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Case Report

Trip to immunity: resistant cytomegalovirus infection in a lung transplant recipient

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SUMMARY

We report the case of a young female lung transplant recipient with difficult-to-treat cytomegalovirus (CMV) disease. While treatment with intravenous (IV) ganciclovir failed due to antiviral drug resistance, a trial with foscarnet resulted in severe side effects. In addition, the patient received IV CMV-specific immune globulins as adjunctive therapy and leflunomide as experimental therapy. In this context, CMV-specific immune monitoring was performed and was successfully implemented in management decisions. The patient was screened for acquisition of an adaptive immune response, and antiviral prophylaxis and therapy was tailored according to results. This report highlights the impact of CMV-specific immune monitoring on individualized therapy for appropriate prophylaxis and management of CMV infection and diseases.

1. Introduction

Cytomegalovirus (CMV) is the most prevalent opportunistic infection that occurs in lung-transplant recipients. The most important risk factor is CMV serological status, with D+/R- recipients having the highest risk. CMV infection may evolve to CMV disease with life threatening tissue invasive disease. CMV-induced immunosuppression may lead to infection with other opportunistic organisms and CMV infections have been associated with acute and chronic rejection.[1] CMV-specific immune monitoring may help to identify the time point of acquisition of adaptive immune response and therefore to tailor antiviral prophylaxis and therapy.

2. Case report

We report the case of a 42-year-old female solid organ transplant recipient who underwent a double lung transplantation for cystic fibrosis. The recipient was CMV-seronegative and received a graft from a CMV-seropositive donor.

The initial postoperative course was free of adverse events. The recipient received a center-specific immunosuppressive regimen with tacrolimus, mycophenolate mofetil (MMF), and prednisolone. As preemptive therapy of CMV infection is part of routine care in our center, the patient did not receive antiviral prophylaxis with valganciclovir. Patients are monitored weekly for CMV infection and treatment can be initiated immediately.

Shortly before hospital discharge, CMV PCR was positive for the first time (206 copies/ml). On the day of discharge, CMV DNA had increased to 4300 copies/ml and treatment was initiated. Treatment with valganciclovir 900 mg twice daily was started and MMF was discontinued. The CMV viral load was successfully decreased over the course of 4 weeks of rehabilitation (Figure 1). No symptoms of CMV disease had occurred this far.

Upon routine visit to our outpatient clinic 2 months after transplantation, CMV DNA was <150 copies/ml. CMV-specific immunity was assessed using two commercially available ELISA interferon gamma release assays (IGRAs) predicting CMV-specific T-cell responses and by flow cytometry analysis, investigating the number of interferon gamma (IFN-γ)-producing T-cells. All three

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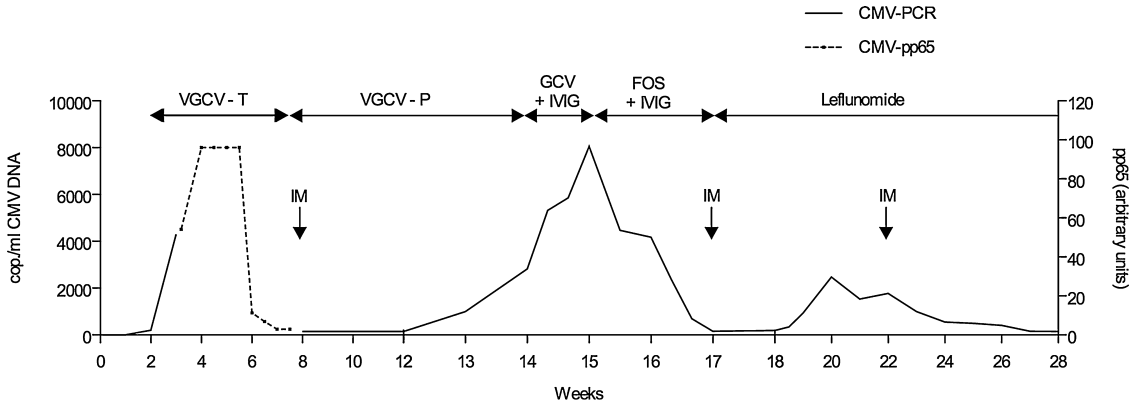

Figure 1. Course of CMV burden and therapeutic interventions. VGCV-T, valganciclovir at a therapeutic dose of 900 mg twice daily; VGCV-P, valganciclovir at a prophylactic dose of 450 mg twice daily; GCV, intravenous ganciclovir 5 mg/kg body weight twice daily; FOS, foscarnet (60 mg/kg body weight three times daily); IVIG, intravenous CMV-specific immune globulin (Cytotect 100 international units (IU)/kg body weight); IM, immune monitoring.

methods demonstrated a lack of CMV-specific immunity (frequency (f) of IFN-γ-producing T-cells per 10 000 T-cells: Δf CD8+/Δf CD4+ 6.6/1.8). Valganciclovir was continued at a prophylactic dose of 450 mg twice daily and CMV DNA remained low at <150 copies/ml over the following month.

However, 3 months after transplantation, the viral load increased to 2820 copies/ml and the patient was admitted to our center for further management. The patient reported signs of a viral syndrome for the first time, with malaise, fatigue, and shivering. The patient was treated with intravenous (IV) ganciclovir (5 mg/kg body weight twice daily) and IV CMV-specific immune globulins. No evidence of tissue invasive diseases was found.

