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Adoptive Immunotherapy for Cytomegalovirus (CMV) Disease in Immunocompromised Patients

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Cytomegalovirus (CMV) reactivation in immune compromised patients such as those undergoing hematopoietic progenitor cell transplantation (HPCT) and those with HIV infections can cause severe morbidity and mortality despite treatment with appropriate antiviral agents. The recovery of Cytomegalovirus (CMV) specific cytotoxic T lymphocytes (CTL) plays an important role in the reconstitution of CMV specific immunity in immunocompromised patients. Recent studies have reported that CMV reactivation can be successfully treated by adoptive transfer of CMV-specific T cell clones from CMV seropositive donors expanded *in vitro* with CMV infected fibroblasts or lysates of CMV infected cells. Other studies have used immune dominant CMV proteins or peptides to expand CMV-specific cytotoxic T lymphocytes. This review describes the clinical manifestations of CMV disease in immunocompromised patients, recent advances of antiviral therapy for CMV disease, the principals of the induction of cellular immune response to CMV, and the clinical application of CMV immunotherapy.

Key Words: Cytomegalovirus, immunotherapy, cytotoxic T lymphocytes, CMV pp65

Cytomegalovirus (CMV) Infection

Cytomegalovirus (CMV) is a ubiquitous β-herpesvirus and is principally present in peripheral monocytes from 50% to 90% of normal individuals. Myeloid precursors in the bone marrow are the principal target of CMV and CMV can be retained during differentiation to monocytes. After CMV infection, the viral genome is expressed sequentially, giving rise to production of immediate early (0-2 hour), early (< 24 hour) and late (> 24 hour) viral proteins in host cells.[1] Mature CMVpp65 protein which is an early type of protein is transferred into cells with the CMV virion at the onset of infection. CMV pp65 is present even before the initiation of viral gene expression.[2,3] For this reason, CMVpp65 matrix protein plays an important role in the early diagnosis of CMV infection and may serve as the main target of the CD8+ cytotoxic T lymphocyte (CTL) response against CMV, even without viral gene expression.[4]

CMV Disease in Immune Competent Hosts

Primary CMV infection in healthy hosts is usually asymptomatic, but it can present with clinical manifestations that are very similar to infectious mononucleosis due to primary Epstein Barr virus (EBV) infection. These manifestations include fever, myalgia, cervical lymphadenopathy and hepatitis.[5,6]

In some cases the primary CMV infection may be congenital. Although about 5-20% of congenital CMV infections are symptomatic at birth, congenital CMV infections is associated with high mortality and serious sequelea such as mental, visual and hearing impairment.[7]

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CMV Disease in Immunocompromised Patient

CMV disease in immunocompromised patients can be very serious. In transplant recipients the risk of HCMV disease is 3-5 times greater in a seronegative than a seropositive recipient due to the transmission of CMV with grafts from a seropositive donor. In renal transplant recipients HCMV infections have been reported to be associated with graft rejection and renal artery stenosis,[8,9] in heart transplant patients with accelerated coronary artery stenosis,[10] and in liver transplant patients with 'vanishing bile duct' syndrome.[11] However, none of these associations have been definitively established as being caused by CMV, and each is somewhat controversial.

In BMT recipients, 30-50% of CMV infections cause serious clinical manifestations including pneumonitis. Interstitial pneumonitis occurs in 10-15% of allogeneic BMT recipients and prior to the advent of antiviral therapy had a mortality rate of 80%. The relationship between HCMV disease and GVHD is controversial. Some people hypothesize that HCMV infections lead to more severe GVHD, but others believe that GVHD leads to CMV disease.[12] 'Pre-emptive' Ganciclovir (GCV) treatment in CMV-PCR positive patients reduces the incidence of CMV disease in the first 100 days after BMT. However, late CMV disease (after the first 100 days) still remains a serious problem in BMT recipients and implies that GCV delays the reconstitution of immunity to HCMV.[13]

HCMV disease is one of the most frequent opportunistic infections in patients with advanced HIV infection, of whom 40% develop life threatening HCMV disease. The most common manifestation of HCMV infection in patients infected with HIV is retinitis which prior to the development of effective antiviral therapy was seen in up to 25% of patients with AIDS.[14] A syndrome recognized subsequent to the use of HAART is 'immune recovery vitritis'. This syndrome is characterized by posterior segment inflammation and occurs as the CD4 count reconstitutes due to antiretroviral therapy in patients with previously treated but inactive CMV retinitis. Although this has been attributed to infiltrating T cells reacting to HCMV antigens in the eye, this mechanism has not yet been proven. This inflammatory condition responds to steroids alone.[15]

A recent study reported that HCMV was associated with the smooth muscle cell proliferation responsible for coronary artery restenosis following angioplasty, but other subsequent reports have failed to confirm this association[16,17] and other microbial agents (particularly Chlamydia pneumoniae) have now been reported to be associated with atherosclerotic lesions in humans.[18,19]

Antiviral Therapy for CMV Disease

Antiviral agents

Several drugs are now available for the treatment of HCMV disease. Aciclovir (ACV) has little *in vitro* activity against HCMV, which unlike Herpes Simplex virus does not possess a thymidine kinase (TK). Another nucleoside analogue, GCV, is monophosphorylated in infected cells by the UL 97 gene product of HCMV and is active against HCMV. The most serious side effect of GCV is myelotoxicity including leucopenia and thrombocytopenia. Males also experience azoospermia.[20] Valganciclovir, the valyl ester prodrug of GCV, has much greater oral bioavailability. It seems likely that valganciclovir will become an oral substitute for GCV in many setting.[21]

