



# Tissue Expression of Ligand to Programmed Death Receptor 1 (PD L1) in Endomyocardial Biopsies of Patients after Heart Transplantation - Association with Allograft Rejection, Pilot Study

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## Abstract

**Background:** Data suggest that PDL1 expression in donor tissue can down-regulate recipient alloreactive T cell responses by interaction with the PD-1 receptor expressed on alloreactive T cells, thus limiting the local inflammation leading to rejection and vasculopathy. The aim of the study was to assess the relation between PDL-1 expression in Endomyocardial Biopsies (EMBs) of adult patients after heart transplantation and allograft rejection.

**Patients and Methods:** The study included 15 patients with diagnosed Antibody-Mediated Rejection (AMR), 12 patients with diagnosed grade 2 R Acute Cellular Rejection (ACR), and 16 patients with no AMR or ACR. PDL-1 and C4d immunohistochemical stainings were performed in all EMBs of transplanted hearts included in the study. Graft rejection was evaluated according to ISHLT criteria. Patients with diagnosed allograft rejection had also assessed subsequent control biopsies until the microscopic image normalized. The non-parametric Student's t test was used to compare the results.

**Results:** Six patients were diagnosed with AMR 1 (I), 4 with AMR 1 (H) and 5 with AMR 2. Among all 15 patients with AMR, 5 presented additionally grade 1R ACR features. The difference in PDL-1 expression within the groups was very different from the rest (patients with simultaneous AMR and ACR had a higher percentage of marker tissue expression), while the individual groups did not differ statistically from each other. In biopsies with higher PD L1 expression, lesions were already minimized at the next control biopsy.

**Conclusion:** It seems that higher tissue expression of PDL-1 may be associated with a faster graft rejection resolution; however, it is not related to the type of rejection.

**Keywords:** PDL-1; Heart transplantation; Allograft tolerance

## Background

Transplantation of parenchymal organs, mainly liver, kidneys and heart, is currently recognized as the most effective method of treating end-stage failure of these organs. The quality of life of transplant patients is almost the same as before surgery. Unfortunately, we are still unable to fully control the immune processes that lead to the loss of graft, despite the use of immunosuppressive agents, new antibodies, fusion proteins and new low molecular weight drugs. The problem of induction of transplant tolerance, i.e. the induction of permanent acceptance of the transplanted organ, without the need of chronic immunosuppressive therapy (or with its minimization), while maintaining immunocompetence, is an important issue in the treatment of end-stage organ failure by transplanting their allogeneic counterparts. Immune regulators are responsible for the phenomenon of transplant tolerance: macrophages, CD4 CD25 FOXP3 (Treg) and B lymphocytes (Breg) [1-3]. In the case of a subgroup of regulatory cells belonging to macrophages, i.e. alternatively activated M2 macrophages, it was determined that their mechanism of action is to inhibit the proliferation of polyclonal, activated allogeneic T lymphocytes [4]. This effect is associated with induction of Indolamine 2,3-Dioxygenase (IDO), which leads to increase expression of programmed death ligand 1 (PD-L1 - Programmed Death Ligand) [5]. The receptor for this protein is the PD-1 receptor, or the

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CD279 protein. It is a transmembrane glycoprotein belonging to the CD28: B7 family encoded in humans by the *pdc1* gene whose locus is 2q37.3. This protein is induced on CD4 and CD8 T cells, B cells, NK cells, monocytes and activated dendritic cells. It has two ligands, PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273), present on antigen presenting cells. PD-1 ligation with PD-L1 or PD-L2 inhibits the signal transmitted from activated T lymphocytes, and also reduces the expression of pro-inflammatory cytokines and anti-apoptotic molecules. All these phenomena lead to inhibition of the activation of the immune system, which enables lymphocyte immune tolerance in relation to, among others for transplanted organs [6-9]. It is now believed that tissue expression of PDL-1 in a donor organ is necessary to prevent chronic allograft rejection and other *in situ* graft diseases. Few studies have been performed on *in vivo* transplanted organs. Available literature data come only from mouse model analysis [10-14]. Kaul et al. [13] hypothesized that the increased interstitial expression of PDL-1 in a transplanted heart in mice in which cellular rejection features are present would favor a faster resolution of this process. To date, there are no human studies in this area, while work on the relationship between the PD-1/PDL-1 system and humoral rejection has not yet been carried out. The aim of our study was to evaluate the relationship between PDL-1 antigen expression in endomyocardial biopsies of patients after heart transplantation, and the development/resolution of Acute Cellular Rejection (ACR) and humoral rejection (AMR). This is the first study ever conducted on human tissues. It is also a pilot study and is an introduction to a trial on a larger group of patients.

## Patients and Methods

### Patients

The retrospective analysis of 60 Endomyocardial Biopsies (EMBs) from 43 adult patients, 12 (28%) female and 31 (72%) males, aged from 17 up to 78 years old, who underwent heart transplantation in one institution (The Department of Cardiac Failure and Transplantation, Institute of Cardiology, Warsaw). The immunosuppression protocol for heart recipients consists of combination therapy with calcineurin inhibitor - Cyclosporine (CsA) has been introduced till 2009, currently Tacrolimus is applied, Mycophenolate Mofetil (MMF) and corticosteroids. These medications are obligatory during first year after transplantation. If patients' general condition remains good and no rejection is observed, doses of each drug are reduced. In recipients who had presented with kidney failure and/or needed assist devices before transplantation, IL-2 inhibitors are given as induction of immunosuppression. ACR grade 2R is treated with increased doses of either Encorton or Methylprednisolone. AMR alone in patients without clinical symptoms is not treated, "watchful waiting" attitude with additional EMB is optimal management. In "Mixed Rejection" (AMR and ACR) cases increased doses of corticosteroids or exchange of MMF into Everolimus are usually applied.

