General Immunocompetence of Neonatally–Treated Mice That Accept Third–Party Cardiac Allografts

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Introduction

•Heart transplantation is a highly successful treatment for infants with congenital heart disease that would otherwise be lethal. After transplantation, patients must be placed on a lifelong immunosuppression regimen to prevent graft rejection. However, this can result in substantial morbidities due to adverse side effects.

•The induction of tolerance towards donor antigens would avoid or minimize the need for immunosuppression. The 'Medawar model' of neonatal tolerance demonstrated that immune tolerance can be induced in neonatal mice by injection of donor strain cells. Tolerance is manifested by long–term acceptance of skin grafts from the same donor strain as the tolerizing cells while skin grafts from other third–party strains are rejected.

•Our lab has shown that the Medawar model of tolerance can be applied to cardiac transplants (Figure 1).

- •Neonatal C3H (H–2^k) mice injected with cells from F1 [C3H x BALB/c (H–2^d)] mice will accept BALB/c heart grafts as adults. Moreover, recipient mice will also accept heart grafts from third–party strains.

•We question whether this acceptance of both donor and third–party grafts is due to general immunodeficiency induced by the cell inoculation.

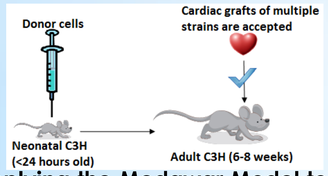

Figure 1. Applying the Medawar Model to heart transplants. Neonatal recipient mice are injected with cells of the donor strain. As adults, the recipient mice can accept heart grafts from multiple strains of mice.

•Previously, our lab has shown that mice treated neonatally with F1 fetal liver cells (FLC) are not immunodeficient and can accept third–party heart grafts

•In this study, we examined the response of neonatally–treated mice after triggering the immune system to determine whether they were immunocompetent or immunodeficient following cell inoculation with F1 bone marrow and/or splenocytes.

Methods

•C3H neonates were treated with bone marrow and splenocytes (BM/SPL) (n = 6 treated, n = 4 untreated) or just splenocytes (SPL) (n = 17 treated, n = 7 untreated) harvested from F1 [C3H x BALB/c] mice.

•Untreated C3H neonates were used as controls.

•As adults, the mice were subject to a delayed–type hypersensitivity (DTH) where ear thickness was measured to assess the immune response after exposure to an allergen (Table 1).

•Several weeks later, the same mice were injected with toxoids to assess humoral and cellular response using ELISPOT and BrdU ELISA (Table 2).

Day	Treatment
1	50 µL of 3% oxazolone applied to shaved flank
2	50 µL of 3% oxazolone applied to shaved flank
6	10 µL of 1% oxazolone applied to left ear 10 µL of vehicle control applied to right ear
7	Measurement of thickness of both ears at 24 hours
8	Measurement of ears at 48 hours
9	Measurement of ears at 72 hours

Table 1. DTH treatment procedure. Oxazolone was dissolved in 4:1 acetone:olive oil solution. Vehicle control consisted of solution without oxazolone. Measurements were made using a thickness gage.

Type of Injection	n =
Diphtheria toxoid (DT) and tetanus toxoid (TT)	4 SPL treated, 3 BM/SPL treated, 6 untreated
PBS	3 SPL treated, 3 BM/SPL treated, 4 untreated

Table 2. Details for i.p. vaccinations. On day 1, mice were injected with 1 µg TT, 1 µg DT, and 100 µL Complete Freund's Adjuvant (CFA) in a 200 µL injection or 100 µL of 1X PBS and 100 µL CFA in a 200 µL injection. 2 weeks later, the mice received the same injections with 1X PBS replacing CFA.

Results

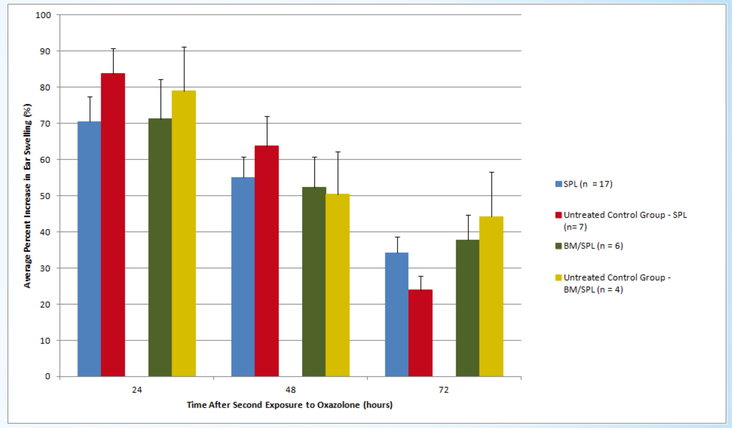

Figure 2. Mean increase in ear thickness. Percent increase was determined by comparing the thickness in the left ear (oxazolone treated) with that of the right ear (vehicle control).

•Statistical analysis was performed using the two–tailed Mann–Whitney test and two–tailed unpaired t–test. Results showed no significant difference ($p > 0.17$ for all tests) in the response between treated and untreated mice at any time. There was also no significant difference between the response of both treated groups.

Summary

•Our results provide evidence that acceptance of third–party cardiac grafts by neonatally–treated mice cannot be explained by immunodeficiency induced by the tolerizing cells.

•Our DTH results are in concordance with those found in the FLC study.

Future Directions

•Humoral and cellular assays are ongoing.

•Some of the mice from our study will receive heart transplants in order to further investigate mechanisms of acceptance.

Acknowledgements

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