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BRIEF REPORT

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Late onset cytomegalovirus disease in liver transplant recipients: de novo reactivation in recurrent hepatitis C virus hepatitis

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Abstract Late onset cytomegalovirus (CMV) disease (occurring more than 1 year post-transplant] was documented in two liver transplant recipients with recurrent hepatitis C virus hepatitis in the absence of factors known to precipitate CMV disease, i. e., primary acquisition of CMV, allograft rejection, augmented immunosuppression, concomitant infections, or blood transfusions. Both patients had CMV enteritis (with CMV adrenalitis in one case]; however, other symptoms and signs of overt CMV infection, i. e., fever, leukopenia, or atypical lymphocytes, were lacking. Hepatitis C virus is an immunomodulatory virus; impaired CMV-specific T-cell responses may have accounted for the predisposition of our patients to unprovoked, late onset CMV disease. Given the high incidence of hepatitis C virus recurrence after liver transplantation, awareness of the occurrence and recognition of the unusual presentation of CMV disease in this setting is both clinically relevant and significant, particularly since CMV is treatable if recognized promptly.

Key words Cytomegalovirus, liver transplantation · Liver transplantation, cytomegalovirus · Late onset infection, cytomegalovirus, liver transplantation

Introduction

Cytomegalovirus (CMV) remains the foremost opportunistic pathogen and a significant cause of morbidity in organ transplant recipients. CMV is generally an early occurring infection in transplant recipients; most CMV infections occur between 3 and 12 weeks post-transplant. The characteristic timing of onset and clinical presentation (viral syndrome with or without organ involvement] readily lends itself to consideration of CMV in the evaluation of a febrile transplant recipient during this time period. Late onset CMV (occurring > 1 year post-transplant), on the other hand, is an unusual event [1, 12, 15]. Of nine such cases described in the literature on renal transplant recipients, late occurring primary acquisition of CMV or augmented immunosup-pression were the predominant precipitating events. More recently, symptomless CMV infection in renal transplant recipients was shown to present as late-acute allograft rejection [14]. These patients did not respond to conventional antirejection therapy; however, ganciclovir resulted in stable improvement in graft function in 17 of 21 patients [14].

Late onset CMV is not a well-described clinical entity in liver transplant recipients. Although a few cases of late acute rejection with concomitant CMV infection have been described [2], we are unaware of any reported cases of late onset CMV disease in liver transplant recipients occurring in the absence of allograft rejection. We describe two liver transplant recipients with late onset CMV disease occurring in the absence of the aforementioned precipitating events. Their unique (somewhat atypical) but strikingly similar clinical presentation prompted us to report these cases, particularly since CMV is a treatable infection if suspected and promptly recognized.

Materials and methods

Detection of CMV

CMV antigenemia (pp65 assay) was employed for the detection of CMV in the buffy coat samples [6, 17]. Urine and tissue samples were cultured using the shell vial (early antigen) assay.

Memory T-helper responses of peripheral blood mononuclear cells

CMV antigen was prepared as previously described using a glycine buffer extraction method following infection of human foreskin fibroblasts with the AD169 strain of CMV [11]. Peripheral blood mononuclear cells were isolated from whole blood on a Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) density gradient and cultured at a concentration of 10^5 cells/well in 96 round bottom microtiter plates in triplicates with RPMI and 5% human serum. Cultures were stimulated with CMV antigen (1:100 dilution) and tetanus toxoid antigen (4 μg/ml) for 5 days and pulsed with 1mCi ^{3}H-thymidine per well for 18 h; they were then harvested and counted in a liquid scintillation counter [7]. A response was considered positive when the stimulation index (SI = cpm with antigen/cpm media) was greater than 3 and the cpm were above 1200.

Peripheral blood mononuclear cells obtained 3–5 weeks after the diagnosis of CMV disease from the two cases were tested for T-helper responsiveness to CMV antigen and to tetanus toxoid. Both patients exhibited a positive response to CMV antigen (case 1: 1827 ± 149 cpm background vs 1461 ± 563 cmp, SI = 3; case 2: 810 ± 4 cpm background vs 4933 ± 1340 cmp, SI = 6) and to tetanus toxoid.

