV antigenemia was not seen for the studied period.

Under the therapies described, the peripheral blood lymphocyte counts in CMV antigenemia-positive patients and antigenemia-negative patients were 812 ± 475/μl and 2066 ± 1338/μl, respectively ($P = 0.01$). The serum IgG levels in CMV antigenemia-positive patients and antigenemia-negative patients were 1433 ± 455 and 1474 ± 513 mg/dl, respectively ($P = 0.85$) (Table 1).

Five of ten patients with CMV antigenemia demonstrated symptomatic CMV disease (all cases of fever, two cases of liver involvement, or two cases of interstitial pneumonia and one case of unknown organ involvement), whereas the remaining five patients were asymptomatic. CMV disease developed after 3–5 weeks of the initiation of intravenous mPSL pulse (case 1) or intravenous CYP pulse (cases 9, 12, 13, and 14). The number of CMV antigen-positive cells in patients with symptomatic CMV infection was higher than that in patients with asymptomatic antigenemia (Table 1).

Four patients with CMV antigenemia simultaneously demonstrated to be positive for *Pneumocystis jiroveci* in induced sputum examined by PCR analysis in addition to elevated level of the serum β-D-glucan, who presented with clinical sign of suspicious *Pneumocystis* pneumonia (the finding of geographical ground-glass opacities on chest computed tomography). Lymphocytopenia with a count lower than 1000/μl was observed in three of these CMV antigenemia-positive and *Pneumocystis jiroveci*-positive patients. Two patients with PM/DM (cases 8 and 11) died of complication within 60 days after commencement of trimethoprim and sufamethoxazole treatment (Table 2).

In seven of ten patients with CMV antigenemia a 14-day course of treatment with ganciclovir resulted in a decrease to zero in antigen-positive cells and an improvement of the clinical manifestations. Three patients died despite the decrease in CMV antigen-positive cell counts due to ganciclovir treatment. One patient with SLE and thrombotic thrombocytopenic purpura (case 1) died of sepsis, one patient with PM/DM (case 11) died of the exacerbation of interstitial pneumonia, and one patient with PM/DM (case 8) died of bacterial pneumonia (Table 2 and Fig. 1).

Discussion

Many inflammatory CTDs such as SLE are complicated by various opportunistic infections or diseases due to the primary disease itself or a compromised immune function caused by the treatment drugs. Many recent reports have indicated that opportunistic infections complicating inflammatory CTDs are generally due to a compromised immune function caused by the administration of high-dose steroids

Table 1. Background characteristics of 23 patients with inflammatory connective tissue diseases under intensive immunosuppressive therapies

