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Indoleamine, 2-3 Dioxygenase Activity Could Be an Early Marker of Graft Rejection in Heart Transplantation

N. Suarez-Fuentetaja, N. Domenech-Garcia, M.J. Paniagua-Martin, R. Marzoa-Rivas, E. Barge-Caballero, Z. Grille-Cancela, J. Pombo-Otero, J. Muñiz-García, A. Castro-Beiras, M.G. Crespo-Leiro

Abstract

Background. The indoleamine, 2-3 dioxygenase (IDO) is an inducible intracellular enzyme with immunosuppressive effects mainly on lymphocyte populations. It has been postulated that indirect determination of IDO serum activity may be a marker of renal graft rejection, but its potential usefulness in heart transplantation (HT) is unknown.

Methods. This longitudinal study included 98 HT patients (83% males) who survived ≥1 year. Mean age was 54.14 ± 11.57 years. Serum IDO activity was analyzed one month after HT by means of high performance liquid chromatography and correlated with the cumulative incidence of acute rejection (AR) during one-year follow-up. AR was defined as biopsy-proven ≥ ISHLT grade 2R rejection or empirically treated non-biopsy-proven rejection. The study sample was divided into two groups: AR group (n = 51), including patients who experienced at least one AR episode during the first year after HT; No-AR group (N = 47), including the remaining patients.

Results. Mean serum IDO activity one month after HT was significantly higher (P = .021) in the AR group (3.32 ± 1.56) than in the no-AR group (2.62 ± 1.35). No significant association between serum IDO activity and gender (male: 3.1 ± 1.56, women: 2.43 ± 0.99, P = .092), recipient age (r = −.07, P = .943) or donor age (r = 0.108, P = 0.293) was observed. By means of binary logistic regression, an odds ratio of 1.4 [CI 95%: 1.033-1.876, P = .03] per unit increase of act-IDO was estimated, with no significant modification upon forced adjustment for age and sex. Mean glomerular filtration rate 1 month after HT was 67.01 ± 28.51 mL/min/m^2. No significant correlation between this parameter and serum IDO activity was observed (r = .160, P = .117).

Conclusions. Our study suggests that serum IDO activity one month after HT might be associated with a higher risk of AR during one-year follow-up. This association seems to be independent of recipient gender, age or renal function.

Indoleamine 2,3-dioxygenase (IDO) is an inducible enzyme present intracellularly in several cell types, including monocytes, macrophages, and dendritic cells (DCs).[1] It shows immunosuppressive effects mainly on lymphocytes.[2 and 3] This activity can be explained by two hypotheses, which are not mutually exclusive: (1) IDO catalyzes the first limiting step of the tryptophan metabolic pathway (trp), an essential amino acid for protein synthesis and a control point in the G1 phase of the lymphocyte[1 and 2] cell cycle making them susceptible to apoptosis; and/or (2) the production of proapoptotic metabolites, such as kynurenines (kyn), confers a state of immunosuppression on the microenvironment[4] (Fig 1). The concentrations of kyn (and its derivatives) necessary to induce the same effects on populations of macrophages or DCs is about 10 times higher. This factor mostly affects the Th1 versus the Th2 population, appearing to be related to Th2 lymphocyte differentiation.[1] Interferon gamma (IFNγ) is the main inducer of IDO activation.[5] Curiously stable expression of IDO is enhanced by interleukin (IL)-10,[6 and 7] but IDO is also affected by other stimuli such as IL-1, CD40, lipopolysaccharide, and superoxide anions.[8] Increased IDO activity has also been observed in DCs by "reverse signaling" mediated by binding of CTLA-4 on the lymphocyte surface to CD80 and CD86 on the DC surface[6] and with the fusion protein CTLA-4-Ig[9] (Fig 1).