### Endomyocardial biopsy (EMB)

All EMBs were pathologically processed, analyzed and stored in one institution (The Department of Pathology, The Children's Memorial Health Institute, Warsaw). Each myocardial specimen comprised of 1 to 5 tissue samples 2 mm to 5 mm in size which had been taken from right ventricle with a biptome percutaneously. All samples were fixed in 4% formalin and embedded in paraffin. The paraffin 4  $\mu$ m sections were routinely stained with hematoxylin and eosin (H and E). To the study 15 patients with diagnosed Antibody-Mediated Rejection (AMR), 12 patients with diagnosed grade 2 R

Acute Cellular Rejection (ACR), and 16 patients with no AMR or ACR were enrolled. In patients diagnosed with allograft rejection (AMR or ACR), biopsies with both histopathological changes and subsequent control biopsies were assessed until the microscopic image normalizes. In all EMBs immunohistochemical stainings were performed using anti-PDL-1 antibody (VENTANA PD-L1 (SP263) Assay) and C4d (Biomedica groupe, dilution 1:40). Biopsies and additional stainings were evaluated in a light microscope by two independent pathologists. The PDL-1 reaction was expressed as a percentage of the available myocardial tissue that showed positive marker expression. The degree of ACR and AMR was assessed according to ISHLT criteria. The non-parametric Student's t test was used to compare the results.

## Results

Six patients were diagnosed with AMR 1 (I), 4 with AMR 1 (H) and 5 with AMR 2. Among all 15 patients with AMR, 5 had additionally grade 1R ACR features. The difference in PDL-1 expression within the groups was very different from the rest (patients with simultaneous AMR and ACR had a higher percentage of marker tissue expression), while the individual groups did not differ statistically from each other. In biopsies with higher PD L1 expression, lesions were already minimized at the next control biopsy.

## Discussion

Programmed Death receptor 1 (PD-1) after binding to PD-L1 ligands inhibits the activation of the immune system, which allows immune tolerance of lymphocytes to transplanted organs [6,10]. In oncological treatment, monoclonal antibodies associated with the PD-1/PD-L system are used mainly in patients with non-small cell lung cancer [15-17]. The results of studies in mice presented in the literature indicate that increased tissue expression of PDL-1 in the transplanted heart is associated with a faster descent of the process of acute cellular rejection [13]. Our preliminary study is the first one performed in human patients, which focused on the relationship between the PD-1/PDL-1 expression and type of rejection. To date, only the relationship between tissue PDL-1 and ACR expression and/or chronic rejection (graft vasculopathy) has been studied [10,11,18]. We are the first to assess PDL-1 expression in relation to different types of allograft rejection *in vivo* (Figure 1). The biopsies selected for the study came from 2 weeks to 11 years after transplantation, which allowed us to analyze whether the time after transplantation affects PDL-1 expression. Unfortunately, in humans, in contrast to

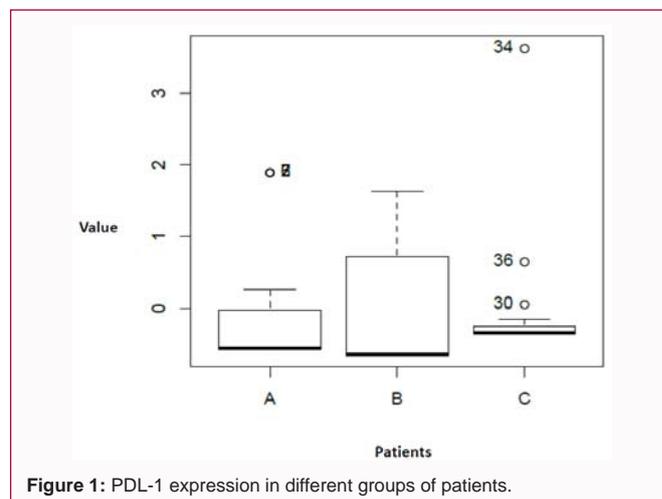


Figure 1: PDL-1 expression in different groups of patients.

mouse models, the immune response is influenced by many factors, including the immunosuppressive treatment used, which could affect the results. The patient with diagnosed AMR 1 (I) and zero PDL-1 expression in two consecutive biopsies maintained a positive C4d reaction, and developed the features of ACR grade 1 in forth biopsy. The lesions disappeared only in 5 biopsies. While in a patient with concomitant AMR 1 (I) and ACR grade 1 and a relatively high percentage of PDL-1, the C4d reaction normalized already in the next biopsy, while the symptoms of acute cellular rejection resolved in the third biopsy. However, these are individual cases. We were unable to confirm statistically significant differences between the groups by any test, whether parametric or nonparametric. The results obtained in individual groups were very different, but the general trend was similar in all groups. Based on individual observations, it seems that higher tissue expression of PDL-1 may be associated with a faster graft rejection resolution; however, it is not related to the type of rejection. It would be consistent with observation by Kaul et al. [13] we also did not observe a difference in PDL-1 expression depending on the time after transplantation. However, our group is unique - none of the patients were clinically diagnosed with vasculopathy, while relatively many patients (6) were diagnosed with AMR 1 (I) many years after transplantation (in one patient 11 years after surgery). This is a rare phenomenon because the probability of rejection is highest in first year. Our study is experimental; we were the first in the world to determine PDL-1 expression in adult patients after heart transplantation. We selected groups based on the diagnosis of different types of rejections, which has never been done before. However, it is difficult to draw conclusions, as our group is small, but in larger study it may appear meaningful. Further research on larger patient groups is necessary.

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