Enzyme-Linked Immunospot Assay as a Complementary Method to Assess and Monitor Cytomegalovirus Infection in Kidney Transplant Recipients on Pre-emptive Antiviral Therapy: A Single-Center Experience

E. Favi[a], R. Santangelo[b], S. Iesari[c], M. Morandi[b], G.E. Marcovecchio[b], E.M. Trecarichi[d], M.P. Salerno[e], M. Ferraresso[a,f,*], F. Citterio[e], and J. Romagnoli[e]

[a]Kidney Transplantation, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy; [b]Microbiology, Fondazione Policlinico Universitario A. Gemelli, Rome, Italy; [c]Organ Transplantation, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Coppito, L'Aquila, Italy; [d]Infectious Diseases, Fondazione Policlinico Universitario A. Gemelli, Rome, Italy; [e]Kidney Transplantation, Fondazione Policlinico Universitario A. Gemelli, Rome, Italy; and [f]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

ABSTRACT

Background. Cytomegalovirus (CMV) disease represents a major cause of post-transplantation morbidity and mortality. To estimate the risk of infection and monitor response to antiviral therapy, current guidelines suggest combination of viral load monitoring with direct assessment of CMV-specific immune response. We used enzyme-linked immunospot (ELISpot) for the evaluation of CMV-specific T-cell response in kidney transplant recipients with CMV viremia and investigated how information gained could help manage CMV infection.

Methods. Seventeen patients on pre-emptive antiviral therapy and CMV quantitative polymerase chain reaction (qPCR) $\geq$500 copies/mL (first episode after transplantation) were assessed using ELISpot and divided into Weak (9 patients with baseline ELISpot $<$25 spot-forming colonies [SFCs]/200,000 peripheral blood mononuclear cells [PBMCs]) and Strong Responders (8 patients with baseline ELISpot $\geq$25 SFCs/200,000 PBMCs). CMV-specific T-cell response, infection severity, viral load, and antiviral therapy were prospectively recorded and compared between groups at 1, 2, and 24 months of follow-up.

Results. Demographic and transplant characteristics of Weak and Strong Responders were similar. No episodes of CMV disease were observed. Weak Responders were more likely to experience CMV syndrome (56% vs 36.5%) and late virus reactivation (56% vs 25%) than Strong Responders. Weak Responders showed higher baseline median viral loads (19,700 vs 9265 copies/mL) and needed antiviral therapy for longer (179 vs 59.5 days). T-cell response showed 2 main patterns: early and delayed.

Conclusions. ELISpot provides prognostic information about infection severity, risk of late reactivation, and response to therapy. Randomized trials, evaluating the need for antiviral therapy in kidney transplant recipients with asymptomatic infection and effective virus-specific T-cell immune response, are warranted.

DUE TO chronic exposure to powerful immunosuppressive agents, transplant recipients are extremely susceptible to drug-related toxicity, malignancies, and infections [1]. Among the latter, Cytomegalovirus (CMV) disease represents a major cause of morbidity and mortality

*Address correspondence to Mariano Ferraresso, MD, PhD, Associate Professor General Surgery, Department of Clinical Sciences and Community Health, University of Milan, Via Della Commenda 12, 20122 Milan, Italy. E-mail: mariano.ferraresso@unimi.it

0041-1345/17
http://dx.doi.org/10.1016/j.transproceed.2017.07.001

[2]. Main factors determining the risk of CMV infection after transplantation are as follows: donor/recipient CMV serostatus, net state of immunosuppression, and antiviral prophylaxis [2,3]. In the transplantation setting, CMV may occur as primary infection, secondary infection, or superinfection. In all these cases the virus can elicit a very aggressive immune response and cause profound immunomodulation [2–4]. To prevent tissue-invasive CMV disease, 2 strategies have been proposed: antiviral prophylaxis and pre-emptive therapy. The main benefit arising from pre-emptive therapy is that only a few patients receive antiviral medications and for a shorter period [5]. However, there is a need for frequent viral load monitoring and the risk of CMV disease in the early post-transplantation phase remains significant [6]. To date, randomized clinical trials comparing antiviral prophylaxis to pre-emptive antiviral therapy are scarce and report conflicting results [7]. Whole blood real-time quantitative polymerase chain reaction (qPCR) is the most reliable diagnostic test for CMV infection and it is routinely used for monitoring response to antiviral therapy in the immunocompromised host [8]. Yet, qPCR can only provide indirect information about the severity of the disease. According to current guidelines, viral load monitoring should be combined with clinical evaluation and direct assessment of CMV-specific immune response [9].

