Cell Types and Events in Graft Infiltrates that Lead to Acceptance or Rejection of Cardiac Allografts in Neonatally-Tolerized Mice

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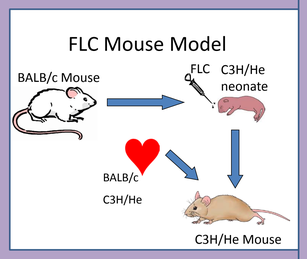
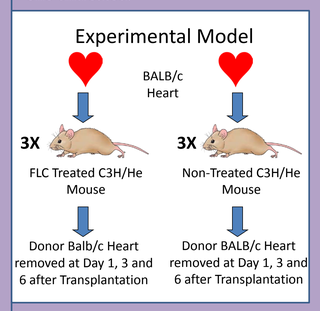
Introduction

Acute rejection of an organ graft is often associated with infiltration by T cells and other cell types, causing damage to the blood vessels and tissues. Long-term acceptance of organ grafts without immunosuppressive drug therapies is a major goal of transplantation research. Many strategies have been explored to induce transplant tolerance in animal models. One strategy to induce cardiac allograft acceptance involves injecting neonatal C3H/He mice (H-2^k) with allogeneic fetal liver cells (FLC) from BALB/c mice (H-2^d). Typically, this induces acceptance of BALB/c cardiac grafts in >90% of recipients, while 100% of untreated mice reject grafts. Our lab is currently studying the mechanisms of graft acceptance in this model. The objective of this project was to investigate how the cell types and events in graft infiltrates differ between FLC-treated and untreated mice, in an attempt to understand the processes leading to cardiac graft acceptance.

Methods

Fetal livers were collected from BALB/c mice (H-2^d) at approximately 15 days gestation and made into a single cell suspension for injection. Three neonatal C3H/He mice (H-2^k) were injected with FLC within 24 hours of birth and three other C3H/He neonates were left untreated. At approximately six weeks, the mice underwent heterotopic heart transplantation from BALB/c donors. Two mice were sacrificed one day after transplantation: one FLC- treated and one untreated.





The BALB/c donor and C3H/He native hearts were collected along with other C3H/He organs. This process was repeated at days 3 and 6 post-transplant for the remaining mice. The six donor hearts and a naive BALB/c control heart were made into cryosections. The cryosections were labelled either directly or indirectly with antibodies to identify different cell types by confocal microscopy.

Antibody Chart

Target	Label	Isotype	Clone
CD3 (T Cells)	Alexa Fluor 647	Rat(SD) IgG_{2b}, κ	17A2
CD4 (Helper T Cells)	Alexa Fluor 647	Rat(DA) IgG_{2a}, κ	RM4-5
CD8a (Cytotoxic T Cells)	Biotin	Rat IgG_{2a}, κ	53-6.7
CD11b (Macrophages)	Biotin	Rat IgG_{2b}, κ	M1/70
CD11c (Dendritic Cells)	Purified	Ar Ham IgG_1, λ_2	HL3
Foxp3 (Regulatory T Cells)	Biotin	Rat IgG_{2a}, κ	FJK-16s
CD45R (B Cells)	Biotin	Rat IgG_{2a}, κ	RA3-6B2

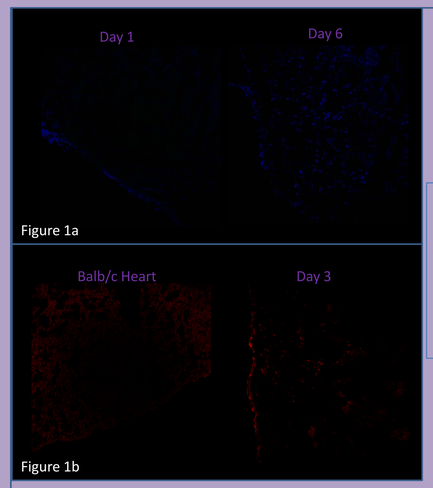


Figure 1: CD3-labelled T cells and CD11b labelled macrophages in rejecting grafts and un-transplanted heart

Results

Dendritic cells (CD11c) and B cells (B220) were infrequent and were found in both treated and untreated groups (not shown). T cells (CD3) and macrophages (CD11b) were relatively numerous in graft cryosections in both treated and untreated groups. In the grafts of untreated mice, T cells increased substantially from Day 1 to Day 6 (Figure 1a). T cells appeared to increase in rejecting grafts far more extensively than in grafts in FLC-treated mice (Figure 2a and 2b). An accumulation of macrophages was also seen in both rejecting and accepted grafts; no macrophages or T cells were observed in naive (untransplanted) BALB/c heart (Figure 1b). This infiltration by T cells and macrophages indicates an inflammatory response and the possibility of acute graft rejection, so further staining was done to characterize the types of T cells in the infiltrates. Immunostaining with antibodies against CD8 (Figure 3a and 3b), CD4 (Figure 4a and 4b), and Foxp3 (Figure 5a and 5b) showed that many T cells in grafts in untreated controls were cytotoxic T cells (CD8), whereas a majority of T cells in accepted grafts of FLC-treated mice were helper T cells (CD4). No obvious difference in Foxp3-expressing cells was observed between the groups; regulatory T cells (Foxp3) were found to be present at all time points with a slight increase in Day 3 for both groups.

