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Enhanced Indoleamine 2,3-Dioxygenase (IDO) Expression in Transgenic Mice Inhibits T Cell-Mediated Allograft Rejection.

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IDO is the rate-limiting enzyme in the kynurenine pathway of tryptophan metabolism and has been shown to have inhibitory effects on T cell responses. To evaluate the effects of cells expressing IDO on allogeneic T cell responses *in vivo*, we used mice that were transgenic for IDO (Tg-MI). In this study, we have used the H-Y (male specific) alloantigen to investigate the suppressive effect of IDO on T cell responses. Most female Tg-MI mice and normal (IDO-wildtype) mice accepted primary skin grafts from syngeneic male Tg-MI mice, whereas Tg-MI and normal females rejected syngeneic male grafts from normal mice by day 25. All female recipients received a second skin graft from non-transgenic (non-Tg) B6 male 117 days after the first to determine if enhanced IDO expression by primary graft promoted allograft tolerance. The majority of females that received primary grafts from Tg-MI mice also accepted secondary B6 male allograft while most females that received primary grafts from non-Tg donors rejected secondary B6 grafts. These outcomes suggest that enhanced IDO expression on donor skin cells suppresses T cell responses to allograft and promotes long-term tolerance.

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**Enhanced Indoleamine 2,3-Dioxygenase (IDO) Expression in Transgenic Mice  
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Key words: IDO, immunosuppression, IDO-transgenic mice, T cells, and skin grafts.

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**Abstract.**

IDO is the rate-limiting enzyme in the kynurenine pathway of tryptophan metabolism and has been shown to have inhibitory effects on T cell responses. To evaluate the effects of cells expressing IDO on allogeneic T cell responses *in vivo*, we used mice that were transgenic for IDO (Tg-MI). Previous studies have shown that adoptive transfer of alloreactive donor T cells into Tg-MI recipient mice resulted in a reduction of donor T cell numbers. In the present study, we have used the H-Y (male specific) alloantigen to investigate the suppressive effect of IDO on T cell responses. Most female Tg-MI mice and normal (IDO-wildtype) mice accepted primary skin grafts from syngeneic male Tg-MI mice, whereas Tg-MI and normal females rejected syngeneic male grafts from normal mice by day 25 ( $p < 0.001$ ). All female recipients received a second skin graft from non-transgenic (non-Tg) B6 male 117 days after the first to determine if enhanced IDO expression by primary graft promoted allograft tolerance. The majority of females that received primary grafts from Tg-MI mice also accepted secondary B6 male allograft while most females that received primary grafts from non-Tg donors rejected secondary B6 grafts ( $p < 0.05$ ). These outcomes suggest that enhanced IDO expression on donor skin cells suppresses T cell responses to allograft and promotes long-term tolerance.

## **Introduction**

IDO is the first and rate-limiting enzyme in the kynurenine pathway of tryptophan metabolism. Because tryptophan is an essential amino acid, it has been proposed that local depletion of tryptophan starves, stresses, or otherwise signals within a responding T cell to promote cell cycle arrest and inhibit T cell proliferation. Based on the tryptophan depletion hypothesis, *in vitro* studies have shown that macrophage colony-stimulating factor (MCSF)-matured human monocytes [1], and human dendritic cells (DCs) [2] that express IDO, are capable of inhibiting T cell proliferation by rapid tryptophan depletion. In murine studies, MC57G, a fibrosarcoma cell line, that had been transfected to produce a cell line that over-express IDO, inhibited T cell responses in cell culture systems [3] and *in vivo*, adoptive transfer of alloreactive donor T cells into Tg-MI mice demonstrates that IDO activity suppresses T cell responses [3]. Furthermore, studies have shown the protective role of IDO in preventing allogeneic fetal rejection during gestation by tryptophan catabolism [4]. In the present study, we investigate a functional immunoregulatory role for cells expressing IDO by evaluating the effects of these cells on T cell responses *in vivo*.

## **Materials and Methods**

### *Mice*

All mice used were bred under specific pathogen-free conditions at the Medical College of Georgia. IDO-transgenic mice (Tg-MI) on (CBA x B6) F1 background express IDO under control of the MHC Class II promoter. Tg-MI mice were

previously generated and IDO protein expression and activity were assessed as previously described [3]. IDO wildtype mice are on a CBA x B6 (F1) background.

#### *Skin Grafts*

Tail skin from 6-8 week old male mice was grafted on 6-8 week old female recipient mice as described [5]. Second skin graft (B6 male) were applied day 117 after first grafting. A graft was considered rejected when only 10% or less of the graft remained viable. Statistical Analysis of graft survival was made using the log rank test, with a p-value of <0.05 considered significant.

