Evaluation of virus-specific cellular immune response in transplant patients

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Author contributions: Costa C and Cavallo R designed and wrote the manuscript; Saldan A contributed on method description.

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Abstract

Virus-specific immune responses have a major impact on the outcome of the infection. Viral agents that are characterized by latency, such as herpesviruses and polyomaviruses, require a continuous immune control to reduce the extent of viral reactivation, as viral clearance cannot be accomplished, independently from the anti-viral treatment. In transplant patients, morbidity and mortality related to viral infections are significantly increased. In fact, the key steps of activation of T-cells are major target for anti-rejection immunosuppressive therapy and anti-viral immune response may be altered when infected cells and cellular effectors of immune response coexist in a transplanted organ. The role of cellular immune response in controlling viral replication and the main methods employed for its evaluation will be discussed. In particular, the main features, including both advantages and limitations, of available assays, including intracellular cytokine staining, major histocompatibility complex - multimer-based assays, Elispot assay, and QuantiFERON test, will be described. The potential applications of these assays in the transplant context will be discussed, particularly in relation to cytomegalovirus and polyomavirus BK infection. The relevance of introducing viro-immunological monitoring, beside virological monitoring, in order to identify the risk profile for viral infections in the transplant patients will allows for define a patient-tailored clinical management, particular in terms of modulation of immunosuppressive therapy and anti-viral administration.

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Key words: T-cell; Immune response; Viral replication; Interferon-γ; Transplantation

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INTRODUCTION

Virus-specific immune responses have a major impact on the outcome of the infection. Viral infections are usually followed by the complete clearance of the virus. However, in some cases, immune response is not able to eliminate completely the virus, thus the infection may become persistent. In general, cellular immune response plays a more relevant role in the viral clearance, whereas humoral immune response protects against the reinfection. Immune response may vary according to genetic substrate of the individual and the degree of immunocompetence.

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**Intracellular cytokine staining**

Most studies have analyzed virus-specific T-cell responses using ICS for IFN-γ using flow cytometry. Moreover, ICS allows for evaluation of polyfunctionality of T cells. Whole blood or isolated peripheral blood mono-

nuclear cells (PBMCs) are stimulated with virus-specific peptides or viral lysates. The assay is not restricted for human leukocyte antigen (HLA) when viral lysate is used and there is no need to know HLA type. Stimulated cells are stained with monoclonal antibodies directed against IFN-γ. The method is rapid, with a short incubation time and results being available within 24 h; it allows for identification of CD4+ and CD8+ T cells. The major drawbacks are the need for a flow cytometer and the lack of standardization. Among the advantages, there is the possibility to freeze the cells and send them to reference laboratory for testing.

**MHC - multimer-based assays**

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**Elispot**

The ELISPOT assay determines the number of T-cells secreting IFN-γ in specific response to a viral agent. Following stimulation with viral peptides or viral lysates, the produced IFN-γ is captured by a specific antibody, and then quantified using a labeled antibody. The assay evaluates both CD4+ and CD8+ responses and there is no need to know HLA, as well as to use a flow cytometer. There is the possibility to freeze the cells and send them to reference laboratory for testing. Among the disadvantages, the method requires a relatively high volume of blood (approximately 7-10 mL) and cannot differentiate between CD4+ and CD8+ cells. Although some approaches to standardize the assay, it is still not standardized. Results are available within 24-30 h. A mitogen control assay can determine general T-cell responsiveness. Commercially available kits for virus-specific ELISPOT assays are available (AID, Autoimmun Diagnostika GmbH, Strassberg, Germany).

**QuantIFERON-CMV**

This is a CMV-specific ELISA-based IFN-γ release assay and is commercially available (Cellestis Inc., Melbourne, Australia). It can be easily performed starting from low blood volume (3 mL) and results are available after

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**METHODS FOR EVALUATING VIRUS-

SPECIFIC CELLULAR IMMUNE RESPONSE**

Evaluation of virus-specific T-cell responses can be made by different methods (Table 1), including intracellular cytokine staining (ICS), major histocompatibility complex (MHC)-multimer-based assay, Enzyme-linked Immunospot (ELISPOT) assay and QuantIFERON-cytomegalovirus (CMV) assay (specific for CMV). Beside these virus-specific assays, it is also available a non-specific test, the ImmuKnow assay, that is used to evaluate the overall CD4+ immune response.

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**QuantIFERON-CMV**

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30-40 h. It evaluates only CD8+ responses. The assay may yield nonresponse results in the presence of global immunosuppression (nonresponse to mitogen) as in transplant patients. Test sensitivity decreases in lymphopenic patients because an appropriate number of T-cells are required for the production of IFN-γ.

**ImmunKnow assay**

The Cylex ImmunKnow assay (Cylex Inc., Columbia, MD, US) is a specific assay, which is commercially available. It measures the overall immune response and is indicative of immunosuppression. It determines the amount of ATP produced in response to whole-blood stimulation by an aspecific mitogen (phytohemagglutinin).