Case reports

Case 1

A 45-year-old male underwent liver transplantation for end-stage liver disease due to hepatitis C virus (HCV). The patient and the donor were seropositive for CMV. Postoperative immunosuppression consisted of tacrolimus and prednisone (which was withdrawn 3 months after transplantation). Fifty-six days post-transplantation, CMV viremia and viruria developed for which the patient received a 21-day course of intravenous ganciclovir. Histopathologically recurrent HCV hepatitis developed 18 months post-transplant and interferon-alpha (3 million units thrice weekly) was initiated as therapy. Twenty-two months post-transplant, the patient was admitted with a 3-week history of nausea, loss of appetite, fatigue, and weight loss of unexplained etiology. There were no documented febrile episodes. Endoscopy for the evaluation of his gastrointestinal symptoms revealed erosive enteritis involving the antrum and the duodenum. Viral cultures of the tissue biopsies from these sites revealed CMV. CMV viruria was also detected, but CMV antigenemia was negative. A corticosyntropin stimulation test documented coexistent adrenal insufficiency. The patient received a 21-day course of ganciclovir and a 30-day course of hydrocortisone with gradual resolution of his symptoms. A corticosyntropin stimulation test 1 month after the end of corticosteroid therapy was normal.

Case 2

A 42-year-old male underwent liver transplantation for end-stage liver disease due to HCV. The patient was seropositive for CMV prior to transplantation and received a seronegative donor allograft. Postoperative immunosuppression consisted of tacrolimus, azathioprine, and prednisone. Forty-three days post-transplant, CMV viruria without CMV disease was documented and the patient received a 7-day course of pre-emptive ganciclovir prophylaxis, as previously reported [16]. Eight months after transplantation, *Parvovirus* B–19 infection of the bone marrow was documented and treated successfully with intravenous immunoglobulin. Nine months post-transplant, recurrent HCV hepatitis was diagnosed and treated with interferon-alpha. Twenty-one months after transplantation, persistent nausea and weight loss of unclear etiology prompted an upper endoscopy. Viral culture of a biopsy of the antrum revealed CMV. The buffy coat for CMV antigenemia was negative. The patient received a 3-week course of intravenous ganciclovir with resolution of his symptoms.

Discussion

Tissue invasive CMV infection was documented nearly 2 years post-transplantation in two liver transplant recipients with recurrent HCV hepatitis in the absence of previously known precipitating factors for CMV infection. It is a well-recognized fact that primary CMV infection is associated with a greater severity of symptomatic illness than reactivation infection. Indeed, 78% of cases of late onset CMV disease reported in renal transplant recipients were in patients with primary CMV infection that preceded their late onset CMV disease [1, 12, 15]. Blood transfusions and sexual transmission have been proposed as the mode of acquisition of CMV in these late occurring cases of primary CMV infection [1]. Allograft rejection and/or intensified immunosuppression for its treatment may lead to reactivation of CMV infection at any time post-transplant. It has also been proposed that tumor necrosis factor alpha can reactivate latent CMV and any stimulus promoting tumor necrosis factor alpha release, e.g., sepsis, and that it therefore has the potential to reactivate CMV [4]. Our patients are noteworthy since CMV disease occurred during a period of chronic, stable, exogenous immunosuppression in the absence of primary acquisition of CMV, allograft rejection, intensified immunosuppression, or other concomitant infections.

Both patients were seropositive for CMV prior to transplantation and underwent reactivation of CMV infection 43 and 56 days post-transplantation, respectively. There was no history to suggest sexual transmission of a new strain of CMV, and neither patient had received blood transfusions within a 4-week period preceding their late onset of CMV disease. Our patients were receiving interferon-alpha for HCV hepatitis at the time CMV disease occurred. However, interferon-alpha has not been shown to reactivate CMV infection. On the contrary, some studies have documented a protective effect of interferon-alpha on CMV after transplantation [3]. We therefore believe that their late occurring CMV disease was likely a reactivation and not a primary infection or superinfection with a new CMV strain.