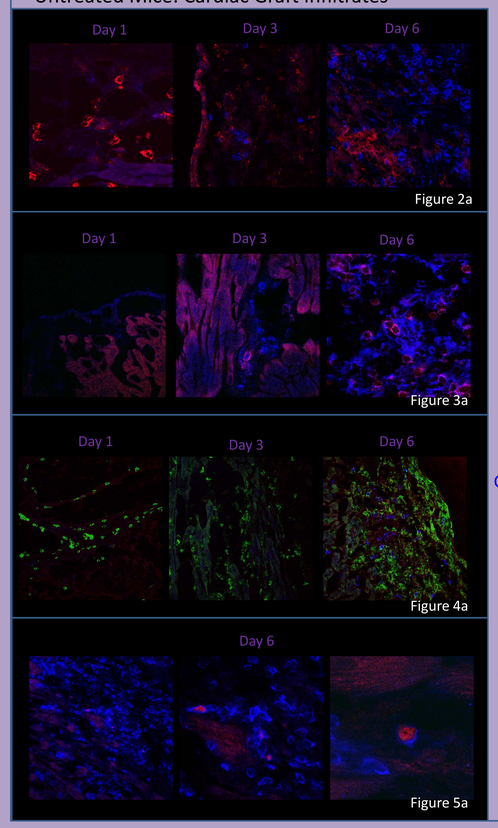
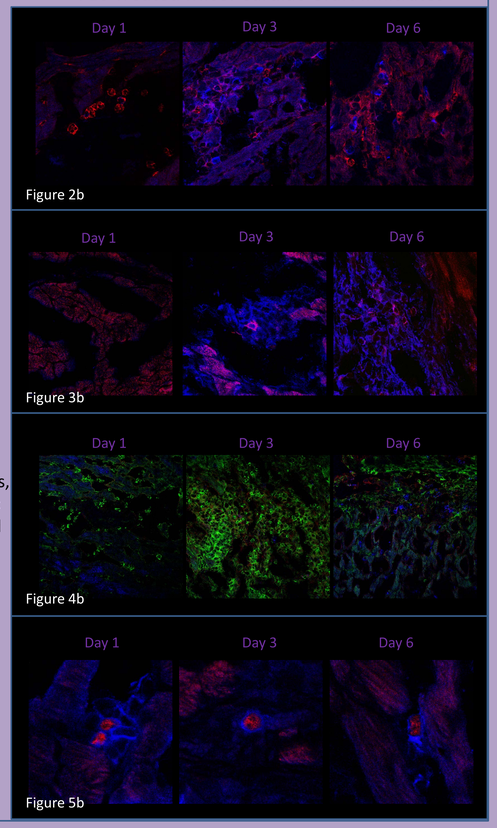


Figure 2: CD3-labelled T cells and CD11b-labelled Macrophages

Figure 3: CD3-labelled T cells and CD8a-labelled Cytotoxic T cells

Figure 4: CD4-labelled Helper T cells, CD11c-labelled Dendritic cells, and CD11b-labelled Macrophages

Figure 5: CD3-labelled T cells and Foxp3-labelled Regulatory T cells



Results (continued)

Other results observed in this qualitative experiment were the apparent cell-to-cell interactions between T cells and macrophages in both grafts from FLC-treated and untreated mice (Figure 6a). In a small number of cases, interactions occurred between helper T cells (CD4) and dendritic cells in Day 6 of the untreated control (Figure 6b).

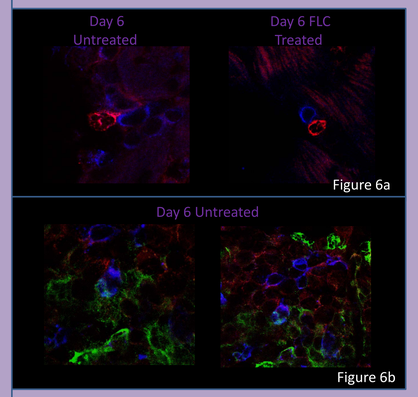


Figure 6: CD4-labelled Helper T cells, CD11c-labelled Dendritic cells, and CD11b-labelled Macrophages

Conclusion

Infiltration of allografts by T cells and macrophages indicates an inflammatory and/or immune response (ie, acute allograft rejection). This T cell-mediated response was observed in both grafts from FLC-treated and untreated mice but the extent to which the T cells increased in accepted grafts was less than in the rejecting grafts. Infiltrating T cells in the grafts of FLC-treated mice could probably be attributed to the alloantigen-independent response of ischemia-reperfusion caused during transplant surgery. In addition, in the untreated mice alloantigen-dependant events can stimulate rejection of the allograft and tissue damage through the activation of donor-specific cytotoxic and helper T cells. Qualitative assessment of graft infiltrating cells suggests less cytotoxic T cells in FLC-treated recipients when compared to rejecting grafts in untreated controls. Further analysis of cellular interactions and events in the graft infiltrates may provide further information on the mechanisms of graft acceptance in this model.

Future Research

- Examine damage to the blood vessels and tissue by immunostaining with antibodies against active Caspase-3 and Complement .
- Analyze the cell types and interactions of FLC-Treated Hearts surviving past 6 days.
- Examine spleen and lymph nodes collected from FLC-treated and untreated mice for cell types and interactions.
- Determine if there is a difference in number of alloreactive regulatory T cells in the graft infiltrates of FLC-treated and untreated mice.

Acknowledgments

I would like to thank AHFMR for the funding and allowing me this opportunity to work in research. As well I would like to thank Dr. Lori West for guidance and giving me the chance to work in her lab. Thanks are given to Tao for doing the heart transplants and Roger for helping me with the microscope and all other aspects of my project. Lastly thank you to the entire West Lab Group for making my summer enjoyable. The West Research Group is funded by: Heart and Stroke Foundation of Canada, AHFMR, CIHR, NIH(USA), NSERC/CIHR(CHRP), CRC/CFI, ISHLT, Stollery Children's Hospital Foundation, WCHRI (Women and Children's Health Research Institute).

Further Information

Please contact *giannits@ualberta.ca* . More information on this and related projects can also be obtained at the West Research group website http://www.cardiactransplantresearch.med.ualberta.ca/index.php.