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Review

Approaches for monitoring of non virus-specific and virus-specific T-cell response in solid organ transplantation and their clinical applications

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ABSTRACT

Opportunistic viral infections are still a major complication following solid organ transplantation. Immune monitoring may allow the identification of patients at risk of infection and, eventually, the modulation of immunosuppressive strategies. Immune monitoring can be performed using virus-specific and non virus-specific assays. This article describes and summarizes the pros and cons of the different technical approaches. Among the assays based on non virus-specific antigens, the enumeration of T-cell subsets, the quantification of cytokines and chemokines and the quantification of intracellular adenosine triphosphate following mitogen stimulation are described and their clinical applications to determine the risk for viral infection are discussed. In addition, current specific methods available for monitoring viral-specific T-cell responses are summarized, such as peptide-MHC multimer staining, intracellular cytokine staining, enzyme-linked immunospot and virus-specific IFN-γ ELISA assays, and their clinical applications to determine the individual risk for opportunistic viral infections with human cytomegalovirus, Epstein-Barr virus and polyoma BK virus are discussed. The standardization of the procedure, the choice of the antigen(s) and the criteria to define cut-off values for positive responses are needed for some of these approaches before their implementation in the clinic. Nevertheless, immune monitoring combined with virological monitoring in transplant recipients is increasingly regarded as a helpful tool to identify patients at risk of infection as well as to assess treatment efficacy.

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Abbreviations: SOT, solid organ transplantation; OI, opportunistic infections; HCMV, human cytomegalovirus; EBV, Epstein-Barr virus; BKV, polyoma BK virus; KTR, kidney transplant recipients; LTR, lung transplant recipients; BAL, bronchoalveolar lavage; ATP, adenosine triphosphate; PHA, phytohemagglutinin; pMHC, peptide-MHC; ICS, intracellular cytokine staining; ELISPOT, enzyme-linked immunospot assay; PBMC, peripheral blood mononuclear cells; TCR, T-cell receptor; PTLD, post-transplant lymphoproliferative disorders; CSA, cytokine secretion assays.

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1. Introduction

Immunosuppressive therapies are essential in solid organ transplantation (SOT) but may increase the risk for opportunistic infections (OI), especially induction therapies including lymphocyte depleting agents [1]. Thus, infections are still a major complication after transplantation. In recent years there has been a growing interest in the development of immune monitoring approaches that may allow the identification of infectious risk in SOT recipients and, eventually, the modulation of immunosuppressive strategies. Immune monitoring can be performed using non virus-specific or virus-specific approaches. In this review, the current assays used for monitoring the immune status after SOT are described, summarizing the pros and cons of these approaches as well as their clinical applications to determine the risk for viral infections and the individual risk for viral OI such as human cytomegalovirus (HCMV), Epstein-Barr virus (EBV) and polyoma BK virus (BKV). While numerous studies have focused on immune monitoring for HCMV, monitoring of EBV- and BKV-specific T-cell responses after transplantation remains largely unexplored.

2. Non virus-specific approaches and their clinical applications

A number of such approaches are currently proposed (Table 1).

2.1. Enumeration of T-cell subsets

Measurement of T-cell subsets in peripheral blood by flow cytometry represents a simple and fast assay that can be performed routinely in a fully automated procedure. Whole blood is stained with a combination of fluorophore-labeled monoclonal antibodies (mainly anti-CD3, CD45, CD4 and CD8 surface molecules) and after lysis of red cells, $CD4^+$ and $CD8^+$ T-cell subsets are analyzed using flow-count fluorospheres.

Monitoring of T-cell subsets remains essential for the clinical management of HIV-infected patients [2]. Few studies have evaluated the kinetics of T-lymphocyte subsets and their association with infections after transplantation [3–6]. Patients with low $CD4^+$ T-cell counts at 1 month after heart transplantation showed poor recovery of $CD4^+$ T cells and developed OI, while low (and poor recovery of) $CD8^+$ T-cell counts were associated with the risk of OI in kidney transplant recipients (KTR) [4]. Similarly, a more recent study has confirmed that low T-cell counts at month 1 post-transplant predicts the subsequent occurrence of OI in KTR [6]. Thus, by this approach, patients at risk of developing OI could be identified as early as 1 month post-transplant. Although prospective studies are still needed to validate this method, measurement of T-cell counts may represent a simple and feasible tool for early risk stratification.

2.2. Quantification of cytokines and chemokines

ELISA is the most widely used method for cytokine or chemokine quantification but it measures one analyte at a time in a given sample. Recent developments enable simultaneous quantification of multiple cytokines/chemokines. These assays are available in several formats based on the use of bead sets and flow cytometry (also known as bead-based multiplex assays). Each bead in the array has a unique fluorescence intensity and is coated with a specific capture antibody. The sample is incubated with a combination of different beads and a mixture of fluorescence-conjugated detection antibodies and subsequently acquired on a flow cytometer [7].

Studies have evaluated the possible value of monitoring cytokine production in SOT recipients. One study suggests that in lung transplant recipients (LTR) IL-10 (using ELISA) in bronchoalveolar lavage (BAL) and/or plasma could be a useful parameter to monitor HCMV clearance, which was prolonged in LTR with detectable IL-10 in comparison with LTR with undetectable IL-10 (median 130 days vs. 58 days, respectively) [8]. In another study (using a bead-based multiplex assay), levels of IL-10 in BAL samples from LTR with HCMV infections remained low with an increase of other pro-inflammatory cytokines tested (IL-1β, IL-6, IL-8, TNF-α and MIP-1β) [9].

Other groups have focused on chemokines. Jackson et al. [10], using a solid-phase bead-array assay for Luminex platform, found that urine CXCL9 and CXCL10 were significantly elevated in KTR experiencing BKV infection but not in KTR without infection. However, CXCL10 (using ELISA) in BAL samples from LTR did not exhibit a significant association with OI post-transplantation [11]. By evaluating plasma markers of inflammation at a baseline time point by ELISA, Rollag et al. [12] found that SOT recipients with detectable HCMV DNA in the follow-up had significantly higher levels of CXCL10 than patients with undetectable HCMV DNA. In another study, high levels of CXCL16 (using ELISA) was found in BAL samples from LTR with HCMV disease compared with those detected in patients with asymptomatic HCMV replication [13]. Overall, multicenter evaluation of standardized protocols is required to assess the clinical utility of cytokine/chemokine monitoring after transplantation and also to investigate the impact of immunosuppressive treatments on cytokine or chemokine production.

2.3. ImmuKnow assay

The ImmuKnow assay (formerly by Cylex Inc., Columbia, MD, USA and now by Viracor-IBT Laboratories, Lee's Summit, MO, USA) is a FDA-approved test that measures the intracellular concentration of adenosine triphosphate (ATP) from $CD4^+$ T cells after nonspecific mitogen [phytohemagglutinin (PHA)] stimulation. Whole blood is incubated with or without PHA overnight. $CD4^+$ cells are then selected by monoclonal antibody-coated magnetic beads and lysed to release of intracellular ATP and the addition of