It has been proposed that HCV is an immunomodulatory and an immunosuppressive virus predisposing patients to opportunistic infections [5, 9]. Liver transplant recipients with recurrent HCV hepatitis have been found to have a significantly high incidence of major infections, particularly due to pathogens requiring intact T-cell mediated immunity [16]. One recent study revealed that renal transplant recipients with HCV hepatitis had a 52% incidence of life-threatening infections versus 20% for those without hepatitis [13]. It is conceivable that patients with HCV hepatitis have deficient CMV-specific T-cell responses that may increase their predisposition to CMV disease. Reconstitution of CD4 + T-helper and CMV-specific CD8 + cytotoxic T-cell responses is critical in order to protect transplant recipients from recurrent CMV; patients deficient in these cellular immune effectors may be susceptible to late onset and recurrent CMV infection [10, 18].

Peripheral blood mononuclear cells obtained 3–5 weeks after the diagnosis of CMV disease from the two cases were tested for T-helper responsiveness to CMV antigen and to tetanus toxoid. Both patients exhibited a positive response to CMV antigen (case 1: 1827 ± 149 cpm background vs 1461 ± 563 cpm, SI = 3; case 2: 810 ± 4 cpm background vs 4993 ± 1340 cpm, SI = 6) and to tetanus toxoid. These data indicate that our patients did develop memory T-helper response to CMV following their CMV disease (SI = 5 for case 1 and SI = 11 for case 2). However, their memory response to CMV prior to the occurrence of late onset CMV disease and during the period of stable immunosuppression is not known.

It is noteworthy that symptoms and signs suggestive of overt CMV infection, e.g., fever, leukopenia, were absent in our patients, and the relatively nonspecific presentation of their late onset CMV disease may easily be overlooked in clinical practice. Both patients presented with subacute onset of gastrointestinal symptoms. Although these symptoms could have resulted from interferon therapy, our patients had been receiving and tolerating interferon for several months prior to the diagnosis of CMV enteritis without any adverse effects. Furthermore, resolution of these symptoms was documented after specific antiviral therapy, despite continuation of interferon. Adrenal insufficiency in case 2 could have been a factor contributing to the patient's symptoms. Although CMV as an etiology of the patient's adrenal insufficiency was not histopathologically documented, normalization of his cosyntropin test after antiviral therapy strongly supports an etiologic role of CMV in adrenalitis. CMV adrenalitis is a frequently encountered clinical entity in HIV-infected patients with CMV infection. However, overt adrenal insufficiency due to CMV is rarely documented in transplant recipients since most CMV infections occur during the first 3 months of transplantation, at a time when these patients are usually receiving prednisone as part of the immunosuppressive regimen. Our case, nevertheless, suggests that CMV adrenalitis may occur in transplant recipients with late onset CMV in whom corticosteroid therapy has been withdrawn.

CMV antigenemia (viremia) was not documented in either of our patients. Gastrointestinal CMV infection is one of very few conditions where clinical CMV disease may occur without viremia. In one study, only 19% of the episodes of CMV enteritis were associated with viremia [8].

In summary, late onset CMV disease occurred in two liver transplant recipients with recurrent HCV hepatitis in the absence of an apparent provocative or precipitating factor for CMV. Whether this finding is unique to patients with recurrent HCV hepatitis in the late post-transplant period remains to be proven. Overt manifestations of CMV, e.g., fever and pancytopenia, may be lacking in these patients. End-stage liver disease due to HCV has emerged as one of the leading indications for orthotopic liver transplantation, and recurrent HCV hepatitis develops in nearly half of all such patients. Because of the increasing number of patients with recurrent HCV hepatitis, it is important that the clinically relevant implications of our observations be validated by other researchers.