### ROLE OF VIRUS-SPECIFIC CELLULAR IMMUNE RESPONSE IN TRANSPLANT PATIENTS

T-cell responses, including both CD4+ and CD8+, are critically important for controlling viral replication. This has been demonstrated by the use of adoptive immunotherapy for treatment of CMV and EBV infections and by the higher frequency of viral reactivation in patients treated with anti-lymphocyte agents. Early reconstitution of cellular immune response prevents or reduces the duration of the infection, thus avoiding the onset of disease or relapses. On the other hand, a delayed or reduced response represents the pathogenic base for the occurrence of repeated episodes of infection and symptomatic disease (in the absence of anti-viral treatment)[7-11]. The role of cellular immune response in the context of organ transplantation has been studied particularly for CMV. In particular, it has been evidenced the basic role played by both CD4+ and CD8+ responses[9]. The CD8+ response is prevalent during the acute phase of infection and it provides for an immediate control of viral replication by the killing of cells in which CMV is replicating. The CD4+ response is fundamental for the long-term maintenance of antiviral control. In a study on solid-organ transplant recipients, it has been demonstrated that stable levels of CMV-specific CD4+ cells correlates with the absence of infectious complications, whereas in patients with unstable levels of specific-CD4+ cells, several episodes of viral reactivation occurred[10]. The median frequency of CMV-specific CD8+ cells was significantly higher in a group of 27 heart and lung transplant patients in cases that did not developed CMV-disease[11]. Moreover, in a study on 73 renal transplant recipients, also the median frequency of CMV-specific CD4+ cells was significantly higher in patients that did not developed CMV disease[12,13]. Gerna and coll. proposed the classification of transplant recipients into two groups in relation to the temporal profile for CMV-specific cellular immune response[14]. The Authors considered a group of early responders, in which reconstitution of cellular immune response occurred within 30 d posttransplantation, and a group of late responders, in which cellular immune response reconstitution occurred at > 30 d posttransplantation and/or was reduced. In these patients, the delay in CD4+ response appeared to be particularly critical[15].

Another virus for which specific cellular response has been investigated is polyomavirus BK. It has been evidenced that healthy BKV-seropositive individuals have CD4+ and CD8+ cells specific for BKV major antigens (including large T antigen and capsid viral protein VP1)[16]. The unbalance between viral replication and BKV-specific cellular immune response should represent the common denominator for the pathogenesis of polyomavirus-associated nephropathy, a viral-associated complication potentially leading to the lost of the transplanted organ and mainly occurring in renal transplant patients[17,18]. It has been evidenced that cellular response to large T antigen and VP1 is significantly higher in patients with decreasing viremia in comparison to those with increasing viremia[19]. Moreover, it seems that the level of immune response is correlated particularly to the administration of some immunosuppressive drugs. For example, in a study on kidney transplant patients *in vivo*, responses were inversely correlated with tacrolimus through levels, but not mycophenolate mofetil, prednisone or the overall immunosuppressive dosing[20]. However, the clinical role of BKV viro-immunological monitoring in renal transplant recipient needs to be further investigated, in particular it is likely that it could represent an approach to modulate immunosuppression.

An interesting issue in the context of protective immunity is represented by the role of mucosal immunization. As mucosal surfaces represent the major entry for many human pathogens (including HSV, HIV, respiratory viruses, as well as mycobacteria), induction of mucosal immunity is represented by the role of mucosal immunization. As mucosal surfaces represent the major entry for many human pathogens (including HSV, HIV, respiratory viruses, as well as mycobacteria), induction of mucosal immunity is represented by the role of mucosal immunization.

### Table 1 Methods for evaluating cellular immune response

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MHC: Major histocompatibility complex; HLA: Human leukocyte antigen.
immune system, including both innate and adaptive responses (CD4+ T helper cells, Th17 cells, high avidity CD8+ cytotoxic T lymphocytes, as well as IgA and IgG1 neutralizing antibodies) seem required for an effective protection against pathogens that lead to chronic infections[18,19].

CONCLUSION

The increase in the number of transplant patients and the use of deeply immunosuppressive drugs have lead to the emergence of viral infections that may influence the outcome of these patients. Beside the adoption of protocols for close virological monitoring, several studies have underlined the role of viro-immunological monitoring in the evaluation of patient's risk of infection and disease, decision on treatment by modulating immunosuppression and/or using antiviral, and adoption of other diagnostic strategies. There is no defined method to evaluate the cellular immune response in transplant recipients, as at the moment no assay is standardized and further studies, particularly on procedures, quality controls and references, as well as interpretation of results are required to standardize. However, ELISPOT assay is gaining increasing recognition due to the potential to evaluate both CD4+ and CD8+ responses and other favoring features. Further studies on the clinical role of cellular immune responses are required, in particular with the aim of define modes and temporal profile for viro-immunological monitoring in different transplant contexts and for different viral agents.

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