INDOLEAMINE 2,3-DIOXYGENASE (IDO) AS A NEW IMMUNOLOGICAL MARKER IN KIDNEY TRANSPLANT

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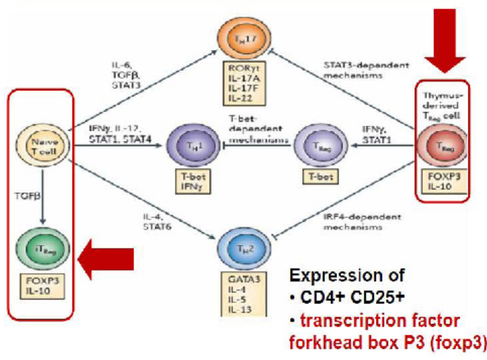
OBJECTIVES

Indoleamine 2,3 dioxygenase (IDO) is an enzyme induced by interferon-ɣ (IFN- ɣ) and toll-like receptors (TLR)-ligands in macrophages, dendritic cells and other cells. IDO degrades the essential aminoacid tryptophan (Trp) to kynurenine (Kyn); enzyme activity can be estimated as Kyn/Trp ratio. T-cell activation is affected by Trp deprivation and accumulation of Kyn.

Activating IDO during immune response counterbalances mechanisms of negative feedback loop of IFNɣ and down-regulates overwhelming immune activation. IDO activation has been reported to be increased during acute rejection and downregulated in vitro by the immunesuppressants used for transplantation (steroids, cyclosporin, tacrolimus, sirolimus and mycophenolate).

Regulatory T cells

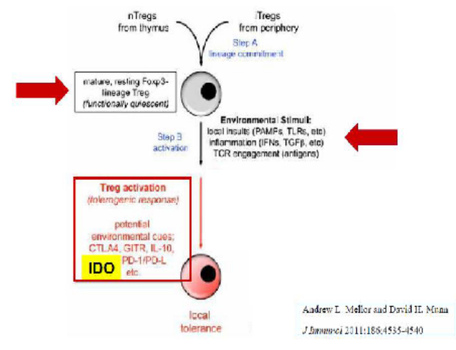


IDO Indoleamine 2,3-dioxygenase

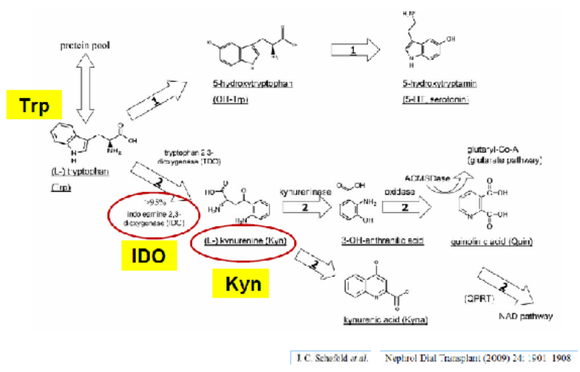
- **Heme containing enzyme**; 45 kD inducible, monomeric protein, encoded by the INDO gene (8p12)

- **Induced by interferons, TNF-α and TLR-ligands**
- Expressed on endothelial cells, smooth muscle cells, fibroblasts, astrocytes, **macrophages, dendritic cells**

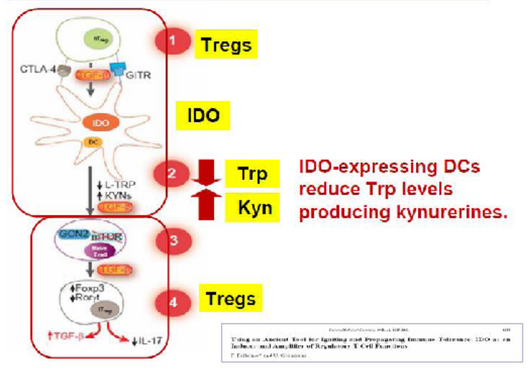
Regulatory T cells



Tryptophan metabolism



Treg and IDO



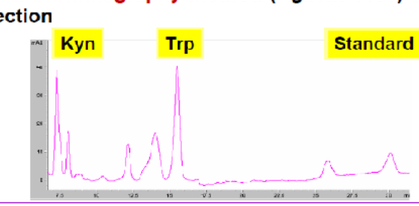
METHODS

Aim of our study was to investigate IDO activity (Kyn/Trp) in 46 samples of peripheral blood mononuclear cells (PBMC) of 16 children with kidney transplant followed in outpatient clinic and of 11 adults (30 samples at 0, 15 and 30 days after kidney transplantation).

IDO activity was assessed in sera as Kyn/Trp ratio, simultaneously determinated using an isocratic RP HPLC method with UV detection.