The enzyme-linked immunospot (ELISpot) assay is a functional immune-enzymatic test used to detect and count antigen-specific T and B lymphocytes [10–12]. Lymphocytes isolated from peripheral blood are incubated and stimulated by specific antigen lysates. Cytokines released by activated cells are directly bound by capture antibodies with emission of a colored and quantifiable signal. ELISpot can detect and characterize activated CMV-specific interferon-gamma–secreting T cells and provides direct evaluation of virus-specific cellular immune response. In combination with viral load monitoring, ELISpot may theoretically allow one to estimate the risk of infection, predict the clinical course of the disease, assess the need for antiviral therapy, and select patients suitable for more aggressive follow-up strategies [13–15].

The aims of this observational study were to evaluate the CMV-specific T-cell immune response in a cohort of kidney transplant recipients with CMV viremia and to investigate how information gained using ELISpot could improve the assessment and the management of post-transplantation CMV infection.

MATERIAL AND METHODS

Study Design and Patient Population

In this prospective single-center observational study with 2 years of follow-up, we enrolled 17 adult kidney transplant recipients with CMV viremia. The study was conducted on patients who signed an informed consent approved by the Catholic University of Rome ethics committee and performed according to the Helsinki Declaration. Inclusion criteria were as follows: (1) living or deceased donor kidney transplant; (2) recipient age ≥18 years; (3) pre-emptive antiviral therapy; and (4) first episode of CMV viremia after transplantation (qPCR on whole blood sample ≥500 copies/mL). Exclusion criteria were as follows: (1) recipient with undetectable CMV immunoglobulin (Ig)G at the time of transplantation; and (2) hematologic disorders affecting lymphocyte function. At the time of enrollment (baseline = first positive CMV qPCR), participants were assessed by clinical examination, full blood count, CMV qPCR, and ELISpot. Investigations were repeated at 1 and 2 months of follow-up. Extra assessments and tests were arranged as per clinical need. ELISpot was performed at baseline and 1 and 2 months of follow-up. According to their CMV-specific T-cell response (single ELISpot assay at baseline), patients were classified as Weak Responders (ELISpot <25 spot-forming colonies [SFCs]/200,000 peripheral blood mononuclear cells [PBMCs]) and Strong Responders (ELISpot ≥25 SFCs/200,000 PBMCs). Data regarding CMV infection and antiviral therapy were recorded for 2 years. The primary objective of the study was to investigate possible relationships between CMV-specific T-cell response and clinical course of the infection (severity, viral load, antiviral therapy, and late episodes of virus reactivation). We also analyzed how CMV-specific T-cell response changed over time and how information provided by ELISpot could be used to improve the management of post-transplantation CMV infection.

Clinical Evaluation

CMV infection was classified as: (1) asymptomatic infection: low-level (<10,000 copies/mL) CMV viremia without any clinical symptoms; (2) CMV syndrome: high-level viremia (≥10,000 copies/mL) or low-level viremia (<10,000 copies/mL) with fever, malaise, leukopenia (<3 × 1000 cell/μL), and/or thrombocytopenia (<150 × 1000 cell/μL); or (3) tissue-invasive CMV disease: symptoms or signs of organ involvement with virus and/or virus products detected in organ specimens or secretions.

CMV DNAemia Evaluation by Quantitative Real-Time PCR

DNA was extracted from 100 μL of anticoagulated whole blood using the NucliSENS easyMAG System (bioMérieux Corporate, Marcy-l'Étoile, France) in accordance with the manufacturer's instructions. An aliquot (5 μL) of the extracted DNA was processed on the ABI Prism 7300 Real-Time PCR System (Applied Biosystem, Foster City, Calif, United States) using the Q-CMV Real Time Complete Kit (Nanogen Advanced Diagnostics Srl, Turin, Italy). Primers and probes were located in the exon 4 region of the CMV Major Immediate Early Antigen (MIEA; HCMVUL123). The assay detection linear range was 500–5 × 10^6 copies/mL.

Immune Response Evaluation Using ELISpot Assay

Peripheral blood mononuclear cells (PBMCs) were separated using Ficoll-Hypaque (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) density gradient centrifugation. An aliquot of 2 × 10^5 T cells resuspended in RPMI-1640 Medium supplemented with 10% human albumin was incubated in duplicate in the anti-interferon-gamma-antibody–coated wells together with a CMV peptide mix (IE1 and pp65-UL83) for 20–24 hours at 37°C in a CO_2 incubator. When stimulated by the antigens, CMV-specific T cells released interferon-gamma, which was then bound by the antibody-coated wells. Cytokine release was detected by enzyme-labelled detection antibodies. Generated blue spots representing the area surrounding a single interferon-gamma–secreting T cell were counted using a computer-assisted image analysis system (AID ELISpot Reader System, Strassberg, Germany). As negative and positive controls we used cells