Patient (case no.)	Age (years)/ sex	Disease	Immunosuppressive therapy	Lymphocyte count (/μl)	Serum IgG level (mg/dl)	CMV antigen-positive cell count (/1.5 × 10 cells)		Antigenemia	Clinical manifestation of CMV infection
1	69/F	SLE, TTP	mPSL pulse[a], PSL 38 mg/day	480	1570	72[c]	746[d]	Pos	Fever, liver involvement (3w)[e]
2	24/F	SLE	mPSL pulse, PSL 52 mg/day	1180	2310	3	1	pos	Asymptomatic
3	65/F	RV	mPSL pulse, PSL 45 mg/day	n.a.	n.a.	0	0	neg	
4	27/F	Overlap syndrome	mPSL pulse, PSL 45 mg/day	2380	2150	1	0	neg	
5	26/M	SLE	mPSL pulse, PSL 60 mg/day	2477	1382	0	0	neg	
6	22/F	Takayasu's arteritis	mPSL pulse, PSL 60 mg/day	3672	1105	0	0	neg	
7	81/F	MPA	mPSL pulse, PSL 48 mg/day	375	1107	0	0	neg	
8	87/M	PM/DM	mPSL pulse, PSL 60 mg/day	120	1940	3	1	pos	Asymptomatic
9	66/F	SSc	CYP pulse[b], PSL 30 mg/day	1860	1190	6	6	pos	Fever, unknown organ involvement (5w)[g]
10	59/M	PM/DM	CyS 300 mg/day, PSL 30 mg/day	2940	850	1	0	neg	
11	59/F	PM/DM	CyS 150 mg/day, PSL 38 mg/day	830	1250	3	1	pos	Asymptomatic
12	56/F	SLE	mPSL pulse, CYP pulse, PSL 40 mg/day	590	1270	4	1	pos	Fever, liver involvement (5w)[g]
13	52/F	ASD	mPSL pulse, CYP pulse, PSL 45 mg/day	740	n.a.	30	6	pos	Fever, interstitial pneumonia (3w)[f]
14	69/M	RV	mPSL pulse, CYP pulse, PSL 40 mg/day	820	920	14	4	pos	Fever, interstitial pneumonia (3w)[f]
15	29/F	SLE	mPSL pulse, CYP pulse, PSL 36 mg/day	1020	1490	2	2	pos	Asymptomatic
16	29/F	SLE	mPSL pulse, CyS 250 mg/day, PSL 40 mg/day	1702	2247	0	0	neg	
17	25/F	SLE	mPSL pulse, CyS 250 mg/day, PSL 30 mg/day	222	1419	0	0	neg	
18	74/M	SSc	PSL 55 mg/day	480	960	2	7	pos	Asymptomatic
19	58/F	Overlap syndrome	PSL 44 mg/day	3100	1330	1	0	neg	
20	47/F	SLE	PSL 60 mg/day	1305	1638	0	0	neg	
21	33/F	PM/DM	PSL 40 mg/day	507	1466	0	0	neg	
22	19/F	Takayasu's arteritis	PSL 40 mg/day	4407	752	0	0	neg	
23	29/F	SLE	PSL 32 mg/day	1702	2247	0	0	neg	

SLE, systemic lupus erythematosus; RV, rheumatoid vasculitis; MPA, microscopic polyangiitis; PM/DM, polymyositis/dermatomyositis; SSc, systemic sclerosis; ASD, adult-onset Still's disease; TTP, thrombotic thrombocytopenic purpura; PSL, prednisolone; mPSL, methylprednisolone; CYP, cyclophosphamide; CyS, cyclosporine A; n.a., not available; pos, positive; neg, negative

[a] Methylprednisolone 500 mg/day for 3 days

[b] Cyclophosphamide 500 mg/day

[c] At the first time

[d] At the second time

[e] CMV disease developed after 3 weeks of mPSL pulse therapy

[f,g] CMV disease developed after 3 weeks and 5 weeks of CYP pulse therapy, respectively

Table 2. Results for *P. jiroveci* pneumonia, treatment for CMV antigenemia and virologic and clinical outcomes in patients with CMV antigenemia

Patient (case no.)	Age (years)/ sex	Disease	Detection of *P. jiroveci*[a]	β-D-Glucan (ng/l)	Suspicious *P. jiroveci* infection[b]	Treatment for CMV antigenemia	Outcomes of CMV antigenemia and CMV disease
1	69/F	SLE, TTP	neg	<20		GCV[c]	Died of sepsis
2	24/F	SLE	neg	<20		GCV, IVIG[d]	Clear
8	87/M	PM/DM	pos	140	*PC*P	GCV	Died of bacterial pneumonia
9	66/F	SSc	pos	156	*PC*P	GCV	Clear and improved
11	59/F	PM/DM	pos	266	*PC*P	GCV	Died of exacerbation of interstitial pneumonia with PM/DM
12	56/F	SLE	neg	<20		GCV	Clear and improved
13	52/F	ASD	pos	251	*PC*P	GCV, IVIG	Clear and improved
14	69/M	RV	neg	<20		GCV, IVIG	Clear and improved
15	29/F	SLE	neg	<20		GCV, IVIG	Clear
18	74/M	SSc	neg	<20		GCV	Clear