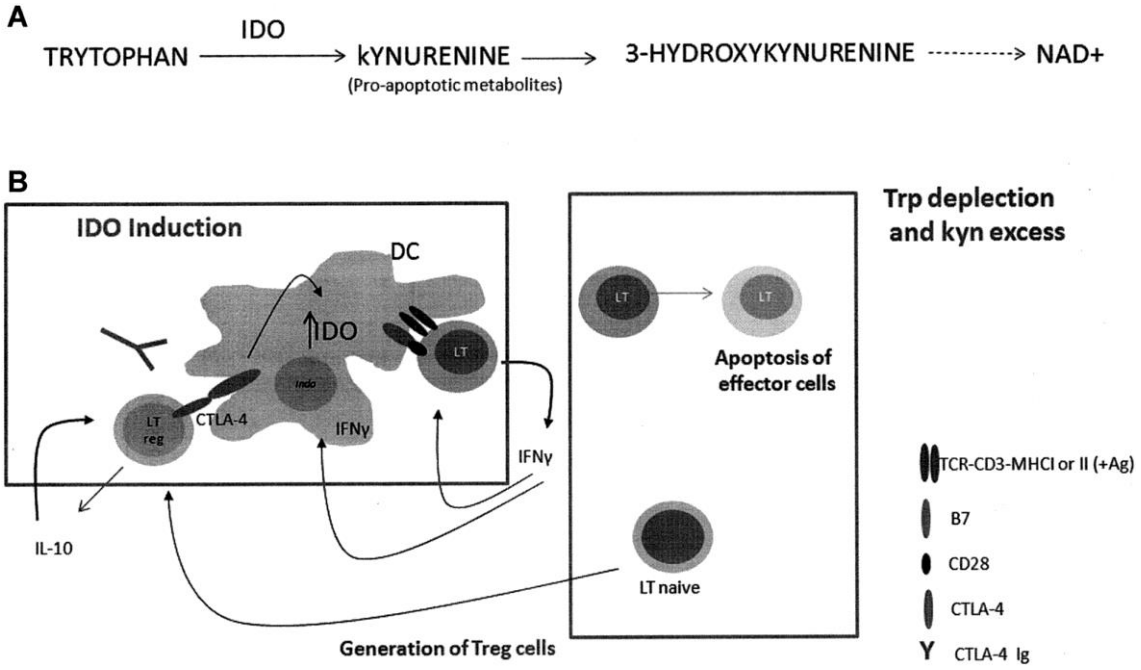

Fig 1. IDO activity. **(A)** Tryptophan metabolism. **(B)** Effects of IDO pathway in Immune response. After antigen, presentation by DCs to T lymphocytes, cytokines production is started by both cell types. IFNγ is the most important inducer of IDO expression by DCs. Other stimuli such as CTLA-4 and IL-10 are able to increase the IDO expression. The tryp privation as well as the excess of kyn in the microenviroment of cells promote T-cell cycle arrest and T-cell apoptosis, as well as the CD4 differentiation in T_{reg} in order to decrease the alloresponse to new antigens. *Ag, antigen; DC,* dendritic cells; *IFNγ,* interferon gamma; Ig, immunoglubulin; IL, interleukin; *Kyn*, kynurenines; LT, T lymphocytes; LT reg, T-regulatory cells; *Trp*, trytophan; IDO, indoleamine 2,3-dioxygenase.

IDO activity (act-IDO) is currently being studied in the fields of oncology, HIV, autoimmunity, and transplantation.[10, 11, 12, 13 and 14] Human kidney transplant patients who show higher levels of act-IDO measured by the kyn/trp ratios in serum and urine samples at one day after transplantation experienced acute rejection episodes (ARE). These authors suggested that act-IDO could be a novel noninvasive biomarker of rejection[15]; its role after heart transplantation (HT) is still uncertain.[4 and 16]

Methods

A longitudinal study was performed in 98 heart transplanted patients who were over the age of 18 years at the time of HT, survived for at least 1 month thereafter, and provided informed consent. The population was subdivided into two groups: "ARE group" experiencing at least one biopsy-proven acute rejection grade ≥2R ISHLT or empirically treated non-biopsy-proven rejection and "no ARE group." An ARE was defined as grade ≥2R ISHLT or as treated rejection.

Serum Samples

Serum samples were obtained at 1 month post-HT, concomitant with clinical follow-up visits. Blood samples were centrifuged (3000 rpm, 15 minutes) and serum separated to be frozen at −80°C until assayed.

