Delivery of Indoleamine 2,3 Dioxygenase for the Induction of Immune Tolerance

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Introduction Indoleamine 2,3-dioxygenase (IDO), an enzyme that catalyzes tryptophan catabolism, has been shown to play critical role in the promotion of tolerance[1]. IDO is expressed in certain cells and tissues, particularly in antigen presenting cells and the placenta[2]. There is evidence that depletion of this essential amino acid increases susceptibility of T cells to apoptosis while some of its resulting metabolites (quinolinic acid and 3-hydroxyanthranilic acid) have a direct cytotoxic effect on effector T cells resulting in reduced immune activation[3]. In addition, IDO-expressing cells preferentially induce proliferation of regulatory T cells. Therefore, we are investigating methods to exogenously deliver IDO in order to manipulate the ratio of tryptophan to kynurenines. This will allow for the induction of regulatory T cells or suppression of effector T cell proliferation in the context of vaccination for autoimmune diseases. **Methods** IDO was purified from E.coli and its purity, activity and endotoxicity well characterized. Briefly, its purity as well as corresponding size was determined by a SDS-PAGE gel. Specific enzyme activity was measured using a spectrophotometric assay to determine the amount of kynurenine formed. Endotoxin levels of the protein were measured using the ChromoLAL method. Cellular response to IDO was then evaluated by adding the enzyme to dendritic cell media at a concentration of 15 µg/mL. Maturation markers (CD80, CD86, MHCII) were assessed via flow cytometry. Next, a proliferation assay was performed by incubating dendritic cells with T cells *in vitro* at a 1:6 ratio. More specifically, dendritic cells from a C57BL/6 mouse were pretreated with Ovalbumin peptide (OVA 323-339) and incubated with CD4+ T cells from an OT II mouse either in the presence or absence of exogenous IDO. Proliferation of T cells was measured using CFSE incorporation. **Results.** The enzyme was determined to have a molecular weight of approximately 45 kDa and more than 90% purity was observed. Specific activity of the enzyme was determined at 400 pmoles/min/µg and it exhibited less than 1EU/mL as determined by the spectrophotometry assay and ChromoLAL method, respectively. Maturation markers evaluation revealed no phenotypic change on dendritic cells and no activation of T cells when no OVA peptide is present. This is observed in figure 1 and figure 2.

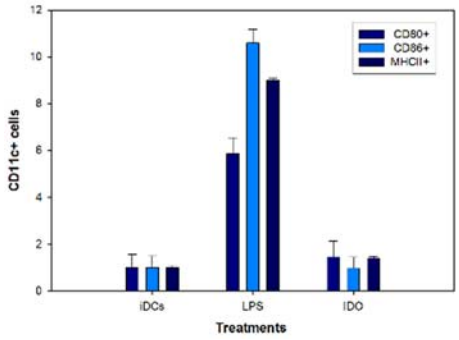

Figure 1. Maturation markers expression on dendritic cells after 24h incubation with exogenous IDO.

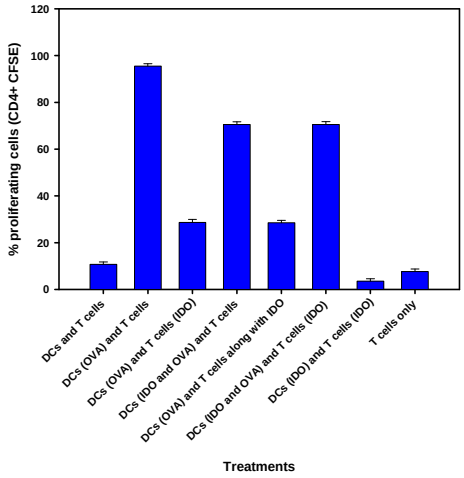

Figure 2. Proiferation assay in the presence or absence of IDO

Conclusion. From this study we can conclude that IDO can be expressed in E.coli with desired characteristics (molecular weight, purity, and endotoxicity) as established by regulatory agencies. In addition, we have determined that IDO does not act as a pathogen associated molecular pattern (PAMP) as it does not induce a phenotypic change on dendritic cells. Lastly, our study shows IDO inhibit T cell proliferation in an antigen specific manner. Future studies include determining the mechanism by which IDO inhibition is observed and evaluation of regulatory T cells formation.

References: [1]M.T. Pallotta and U. Grohmann, *Nature Immunology*, 2011, **12,** 871 [2]P. Terness and G. Opelez, *J. Exp. Med,* 2002, **196,** 447 [3]Mellor, A.L. et al., *Immunol*., 2002 **168**, 3371