ImmunoTools *special* Award 2014

Alda Saldan
Microbiology and Virology Resident

Department of Molecular Medicine
University of Padova
Via Gabelli n.63
35121 Padova, ITALY

Evaluation of the antiviral cell-mediated immunity as a tool to predict CMV infections in the immunocompromised host

Human cytomegalovirus (CMV) is one of the most prominent pathogens, cause of morbidity and mortality, in immunocompromised patients such as solid-organ transplant recipients. Due to a lack in the host's cell-mediated immune response, uncontrolled viral replication can lead to graft damage and severe CMV disease. At present, antiviral prophylaxis and pre-emptive therapy represent the only strategies that can effectively reduce the occurrence of symptomatic CMV infection in transplant recipients. However, both these approaches are affected by important limitations. In fact, prolonged antiviral prophylaxis might expose the patient to the risk of drug toxicity, as well as leading to the selection of resistant viral strains. On the other hand, pre-emptive therapy does not protect against the CMV indirect effects, such as an increased incidence of allograft injury and rejection.

Cell-mediated immune response has been shown playing a pivotal role in controlling CMV replication[1]. Both $CD4^+$ and $CD8^+$ T-cells showing a T_H1 phenotype are required to solve an episode of CMV infection and maintain the viral latency. For this reason immunological monitoring of CMV-specific T-cell immunity has been recommended during the post-transplant period, in order to better determine the therapeutic strategies on an individual basis and early identify the patients at higher risk of CMV infection[2].

Previous studies of our group have already showed that an inverse correlation exists between CMV infection and specific T-cell immunity in solid organ[3,4,5,6] and haematopoietic stem cell transplanted patients[7]. Moreover, our findings indicated that patients with higher frequency of CMV-specific $CD4^+$ and $CD8^+$ T-cells are protected from subsequent events of CMV infection. Nevertheless we noticed that a discrete percentage of the subjects analysed, although exhibiting low levels of specific IFN-γ-secreting T-cells, had no episodes of CMV infection throughout the entire follow-up.

We speculate that other factors may intervene and contribute to the overall anti-CMV protection, like for instance innate immunity and other specific T-cell subsets.

The aim of the present study is to better dissect the anti-CMV cell-mediated immunity, extending the post-transplant immunological monitoring to the determination of mono- and bi-functional CMV-specific T-cells and Natural Killer (NK) cells. To this purpose, we will conduct a longitudinal study on a cohort of lung transplant recipients (LTx), upon obtainment of patients' informed written consent. At fixed time-points (0, +30, +60, +90, +180, +360 days after transplantation) we will determine both CMV viremia and antiviral cell-mediated immunity. CMV DNAemia will be assessed by Real Time PCR on IE1 gene, as reported elsewhere[8], whereas the immunological monitoring will be conducted by flow cytometry. In particular, after purification of peripheral blood mononuclear cells (PBMCs) by Ficoll centrifugation, we will stimulate the lymphocytes with CMV pp65 peptide pools and – in order to increase the detection of activated T-cells - by co-incubation with anti-CD28 and anti-CD49d antibodies. CMV-specific T-cells will be quantified analysing the expression of the CD3, CD4 and CD8 surface markers and the production of intracellular granzyme B and TNF-α. Similarly, expression of CD16 and CD56 and production of granzyme B and TNF-α will allow quantification of NK cells. Comparison between virological and immunological data is expected to highlight whether antiviral innate immunity (NK cells) and polifunctional CMV-specific T cells (i.e. granzymeB$^+$ TNF-α^+ T-cells) play a protective role against CMV infection.

References

1. Emery V.C., QJM, 2011
2. Kotton C.N. *et al*, Transplantation, 2013
3. Abate D. *et al*, JID, 2010
4. Abate D. *et al*, JCM, 2012
5. Costa C. *et al*, Int J Immunopathol Pharmacol, 2012
6. Abate D. *et al*, JCM, 2013
7. Abate D. *et al*, Transplantation, 2012
8. Mengoli C. *et al*, J Med Virol, 2004