Act-IDO analysis by High Performance Liquid Chromatography

Act-IDO was determined by high performance liquid chromatography (HPLC; Agilent 1200), measuring the serum kyn/trp ratio. Samples pretreated with trichloroacetic acid (1:1, v:v) (Sigma-Aldrich) were centrifuged for 4 minutes at 13,000 rpm to precipitate proteins. Subsequently, 25 μL of supernate were mixed with 125 μL of Na_2HPO_4 (Sigma-Aldrich) before injection into the HPLC. Trp and kyn standards (Sigma-Aldrich) processed using the same pretreatment were analyzed in the same pretreatment were

analyzed by HPLC using a C18 reverse phase column. Trp emission was detected by fluorescence after natural excitation to a wavelength of 285 nm with emission at 360 nm. Kyn was analyzed using an ultraviolet detector at 360 nm. Act-IDO in serum was described by the mean value ± standard deviation.

Renal Function

Renal function was determined by the estimated glomerular filtration rate using MDRD-4 from serum values obtained at the same time as act-IDO (first month post-HT).

Statistical Techniques

Statistical analysis was performed by nonparametric tests (the Kruskal- Wallis or Pearson test) or Student *t* test. The association between act-IDO and the AR group during the first year post-HT was estimated by logistic regression analysis, adjusting for potential confounding parameters of donor and recipient sex and age and recipient's renal function. SPSS (SPSS 15.0 Inc, Chicago, Ill, USA) and GraphPad Prism 4 were used for statistical analysis.

Ethical Statements

Written informed consent was obtained from all patients included in the study. Our Ethics Committee approved the protocol.

Results

Characterization of the AR and No AR Groups

The mean age of the studied patients was 54.14 ± 11.57 years and 83% were males. There were 51 patients (52%) with any AR in the first year (AR group) and 47 patients (48%) without AR (no AR group). Table 1 shows demographic and clinical characteristics among both groups.

Table 1. Demographic and Clinical Characteristics

	TOTAL Group	AR Group	No AR Group	*P* Value
n (%)	98 (100)	51 (52)	47 (48)	
Recipient age (ys)	54.14 ± 11.54	55.15 ± 9.71	53.04 ± 13.28	NS
Donor age (ys)	42.48 ± 13.72	44.08 ± 13.8	40.74 ± 13.55	NS
Male gender (%)	83	84	81	NS
Reason for HT (%)				NS
Ischemic	42	41	43	
Dilated	39	45	32	
Valvular	7	6	9	
LVEF (at 12 mon)	63.64 ± 6.23	64.53 ± 6.47	62.69 ± 5.88	NS
Act-IDO (at 1 mon)	2.98 ± 1.49	3.32 ± 1.56	2.62 ± 1.35	0.021
GFR (MDRD-4 at 1 mon)	67.01 ± 28.5	64.02 ± 27	70.32 ± 30.04	NS

LVEF, left ventricular ejection fraction; AR, acute rejection; No AR, no acute rejection; HT, heart transplantation; Act-IDO, indoleamine 2,3-dioxygenase activity; GFR, glomerular filtration rate; NS, not significant; MDRD-4.

Analysis of the Act-IDO at First Month Post-HT

Mean act-IDO was significantly lower among the No AR group versus the AR group; 2.62 ± 1.35 versus 3.32 ± 1.56 (P = .021; Fig 2). No association was observed between act-IDO and recipient gender (male: 3.1 ± 1.56; female: 2.43 ± 0.99: P = .092), recipient age (r = .07: P = .943) or donor age (r = .108: P = .293). Binary logistic regression showed an odds ratio of 1.4 (CI 95%: 1.033-1.876, P = .03) per unit increase in act-IDO unmodified when adjusting for age and sex.