However, in the following days, her viral load increased further to a maximum of 8050 copies/ml. Hence, testing for resistance mutations was performed and treatment was switched to foscarnet (60 mg/kg body weight three times daily), followed by a second course of CMV-specific immune globulins. Ganciclovir resistance was confirmed by sequencing. A point mutation in the UL97-kinase gene resulted in amino acid switch in codon 460 from methionine to valine.

The viral load was reduced to 156 copies/ml within 2 weeks of treatment escalation. However, the patient developed side effects of antiviral treatment, with hypokalemia, hypomagnesaemia, and impaired renal function. Weight gain due to generalized edema, loss of appetite, nausea, and fever occurred. Due to the severity of drug-related side effects, foscarnet was withdrawn. Consequently, symptoms and electrolyte disturbances disappeared and kidney function recovered.

Again, approximately 4 months after transplantation, CMV-specific immunity was reassessed without signs of immunity. The results of the assays were negative and the number of CMV-specific T-cells (Δf CD8+/Δf CD4+ 9.7/6.4) remained low. Due to the lack of immunity, another increase in CMV DNA was anticipated and a therapeutic attempt with leflunomide was initiated. The patient gave informed consent and received a loading dose of 100 mg leflunomide, followed by 40 mg daily. Side effects of leflunomide such as liver damage or myelosuppression did not occur.

Initially, CMV PCR remained low and the patient was discharged. However, shortly after discontinuing foscarnet and starting leflunomide, CMV DNA started to increase again over the following weeks (maximum 2470 copies/ml). In agreement with the patient, we did not initiate antiviral therapy with foscarnet again. Due to nephrotoxicity, cidofovir was not an option in this case. However, a modification of the immunosuppressive regimen was discussed.

In the following days (approximately 5 months after transplantation), the CMV viral load started to decrease and CMV-specific immunity was assessed once more. For the first time, the patient demonstrated an appropriate immune response and the presence of CMV-specific T-cells (Δf CD8+/Δf CD4+ 36.8/46.8). Serological testing revealed positive CMV IgG and IgM levels, indicating the early stages of B-cell-mediated immunity.

Our patient remained on calcineurin inhibitor (CNI)-based immunosuppression, and MMF was exchanged for azathioprine. Leflunomide was maintained as adjunctive therapy.

More than 1 year later, the patient remains healthy without any signs of CMV infection.

3. Discussion

We report a lung transplant recipient with difficult-to-treat CMV disease and focus on the impact of detailed knowledge of immune status for appropriate management. In the context of ganciclovir resistance, side effects of second-line antiviral drugs, and experimental therapies, the need for CMV-specific immune monitoring is highlighted.

Despite receiving a prophylactic dose of valganciclovir followed by a full dose of IV ganciclovir over 1 week, the patient exhibited an increase in viral load, highly suggestive of antiviral drug resistance. Knowledge of resistance mutations is essential for further treatment. Depending on the type of mutation, different levels of antiviral drug resistance must be expected.[1] Foscarnet is the treatment of choice in the case of ganciclovir resistance, but side effects are frequent and may limit its clinical use, as reported in our case.

CMV-specific immune monitoring may help to identify the time point of acquisition of an adaptive immune response and therefore to tailor antiviral prophylaxis and therapy. The value of immunity testing has not been assessed in randomized clinical trials and is not yet applied in clinical routine. Two assays were used in this case. Whereas the QuantiFERON-CMV (Cellestis) assay is used to detect IFN-γ secreted by CD8+ T-cells, the T-Track CMV (Lophius Biosciences) allows quantification of IFN-γ-secreting CD4+ and CD8+ T-cells after specific stimulation. Furthermore, flow cytometry provides quantitative and qualitative characteristics of CMV-specific T-cells. The above mentioned methods have been suggested to be predictive of CMV disease.[2] Moreover, antibody measurements were performed in our patient. Whereas, CMV IgG levels may be influenced by the administration of IV CMV-specific immune globulins, CMV IgM may indicate early humoral immunity. CMV serology testing has no impact on the management of CMV infection. However, lack of seroconversion may be useful for the identification of patients at risk of late-onset CMV disease.[3]

Leflunomide has been reported to have both immunosuppressive and anti-CMV activity.[4] The mechanism of action conferring the antiviral activity remains unknown. In vitro phenotypic assays have indicated that leflunomide is active against both wild-type and ganciclovir-resistant CMV strains,[4] and leflunomide has been used to treat CMV in a few patients.[5]

In our patient, CMV PCR remained low for 2 weeks after the introduction of leflunomide, but then went up again over the next 2 weeks. The following and final decrease in CMV DNA correlated well with the concurrent development of a CMV-specific immune response. Therefore, the reasons for disease control might have been two-fold: the antiviral effect of leflunomide and/or the development of CMV-specific immunity. A CMV-specific immunemodulatory effect by leflunomide has not been reported so far.

In the context of antiviral treatment failure, alternative strategies were discussed. Previous studies have shown that regimens based on mTOR inhibitors might reduce CMV infections. Since our patient demonstrated immunity, she remained on a CNI-based immunosuppressive regimen and remained healthy without signs of CMV infection.

In conclusion, antiviral drug-resistant CMV disease is a rare but important threat. Detailed knowledge of the resistance profile is crucial to guide treatment. Furthermore, the presented case highlights the impact of immune monitoring in affected individuals and raises awareness of the need for a more sophisticated, individualized approach to prevent CMV infections, beyond standard prophylaxis or preemptive therapy. Measures of CMV-specific immunity might help to guide treatment and assess experimental drugs in vivo.

Conflict of interest: All authors declare no conflict of interest. No financial support.

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