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Significance of immune cell function monitoring in renal transplantation after Thymoglobulin induction therapy

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ABSTRACT

Monitoring of immune status in transplant recipients is essential for predicting the risk of rejection or infection. In this study, we assessed the significance of immune cell function in 76 renal allograft recipients after Thymoglobulin induction and initiation of maintenance immunosuppression. Using the Immuknow (Cylex Inc) assay, the amount of adenosine triphosphate (ATP) produced by CD4+ cells in response to phytohemagglutinin (PHA) was measured in patients whole blood. In parallel, the frequency and phenotype of CD4+ T cells were determined by flow cytometry. The Immuknow assay yielded paradoxically high ATP values during the first 3 months post-transplantation, despite very low CD4+ T cell counts. High ATP values were caused by peripheral blood myeloid cells, did not predict rejection, and occurred primarily in transplant recipients who received darbepoietin ($p = 0.017$). CD4+ T cells displayed predominantly an activated/memory phenotype and comprised a subpopulation of CD25+FOXP3+ cells. Over the first 5 months post-transplantation, mean ATP activity gradually decreased, whereas CD4+ T cell counts slowly increased. Low ATP values were predictive of infection ($p = 0.002$). Thus Immuknow results need to be interpreted with caution in patients receiving Thymoglobulin induction therapy. Although low ATP levels identify patients at increased risk for infection, high ATP values fail to correlate with rejection and do not justify increased immunosuppression.

1. Introduction

Extensive evidence indicates that T lymphocytes play a key role in allograft rejection [1–3]. T cells can recognize allogeneic human leukocyte antigens either directly, as whole molecules expressed by donor cells, or indirectly, as peptides processed and presented by autologous antigen presenting cells [4,5]. In experimental and clinical renal transplantation, T lymphocytes have been identified as a predominant cell type infiltrating renal allografts during acute cellular rejection [6]. It has been demonstrated that alloreactive CD4+ T helper cells produce the cytokines required for the activation and differentiation of CD8+ T cells, whereas allospecific CD8+ effector T cells cause parenchymal cell destruction [6,7].

Because they are key mediators of allograft rejection, T cells represent a major target for induction therapy and maintenance immunosuppression. Induction therapy using Thymoglobulin or Alemtuzumab causes a drastic and rapid reduction of the number of lymphocytes, and it has been suggested that this approach may induce transplantation tolerance [8,9]. Although tolerance was successfully achieved in rodent models, it remains an elusive goal in clinical transplantation [10]. A high incidence of early acute rejection was reported in renal transplant recipients receiving vigorous T-cell depleting therapy and no maintenance immunosuppression [11]. Rejection in this setting is characterized by predominant monocyte/macrophage infiltrates and is associated with substantial renal dysfunction. Soluble factors, such as monocyte colony stimulating factor, released by renal tubular cells in response to ischemia-reperfusion injury, were shown to stimulate monocytes and to recruit them at the allograft site [12,13].

The view that lymphocyte depletion agents may induce tolerance in patients has been further challenged by reports describing the phenotypic and functional features of post-depletion T cells [10,14,15]. Such reports showed that, although depletion agents cause a drastic reduction of lymphocyte numbers, these agents do not totally eliminate T lymphocytes. Furthermore, T cells that survived alemtuzumab or Thymoglobulin treatment display a memory phenotype and are immunologically competent. Based on these data, it has been proposed that a relatively small number of T cells may initiate rejection via alloantigen-induced activation, and that

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this effect may be amplified by activation of monocytes and release of cytokines that impair parenchymal cell function [16].

Although lymphocyte depletion therapy does not induce tolerance in clinical transplantation, it results in a lower incidence of early acute rejection and reduces the need for chronic administration of high doses of immunosuppressive drugs [17–19]. However, the use of Thymoglobulin for induction therapy has been shown to increase the risk of infection and malignancy [20–22]. In renal transplantation, reactivation of BK virus is of particular concern because of BK virus–associated renal allograft nephropathy, which has emerged as an important cause of graft loss [23,24]. The risk of infection or rejection in patients treated with Thymoglobulin is in part dependent on the type of immunosuppression considered for long-term therapy after transplantation [22]. It is therefore obvious that monitoring of the patient's immune status and tailoring of immunosuppression according to individual needs are instrumental in maintaining the delicate balance among rejection, infection, and quiescent immune status.

Assessment of immune cell profiles in patients who received Thymoglobulin has been difficult because of persistently low T-cell counts. Recently, the Immuknow assay (Cylex, Columbia, MD) has been used to assess cell-mediated immunity in transplant recipients [25–28]. This test measures the amount of adenosine triphosphate (ATP) produced by CD4+ T cells from whole blood after stimulation with phytohemagglutinin (PHA). It has been reported that low ATP activity occurs in patients at increased risk for infections, whereas high ATP levels predict rejection [25].

