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Affects of immunosuppression on circulating dendritic cells: An adjunct to therapeutic drug monitoring after heart transplantation

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Abstract

Objectives: Recent evidence emerges dendritic cells (DCs) as pharmacological targets of immunosuppressive drugs. Therefore, in this study we monitored DCs in peripheral blood to compare the effects of calcineurin inhibitors (CNI: cyclosporine, tacrolimus) and mammalian target of rapamycin inhibitors (sirolimus, SRL, everolimus, ERL) basis-immunosuppressive therapies in human heart transplanted (HTx) recipients.

Methods: We compared HTx recipients which were converted from either CNI to ERL (severe renal dysfunction, $n=8$), or from SRL to ERL (approval of ERL for HTx, $n=8$) with 20 healthy human controls. Twenty four after the last CNI or SRL dose recipients were treated with ERL/BID on days 1–3. Peripheral blood was collected at trough in the morning before and on day 4 after conversion. Percentages of positive myeloid and plasmacytoid DC (m and pDC) subsets in peripheral blood were analysed by flow cytometry. The status of maturation was further characterised by flow cytometry analysis of % expression of CD83 and % expression of various intracellular cytokines (IL-1β, TNF-α, IL-8, IL-12), respectively.

Results: HTx recipients had higher % positive mDCs regardless the immunosuppressive therapy compared to controls ($p<0.05$). Whereas, % positive pDCs were only significantly lower in recipients converted from CNI to ERL compared to controls ($p<0.05$). The data consolidate the finding that the subset ratio pDCs/mDCs was lower in recipients compared to controls. But after conversion from CNI or SRL to ERL the ratio increased towards pDCs. Percentages of expression of CD83 on mDCs were not different among the recipient groups and controls. Recipients with CNI and SRL had higher % expression of IL-12 and lower % expression of IL-1β compared to controls ($p<0.05$). However, after conversion to ERL % expression of both IL-12 and IL-1β returned to control values in both groups.

Conclusions: The results showed that analysis of immunosuppression of circulating DCs in peripheral blood may be an adjunct to therapeutic drug monitoring to optimize immunosuppressive therapy after HTx.

Keywords: Dendritic cells; Calcineurin inhibitors; mTOR inhibitors; Transplantation; Immunosuppression; Flow cytometry

1. Introduction

Despite the advances of the introduction of new potent immunosuppressants into the clinic the control of

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allograft rejection after heart transplantation (HTx) remains problematic [1].

The immune response to allograft rejection is a complex network of interactions between antigen-presenting cells (APCs) and T cells.

Dendritic cells (DCs) are potent APCs which are derived from bone marrow progenitors and after maturation they acquire the ability to stimulate naïve T cells to differentiate into T-helper (Th)-1 and -2 cells. Circulating DCs in peripheral human blood are distinct into myeloid and plasmacytoid DCs (m and pDCs) which polarize either Th-1 or Th-2 cells, respectively [2]. Maturation of DCs is associated with changes in the phenotype and function of DCs, including up-regulation of co-stimulatory and adhesion molecules, production of chemokines and inflammatory cytokines [3].

Standard chronic basis-immunosuppressive drug therapy for prevention of allograft rejection currently consisted of either one of the calcineurin inhibitors (CNI), cyclosporine (CsA) or tacrolimus (TRL), or one of the mammalian target of rapamycin inhibitors (mTORI), sirolimus (SRL) or its derivate everolimus (ERL), plus an adjunctive anti-proliferative agent, with or without steroids [4].

Over the recent years a large number of investigations studied the effects of CsA, TRL and SRL on DCs phenotype and their functions in peripheral blood mononuclear cells (PBMC) or mixed lymphocyte reactions (MLR) of blood obtained from healthy human volunteers or drawn from animals in different experimental models [5].

Despite these considerable results, up to date there are none or only few reports about the effect of a mTORI or CNI basis-immunosuppression, respectively, on DCs phenotypes or functions in peripheral blood of human transplant recipients. Thus, only one study in HTx recipients shows that CNI therapy inhibits maturation of DC subsets one week after transplantation [6].

However, studies in liver transplanted (LTx) recipients demonstrate the relevance to assess DC phenotype and function in the long-term of CNI therapy post-transplant [7–9].

Therefore in this study, we assessed the DC phenotype and the status of maturation in peripheral blood of chronically immunosuppressed stable HTx recipients for a collective comparison between CNI and mTORI maintenance therapy and human healthy controls.

2. Materials and methods

2.1. Study groups

The study protocol was approved by the local medical ethical committee on human research of the University Leipzig (No. 2004-05-WGK-3). All recipients without rejection or infection were recruited from the Department of Cardiac Surgery, University Leipzig, Heart Center (Leipzig, Germany) and gave informal consent before entering the study. Recipients of this study were on a basis-immunosuppression either with CsA ($n=4$), TRL ($n=4$) or with SRL ($n=8$) before conversion to ERL. Adjunctive immunosuppressive drugs consisted of mycophenolate mofetil (MMF) and of steroids before and after drug conversion (Table 1).

Blood was collected from all recipients before drug administration on the day before and on day 4 after conversion of drug therapy in the morning. Twenty healthy human volunteers who received no medication severed as control subjects. All recipients were monitored in the clinic during drug conversion to treat recipients with ERL as a basis-immunosuppressive drug. Day 4 was chosen before recipients were discharged from the

Table 1
Clinical characteristics of the study subjects

		Conversion			
	HC[a]	CNI[b] to ERL[c]		SRL[d] to ERL	
Age, years	53.1±1.4	58.3±1.7		62.0±1.7	
Gender, m/f	13/7	8/0		7/1	
Time after HTx, months	–	51.1±5.6		55.6±6.2	
Steroids, no. of recipients	–	3	3	4	4
MPA[e] level, μg/ml	–	2.5±0.7	3.1±0.9	3.5±0.6	3.3±0.5
WBC[f], 10^3 cells/μl	5977±292	5131±308	5006±236	5735±326	5975±277
% Lymphocytes	34.8±1.5	21.3±1.4*	23.7±1.3*	17.6±1.5*	21.8±2.1*
% Monocytes	7.7±0.4	11.1±1.4	11.3±0.7	9.9±0.7	7.8±0.5

Statistical significant differences ($p<0.05$, Mann Whitney rank sum test) between HTx recipients and HC are indicated (*).

[a] HC, human healthy controls.

[b] CNI, calcineurin inhibitor; blood CsA and TRL concentrations were measure with EMIT.

[c] ERL, everolimus; blood concentrations were measured with LC–MS.

[d] SRL, sirolimus; blood concentrations were measured with LC–MS.

[e] MPA, mycophenolic acid, plasma concentrations were measured with HPLC.

[f] WBC, white blood cell counts.