Another alternative drug to GCV is Foscarnet (trisodium phosphonoformate) which is a competitive inhibitor of the viral DNA polymerase, and shows no cross resistance with GCV. This agent also must be given intravenously. Adverse effects associated with foscarnet include renal impairment and hypocalcemia.[22]

Antiviral prophylaxis

Ganciclovir has been used to prevent primary CMV infection and reactivation in solid organ transplant and BMT recipients, particularly those at high risk of CMV disease and in AIDS patients with less than 100 CD4 cells/μl. Because of the marrow toxicity associated with GCV, it is typically administered at the time of greatest risk for CMV viremia, but prior to the actual development of viremia. Another approach is to give GCV at the onset of CMV viremia, but

prior to symptomatic CMV disease (preemptive therapy). Real time RT-PCR and PCR for the early detection of CMV viremia is essential for the application of preemptive strategies of antiviral therapy.[23] If marrow suppression is a problem, foscarnet (FCN) can be used in place of GCV at a dose of 60mg/kg, three times a day infused over 2 hours.

Immune Response to CMV Infection

After primary infection CMV persists for the life time of the host. A high frequency of CMV specific CD8+ cytotoxic T lymphocytes (CTL) in peripheral blood seems to protect the host from wide dissemination of CMV after reactivation of latent virsu. Activation of naive CTL involves stimulation through the T cell receptor (TCR), which recognizes viral peptide antigen bound to class I MHC molecules, and requires co-stimulation through CD28, which binds members of the B7 family (CD80, CD86) on the antigen-presenting cell (APC). Activated CMV- specific CTLs undergo clonal proliferation and become effector CTLs that kill CMV-infected cells. After the clearance of an acute virus infection, most virus-specific CTL die by apoptosis, but some activated CTLs survive as a population of memory cells that lack direct cytotoxic activity. However, these memory cells can become cytotoxic and proliferate upon restimulation with CMV antigen. Following primary CMV infection, many virus-specific memory cells appear to be long-lived non-dividing cells that may survive in a partially activated state. The degree of activation is sufficient to maintain surface expression of some, but not all activation markers. Other memory cells may periodically undergo cell division and maintain long-lived clones.

Induction of Cellular Immune Response to CMV

CMV-specific CTLs have been raised from asymptomatic CMV seropositive donors by stimulating their peripheral blood lymphocytes (PBL) *in vitro* using autologous fibroblasts infected with CMV,[24-28] EBV-transformed B cell lines infected with recombinant virus,[29] or HLA-restricted CMV peptides.[30] Initial investigations focused on the immediate early protein (IE1 or pp72) as an important target for cellular immune responses, since the analogous protein is importance in the murine CMV model.[31,32] However, limiting dilution analysis (LDA) of peripheral blood mononuclear cells (PBMC) from asymptomatic volunteers indicated that the CTLs present in the highest frequencies reacted with CMVpp65, and to a lesser degree with CMVpp150, and CMV-IE. Very weak reactivity was also detected against CMVgB.[26,28,33] However, recent data have suggested CMV-IE-specific CTLs are present at higher frequency than previously thought in CMV- seropositive donors.

Recent studies have found that peptides 8 to 10 amino acids in length derived from the CMV proteins pp65 and pp150 can be presented by antigen presenting cells and recognized by CMV specific CD8+ T-cells.[34,35] The peptides directly induce rapid production of INF-γ protein and expansion of CTL from donor's PBMC. Several epitopes restricted to major HLA types have been reported, including, CMV $pp65_{495\text{-}503}$ (NLVPMVATV) for HLA-A*0201 and $pp65_{341\text{-}350}$ (QYDPVALFF) for both HLA-A*2402 and HLA-A 0101.[35-37]

Clinical Application of CMV Immunotherapy

The protective function of CMV-specific CTLs could potentially be established by adoptive transfer of these cells early after transplant. In models involving healthy mice primary CMV infections are readily controlled by the immune response. In contrast, in immunodeficient mice primary CMV infection causes a progressive infection involving the lungs and bone marrow. However, in immunodeficient mice the adoptive transfer of syngeneic polyclonal CD4+ and CD8+ MCMV-specific T cells or CD8+ MCMV-specific cytotoxic T cells alone was sufficient to protect irradiated mice from fatal MCMV infection.[38-42]

Phase I / II clinical trials have revealed that the adoptive transfer of donor derived CD8+ CMV-specific CTL clones is a safe and effective way to reconstitute cellular immunity against CMV after allogeneic marrow transplantation.[43,44] Walter et

al. showed that cytotoxic activity against CMV was significantly increased ($p < 0.001$) after the infusion of CMV-specific CTL clones in 11 patients who were deficient in such activity before therapy.[44] The level of CMV-specific cytotoxicity achieved after the infusions was similar to that measured in the donors. Analysis of rearranged T-cell-receptor genes in T cells obtained from two recipients indicated that the transferred clones persisted for at least 12 weeks. Moreover, the study found that CD4 helper-T cell function was needed for the persistence of transferred CD8+ T cells.[44] Hermann Einsele et al. transfused CMV-specific T cells into 8 hematopoietic progenitor cell transplant recipients with CMV infections resistant to anti-viral agents. Following the infusion the CMV load dropped significantly in all 7 evaluable patients, The maximal reduction of CMV load occurred a median of 20 days (range, 5-31 days) after the CTL infusions despite the cessation of antiviral chemotherapy.[45]

Summary

Recently, some investigators have reported that the transfer of CMV-specific CD8+ T cells is a safe and effective way to reconstitute cellular immunity against CMV after allogeneic hematopoietic progenitor cell transplantation. At present, the development of adoptive immunotherapy for the treatment of CMV infection and disease in immunocopromised patient remains restricted to research settings, but the clinical application of CMV adoptive immunotherapy including CMV peptide and protein vaccination will likely be used more broadly in the near future.

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