#### **Results and Discussion**

As can be seen from Figure 1A, all female Tg-MI recipient mice and most (75%) wildtype female recipient mice accepted primary skin grafts from male Tg-MI mice for over 120 days while most male skin grafts from wildtype mice were rejected by day 25 after grafting ( $p < 0.05$ ), regardless of whether the recipient is IDO-wildtype or –transgenic (Figure 1A and 1B). Responses of the female recipient mice from the primary skin graft experiments were tested using a second graft from male B6 mice at day 117 after first graft. Most female Tg-MI recipient mice that received skin graft from male Tg-MI mice (85%) showed tolerance to the B6 skin graft while majority of the female recipient mice that received skin graft from male wildtype mice rejected by day 20 after second grafting (Figure 1B,  $p < 0.05$ ).

In this study, we address the hypothesis that enhanced IDO expression inhibits T cell responses *in vivo*. We show that skin grafts were protected from rejection when skin

originated from Tg-MI mice and that over-expression of IDO in recipient mice alone is not sufficient to protect graft ( $p=0.5656$ ). This observation suggests that IDO-expressing donor cells are capable of inhibiting H-Y specific T cell responses within the recipient mice. In addition, almost all female recipients (both Tg-MI and wildtype) that received primary skin from male MI mice also tolerated secondary skin grafts from male B6 mice compared to female Tg-MI recipients that had received primary skin from male wildtype mice. This observation suggests that cells expressing IDO from primary skin grafts are capable of tolerizing alloreactive host T cells and later prevent a recall response against a second skin graft.

Data from this study suggests that genetic manipulation of IDO activity may affect T cell responses. However, the IDO-transgenic murine model used in this study fails to identify critical cell types that require IDO activity for immunoregulation. DCs are key players in rendering T cells anergic [2] and, most recently, CTLA4 signaling via B7 molecules has been found to be a potent inducer of IDO in a subset of DCs [6]. Also, CTLA4-Ig treatment resulted in long term survival of pancreatic islet allograft [7]. As CTLA4 naturally occurs on  $CD4^+CD25^+$  regulatory T cells ( $T_{reg}$ ) [8], we propose that the DC- $T_{reg}$  interaction via CTLA4-B7 binding may be a mechanism to induce IDO which leads to tolerance induction. To investigate the immunoregulatory role of IDO-expressing DCs, we plan to generate another IDO-transgenic murine model where IDO expression is targeted to DCs.

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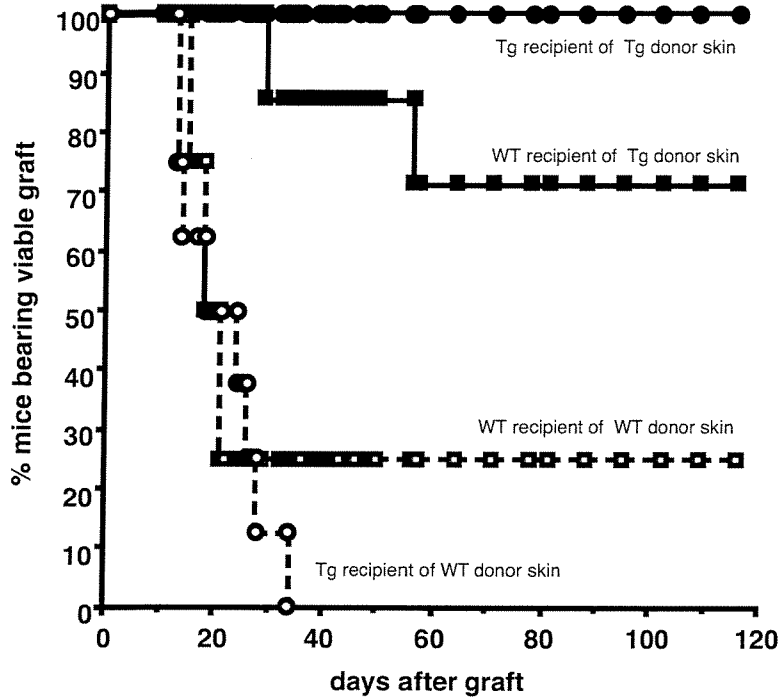
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**Legend:**

**Figure 1. IDO activity protects skin graft and tolerizes T cells.** All recipients are female and all donors are male. (A) Primary skin graft experiments; ● Tg (transgenic) donor → Tg recipients (n=6), ■ Tg → wildtype (WT)(n=7), ○ WT → Tg (n=8), □ WT → WT (n=4). (B) Secondary skin graft experiments; Recipients from A received B6 skin grafts.

## (A) 1° Skin Graft Rejection



## (B) 2° Skin Graft Rejection