SLE, systemic lupus erythematosus; PM/DM, polymyositis/dermatomyositis; SSc, systemic sclerosis; RV, rheumatoid vasculitis; ASD, adult-onset Still's disease; TTP, thrombotic thrombocytopenic purpura; *PC*P, *Pneumocystis jiroveci* pneumonia; GCV, ganciclovir; IVIG, intravenous immunoglobulin; pos, positive; neg, negative

[a] Detection of *Pneumocystis jiroveci* in induced sputum was determined by polymerase chain reaction analysis

[b] Finding of geographical ground-glass opacities was demonstrated by chest computed tomography

[c] Ganciclovir 10 mg/kg body weight/day for 14 days

[d] Immunoglobulin 400 mg/kg body weight/day for 5 days

and treatment with immunosuppressive agents such as CYP, CyS, and methotrexate, rather than the disease itself or an immune function abnormality.[2–11]

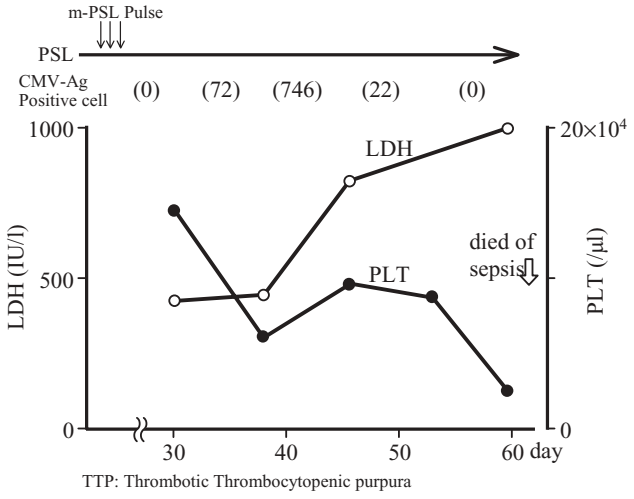
An initial infection with human CMV frequently occurs undetected in childhood, and becomes latent without reactivation in normal adults. However, CMV is reactivated in patients with decreased cellular immunity or diseases of various organs including interstitial pneumonia and liver involvement, which are caused by opportunistic infections, particularly in organ transplant recipients.[16]

The critical step in the pathogenesis of CMV infection, defined as detectable virus assessed by CMV antigenemia assay or quantitative PCR, is the reactivation of latent virus, which is affected by the intensive immunosuppressive therapy. With regard to the background of patients, our study suggested that the use of intravenous CYP pulse, particularly mPSL pulse in combination with additional CYP pulse, is the most important factor associated with occurrence of CMV infection. Ho has reported that relationship between CMV infection (reactivation of latent virus) and CMV disease correlates well with the type of immunosuppressant employed and its effect on cellular immunity.[17] Moreover he reported that in transplant recipients, immunosuppression caused CYP contributes to a high risk of developing CMV infection and that steroids, by themselves, have a minimal effect on reactivation of latent CMV.[17] But the present observations suggested that in patients with inflammatory CTDs, a mean PSL dose of 43 mg daily might also be responsible for the induction of CMV infection.

The most important effect of CMV infection is that it predisposes the patient to the other opportunistic infections with a variety of microbial agents. It is suggested that there is a particularly close association between CMV infection and the development of *Pneumocystis* pneumonia, which is related to an alveolar macrophage function defect induced by the virus.[17] It has been reported that the appearance of *Pneumocystis jiroveci* infection as a complicating opportunistic infection in patients with CTDs correlates with a low peripheral blood leukocyte count and a low serum IgG level.[18,19] In our observations, four of ten CMV antigenemia patients simultaneously developed *Pneumocystis* pneumonia based on detection of *Pneumocystis jiroveci* in induced sputum, increase in level of serum β-D-glucan, and the finding of geographical ground-glass opacities on chest computed tomography. These findings suggested that patients with CTDs under intensive immunosuppressive therapy (intravenous CYP pulse in particular) are highly susceptible to CMV reactivation, and that patients complicated by CMV antigenemia are susceptible to combined opportunistic infections such as *Pneumocystis* pneumonia.