References

1. Boehler A, Schaffner A, Salomon F, Keusch G (1994) Cytomegalovirus disease of late onset following renal transplantation: a potentially fatal entity. Scand J Infect Dis 26: 369–373
2. Cakaloglu Y, Devlin J, O'Grady J, Sutherland S, Portman BC, Heaton N, Tan KE, Williams R (1995) Importance of concomitant viral infection during late acute allograft rejection. Transplantation 59: 40–45
3. Cheeseman SH, Rubin RH, Steward JA, et al (1979) Controlled clinical trial of prophylactic human-leukocyte interferon in renal transplantation: effects on cytomegalovirus and herpes simplex virus infection. N Engl J Med 300: 1345–1349
4. Docke WD, Prosch S, Fietze E, Kimel V, Zuckerman H, Klug C, Syrbe U, Kruger DH, Baehr R von, Volk HD (1994) Cytomegalovirus reactivation and tumor necrosis factor. Lancet 343: 268–269
5. Fishman JA, Rubin RH, Koziel MJ, Periera BJG (1996) Hepatitis C virus and organ transplantation. Transplantation 62: 147–154
6. Grossi P, Minoli L, Percivalle E, Irish W, Vigano M, Gerna G (1995) Clinical and virological monitoring of human cytomegalovirus infection in 294 heart transplant recipients. Transplantation 59: 847–851

7. He H, Rinaldo CR Jr, Morel PA (1995) T cell proliferative responses to five human cytomegalovirus proteins in healty seropositive individuals: implications for vaccine development. J Gen Virol 76: 1603–1610
8. Kusne S, Manez R, Frye BL, St. George K, Abu-Elmagd K, Tabasco-Menguillon J, Fung JJ, Todo S, Rinaldo C, Ehrlich GD (1997) Use of DNA amplification for diagnosis of cytomegalovirus enteritis after intestinal transplantation Gastroenterology 112: 1121–1128
9. LaQuaglia MP, Tolkoff-Rubin NE, Dienstag JL, Cosimi AB, Herrin JT, Kelly M, Rubin RH (1981) Impact of hepatitis on renal transplantation. Transplantation 32: 504
10. Li CR, Greenberg PD, Gilbert MJ, Goodrich JM, Riddell SM (1994) Recovery of HLA-restricted cytomegalovirus (CMV) – specific T-cell responses after allogeneic bone marrow transplant: correlation with CMV disease and effect of ganciclovir prophylaxis. Blood 83: 1971–1979
11. Lindsley MD, Torpey DJ III, Rinaldo CR Jr (1986) HLA-DR restricted cytotoxicity of cytomegalovirus-infected monocytes mediated by Leu-3-positive T cells. J Immunol 136: 3045–3051
12. Linneman CC, Dunn CR, First MR, Alvira M, Schiff GM (1978) Late onset of fatal cytomegalovirus infection after renal transplantation, primary or reactivation infection? Arch Intern Med 138: 1247–1250
13. Rao KV, Ma J (1996) Chronic viral hepatitis enhances the risk of infection but not acute rejection in renal transplant recipients. Transplantation 62: 1765–1769
14. Reinke P, Fietze E, Ode-Hakim S, Prosch S, Lippert J, Ewert R, Volk HD (1994) Late acute renal allograft rejection and symptomless cytomegalovirus infection. Lancet 344: 1737–1738
15. Rubin RH, Tolkoff-Rubin NE (1984) The problem of cytomegalovirus infection in transplantation. In: Morris PJ, Tilney NL (eds) Progress in transplantation, vol. 1. Churchill Livingstone, Edinburgh, pp 89–114
16. Singh N, Gayowski T, Wagener MM, Marino IR (1996) Increased infections in liver transplant recipients with recurrent hepatitis C virus hepatitis. Transplantation 61: 402–406
17. The TH, Bij W van der, Berg AP van den, Giessen M van der, Weits J, Sprenger HG, Son WJ van (1990) Cytomegalovirus antigenemia. Rev Infect Dis 12 [Suppl]:737–744
18. Zeevi A, Morel P, Spichty K, Dauber J (1996) Clinical significance of CMV-specific memory T-helper responses in lung transplant recipients (abstract). American Society of Transplant Physicians Annual Meeting, Chicago