In this study, we monitored immune cell function in 76 renal transplant recipients receiving Thymoglobulin induction therapy and maintenance immunosuppression. Although we confirm the usefulness of the Immuknow test for identifying patients at increased risk of infection, we now report that Immuknow does not predict rejection. Most importantly, we emphasize that Immuknow results need to be interpreted with caution in patients with low CD4+ T cell counts, because the ATP release does not reflect the activation status of CD4+ T cells but rather the contribution of myeloid cells from the patients' peripheral blood.

2. Subjects and methods

2.1. Blood samples

Peripheral blood samples were obtained from 76 patients who received kidney allografts at Columbia University Medical Center between November 1, 2007, and April 30, 2008. Control blood samples were obtained from 65 healthy volunteers. The study was performed according to an approved institutional review board protocol.

2.2. Testing of immune cell function

Immune cell function was measured using the ImmunKnow assay (Cylex Inc., Columbia, MD), a Food and Drug Administration (FDA)–approved test. Briefly, sodium heparin anti-coagulated blood (250 μl per reaction) was incubated with and without PHA for 15–18 hours at 37°C. CD4+ cells were then positively selected using magnetic beads coated with an anti-CD4 monoclonal antibody (Dynal, Oslow, Norway). These CD4 beads were provided by Cylex Inc., as part of the Immuknow kit. Bead-isolated cells were lysed and the amount of ATP released was measured by luminescence, using the luciferin/luciferase detection system (Cylex Inc.). Results were expressed as nanograms ATP per milliliter of blood.

2.3. Flow cytometry

Immunophenotypic characterization of peripheral blood T cells was performed using multicolor flow cytometry. The following monoclonal antibodies were used for cell surface staining: anti-CD3, -CD4, -CD8, -CD19, -CD16/56, -CD25, -CD45RO, -CD45RA, -CD14, and -CD5 (BD Bioscience, San Jose, CA). Anti-myeloperoxidase (anti-MPO; Beckman Coulter, Miami, FL) and anti-FoxP3 (eBioscience, San Diego, CA) antibodies were used for intracellular staining, according to the manufacturers' instructions. Cells were analyzed on a FACSCalibur instrument, using CellQuest Pro software (BD Bioscience, San Jose, CA). Determination of absolute cell counts was performed using TruCount (BD Bioscience) single-platform flow cytometry.

2.4. Induction therapy, maintenance immunosuppression, and prophylaxis against infection

Thymoglobulin (Genzyme, Cambridge, MA) was administered intravenously at 1.5 mg per kilogram of body weight for 4 consecutive days. Doses were reduced in cases of severe thrombocytopenia or neutropenia. Maintenance immunsuppression consisted of tacrolimus, mycophenolpate mofetil, and steroid taper. Prophylaxis against infections consisted of Valcyte (anti-viral), Bactrim (anti-bacterial), and Nystatin (anti-fungal).

2.5. Diagnosis of infection and rejection

All infections required hospitalization. Urinary tract infections (UTI) were confirmed by positive urine cultures, whereas BK and cytomegalovirus (CMV) infections were confirmed by positive PCR results in the serum.

Biopsies were performed when graft dysfunction occurred. Renal allograft biopsy samples were routinely processed for light microscopy and immunofluorescence, including C4d staining. Banff criteria were used to establish and grade the diagnosis of acute cellular or antibody-mediated rejection [29].

2.6. Statistical analysis

The *t* test was used for comparison of means between groups. To analyze the accuracy of the Immuknow test in predicting infection, the receiver operating characteristic (ROC) curve was generated, using SPSS software (SPSS Inc., Chicago, IL).

3. Results

3.1. Dynamics of immune cell function after induction therapy with Thymoglobulin

The Immuknow assay was used for studying immune cell function in 333 samples of blood obtained from 76 renal transplant recipients. Demographic characteristics of the patient population are presented in Table 1. All patients received Thymoglobulin induction therapy.

According to the manufacturer's instructions, immune cell reactivity levels measured with Immuknow in healthy individuals can

Table 1
Patient information

Patient characteristics	
No. of patients	76
Age (y), mean ± SD	50 ± 14
Gender, *n* (%)	
Men	45 (59%)
Women	31 (41%)
First transplantation, *n* (%)	65 (86%)
Retransplantation, *n* (%)	11 (14%)
PRA, *n* (%)	
<10%	67 (88%)
≥10%	9 (12%)
Acute cellular and/or humoral rejection, *n* (%)	11 (14%)
Infection, *n* (%)	18 (24%)
UTI	13
BK virus infection	4
CMV infection	1
Follow-up (mo, median)	10

BK, BK human polyomavirus; CMV, cytomegalovirus; PRA, panel reactive antibodies; SD, standard deviation; UTI, urinary tract infection.