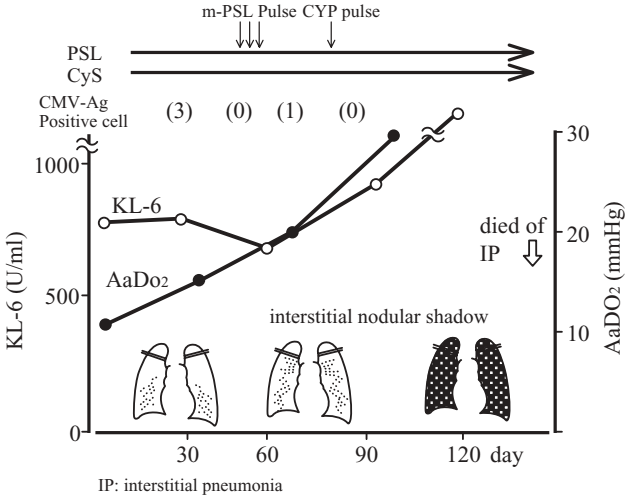
We immediately administer ganciclovir (10 mg/kg body weight/day, for 14 days) to CMV antigenemia patients who rapidly develop fever and CMV disease.[20] Patients without fever and organ symptoms and with an extremely low CMV antigen-positive cell count are first monitored and are then administered ganciclovir when the CMV antigen-positive cell count starts to increase. Patients who show no improvement of organ symptoms and those who show no decrease in CMV antigen-positive cell counts are administered a high-

Case 1. SLE complicated with TTP



TTP: Thrombotic Thrombocytopenic purpura

Case 11. PM/DM complicated with IP
(*P. jiroveci*-PCR positive)



IP: interstitial pneumonia

Case 8. PM/DM complicated with lung cancer
(*P. jiroveci*-PCR positive)

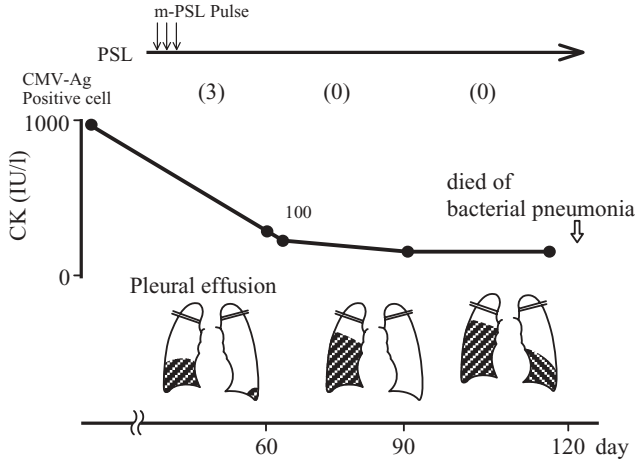


Fig. 1. The course of patients with connective tissue disease who died

dose intravenous immunoglobulin preparation. The above therapeutic regimen has produced satisfactory results.

In addition, CMV infection (reactivation of latent CMV) has been reported to affect the course of SLE.[21,22] In our study, it was considered that the therapy against CMV infection may not cause the exacerbation of CTDs and their complications, and that CMV infection itself might induce the exacerbation of complications of underlying CTDs. However, the relationship between CMV infection and the activity of CTDs was not elucidated.

In summary, patients with refractory inflammatory CTDs develop CMV antigenemia and CMV disease at a high incidence following the administration of intravenous CYP pulse. Cytomegalovirus antigenemia patients are susceptible to the development of concurrent *Pneumocystis* pneumonia. Inflammatory CTD patients who undergo intensive immunosuppressive therapy require a regular CMV antigenemia test. Cytomegalovirus disease, which may be serious particularly in immunocompromised individuals, is improved by early antiviral therapy.

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