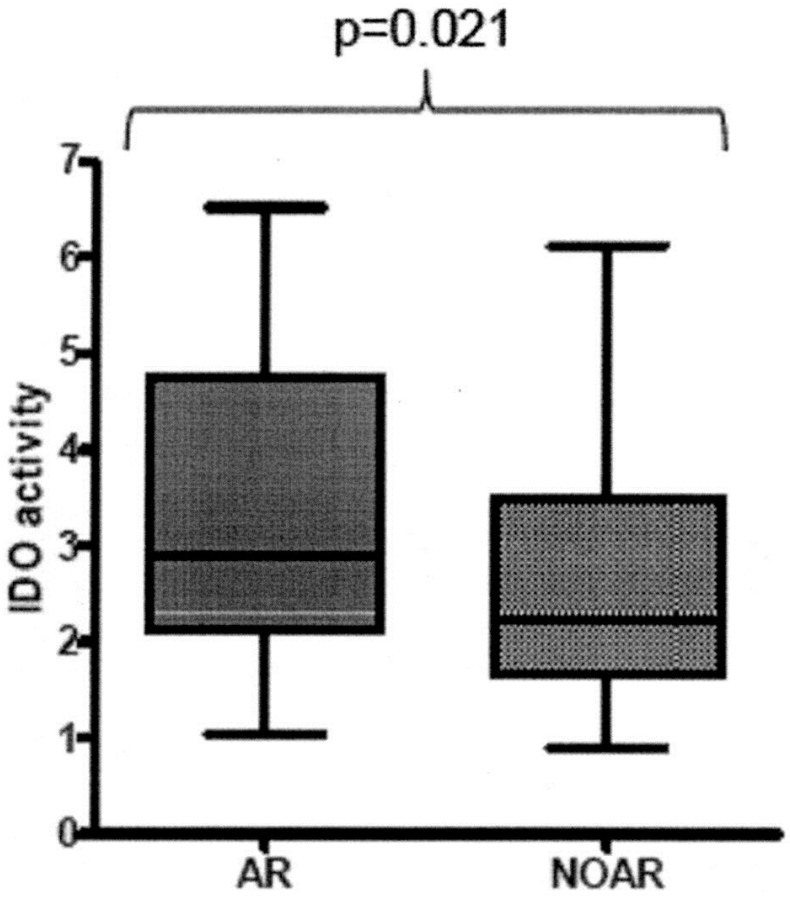

Fig 2. IDO activity in AR and no AR groups. Box plot shows the statistical difference observed between both groups of patients. *AR*, acute rejection; NOAR, no acute rejection; IDO, indoleamine 2,3-dioxygenase.

Act-IDO Was Not Related to Glomerular Function

Mean MDRD-4 was 67.01 ± 28.51. Normal values of >60 were observed in 56% of patients. There was no significant association between serum act-IDO and MDRD-4 at 1 month post-HT ($r = -.160$; $P \pm .117$).

Discussion

We observed an association between act-IDO in serum at 1 month post-HT and a higher risk of AR during the first year post-HT. The association was independent of recipient gender, age, or renal function.

The role of IDO in transplantation tolerance was introduced by Munn et al in 1998, in relation to maternal-fetal tolerance.[13] Subsequently, *Indo* gene transfection into tumor cell lines *in vitro* conferred the ability to inhibit cellular immune responses. [8] Increased act-IDO was noted in an animal model of corneal, lung, kidney, liver, and heart transplantation. [4] Production of proinflammatory cytokines secreted by antigen presenting cells and T cells begins, after transplantation due to responses to the major histocompatibility complex and tissue damage due to surgery. These signals include IFNγ. [5] Pretreatment of mice with DCs differentiated from the bone marrow of the donor (with a vector inducing IDO expression has been shown to induce tolerance to skin grafts. [17] The expression of IDO by DCs regulates the balance of cytokines, which may favor the generation of regulatory T cells (T_{reg}), namely alloantigen-specific T lymphocytes differentiated from effector $CD4^+$ T cells. [17] Such DCs, moreover, have the ability to expand natural T_{reg}. Its relevance has been supported by specific blockade of the enzymatic activity using analog molecules of trp, such as 1-methyl-Trp. Thus, murine models of corneal, skin, and pancreas transplantation have shown decreased time to rejection after treatment with IDO inhibitors. [1] Additionally, animal models have revealed that transduction of tissue-specific vectors for the expression of IDO prolonged graft acceptance, conferring protection against rejection. Despite the tolerance effects

described for act-IDO in animal transplantation models, this enzyme does not appear to have the same effect in human transplantation. Brandacher et al reported that act-IDO did not diminish immune reactions in humans after organ transplantation. [15] We have also observed that act-IDO was higher in serum samples from patients who will develop an AR during the first year. Consequently, act-IDO could be a marker for activation of the immune system against a new organ, and therefore acute rejection.

The limitations of our study were the small sample size and the possibility that differences in immunosuppression between the groups affected act-IDO. Further studies will be needed to clarify the role of act-IDO for noninvasive AR monitoring after HT.

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