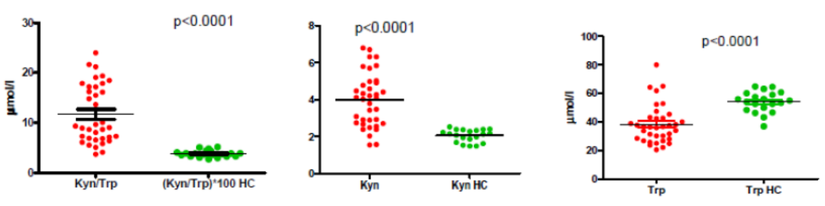
Real time PRC (Taqman) was used to measure mRNA of TLR2, TLR3, TLR4, TLR9 and regulationassociated genes of Treg including forkhead box P3 (Foxp3), Th17-related factors (IL-17), retinoid orphan nuclear receptor (RORc), and TFG-β1 which modulates the differentiation of Th17. Values were normalized using Abelson housekeeping gene mRNA and expressed as fold changes.

- **Measurement in Taqman of mRNA expression of the Treg regulation-associated gene forkhead box P3 (Foxp3) and TGF-β1**
- **IDO activity was assessed in sera as change in the ratio between tryptophan (Trp) and its catabolic product kynurenine (Kyn) simultaneously determined using an isocratic Reverse Phase High Performance Liquid Cromatography method (Agilent 1100) with UV detection**

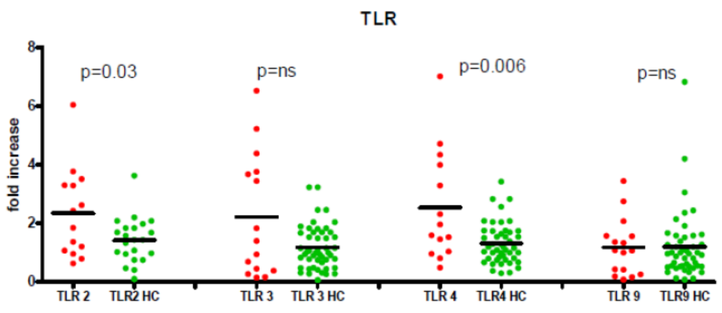


RESULTS

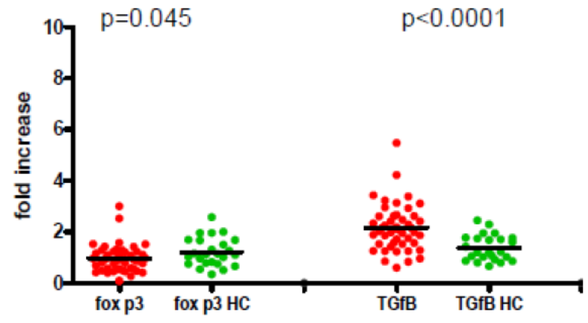
In transplanted patients IDO was significantly activated in comparison to healthy controls (HC) (Kyn 3.97±1.44 vs 2.05±0.33 in HC , p<0.0001; Trp 38.24±13.49 vs 54.02±7.32 in HC , p<0.0001; Kyn/Trp: 11.72±5.84 vs 3.83±0.67 in HC , p<0.0001).



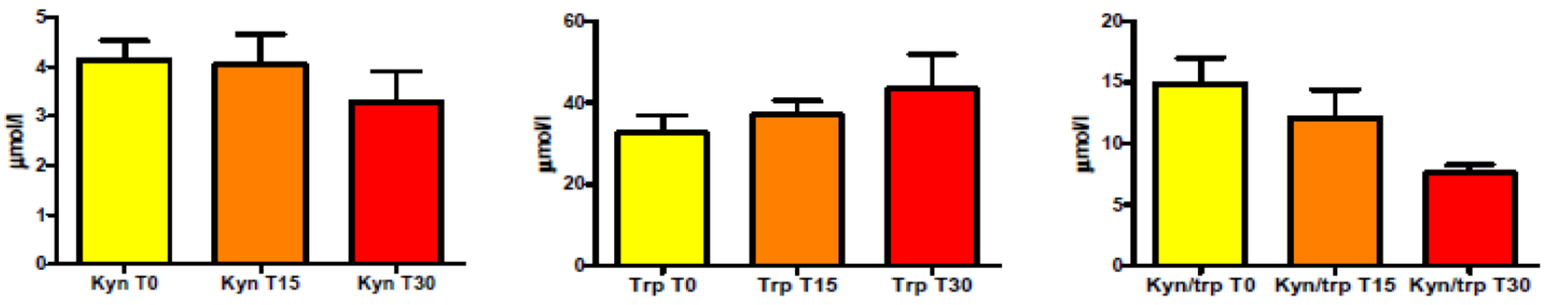
TLR innate immunity pathway was also activated (TLR2 mRNA 4.5±6.1 vs 1.4±0.8 in HC , p=0.03, TLR4 mRNA 4.7±6.8 vs 1.3±0.7 in HC p=0.006).



Foxp3 mRNA was significantly depressed (0.96±0.53 vs 1.21±0.55 in HC , p=0.045) and TGFβ mRNA increased (2.2±0.9 vs 1.4±0.5 in HC , p <0.0001).



In the prospective cohort a trend of reduction of IDO activity was found over the first month after transplantation, with decrease in Kyn/Trp



CONCLUSIONS

IDO is activated in kidney transplant, in agreement with the activation of innate immunity (TLRs) and IFN-ɣ mediated pathways. As anticipated by *in vitro* models, IDO activity decreases under the effect of the immunosuppressants in transplantation. Based on our data IDO activity might be an interesting marker of immunological status in kidney transplant.

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