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The use of ex vivo generated regulatory T cell preparations in a canine lung allograft model

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Allograft survival is dependent on allorecognition due to peptide display differences by a major histocompatibility complex (MHC) distinct from self (major), or presentation of a novel peptide by identical MHC (minor). In the Dog Leukocyte Antigen (DLA)-identical setting without immune suppression, robust lung allograft rejection based on minor antigen differences occurs within 2 weeks in normal recipients. We previously observed an increase in peripheral regulation in DLA-identical mixed hematopoietic chimeras (MC) as compared to normal dogs and saw a further regulation increase in MC with functioning solid organ transplants from their hematopoietic donors¹. As tolerance induced thru regulation of minor antigens via MC could replace immune suppression, but would require a toxic conditioning

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Authorship:

- V. Kraig Abrams drafted and revised the manuscript, conducted the studies, analyzed and interpreted the data. No potential conflicts of interest.
- Billanna Hwang conducted the studies, analyzed and interpreted the data, and revised the manuscript. No potential conflicts of interest.
- Richard A. Nash codesigned the experiment, supervised the studies, interpreted the data, and revised the manuscript. No potential conflicts of interest.
- George E. Georges codesigned the experiment. No potential conflicts of interest.
- Wayne Lamm performed the pulmonary function tests and interpreted the data. No potential conflicts of interest.
- Barry Storer provided statistical support. No potential conflicts of interest.
- David K. Madtes participated in the lung transplants, revised the manuscript and interpreted the data. No potential conflicts of interest.
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regimen, we investigated the administration of ex vivo generated regulatory T cell preparations (Treg preps) as a safer route to tolerance.

In vitro generated, MHC-mismatched alloantigen-primed peripheral blood mononuclear cells contain highly suppressive CD25⁺ cells expressing 10-fold more FOXP3 than CD25⁺ cells in blood⁴. We hypothesized that ‘off-the-shelf’³ recipient-derived major alloantigen-primed CD25-selected cells could regulate incoming minor antigen presentation differences in a DLA-identical lung transplantation model and would extend the duration of allografts compared to that of a historic control group¹. Dogs in both groups received orthotopic lung allografts² without any immune suppression and underwent pulmonary function tests (PFT) to determine allograft duration. Tested controls had early function (median 5 days; range 2–7; n=4) and were electively, serially sacrificed (median 19 days; range 6–33 days; n=7) with allografts from Day 8 onward found engorged at necropsy¹. The experimental group (n=7) underwent the same surgery with only the addition of an intraoperative, intravenous Treg prep infusion immediately after allograft reperfusion. PFT were performed a week posttransplant and monthly thereafter; all evaluable recipients had early allograft function (median 8 days; range 5–8; n=6), while 3 had extended duration with functional allografts on Days 29, 48 and 183.

Treg preps were variable in composition with a higher percentage of CD8⁺CD25⁺ cells found in preps with allograft rejection times as observed in the control group while the highest CD4⁺CD25⁺ percentages were seen in the 2 preps with the longest allograft duration. There was no correlation between allograft duration and cell number infused; the longest duration was with the least number of cells, total and per kg (table 1). The in vitro suppressive capacity of a Treg prep correlated with the in vivo lung allograft duration ($R^2=0.81$; $p=0.01$; $n=6$). Taken together, this suggests that quality of a Treg preparation is paramount to quantity.⁵ Improvements could employ CD8-depletion prior to infusion.

Since alloantigen-primed Treg preps can suppress a DLA-mismatched MLR, a DLA-mismatched-primed Treg prep of recipient origin might prolong DLA-mismatched allograft function if educated against the incoming organ donor’s antigens. This technique would require donor access for over a week and would be clinically difficult. However, if the few cells presenting minor antigen differences in a MHC-matched allograft can be regulated in an organ that contains many passenger leukocytes (lung), then perhaps all cells bearing major antigen differences in organs with few passenger leukocytes (kidney, heart) can be regulated using ‘off-the-shelf’ Treg preps.

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Abbreviations

DLA	Dog Leukocyte Antigen
MC	mixed hematopoietic chimeras

MHC	major histocompatibility complex
PFT	pulmonary function tests
Treg preps	T cell preparations

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Table 1

Ex vivo T regulatory cell characteristics

Ex vivo expanded regulatory T cell characterization including surface phenotyping, percent suppression, and total cells infused for transplantation.

Animal Number	Allograft Duration ^a (Days)	Percent CD4CD25 ^c	Percent CD8CD25 ^c	Percent of MLR ^d	Cells per kg infused ×10 ⁶ ^e	Total cells infused ×10 ⁶ ^e
G894 ^b	ND	13	5	108	10	100
G640	5	34	66	144	6.2	50
H018	6	31	27	73	3.5	45
H004	8	22	51	104	23.6	307
G913	29	33	5	60	3	30
G964	48	40	ND	54	4	76
G850	183	64	5	38	2.4	25

^aFunctional refers to pulmonary function tests done on the isolated left lung allograft. The data are the last day the grafts were functional; the grafts were not functional on the subsequent PFT.

^bG894 experienced a unique drop in hematocrit due to a pulmonary vein thrombosis and was euthanized at Day 6.

^cPercent CD4CD25 and CD8CD25 is of all CD3 cells by flow cytometry.

^dMLRs using the same sources as used to make each alloantigen Treg prep were setup in 96 well format, then set-aside Treg prep cells from the surgery were titrated in triplicate back into the wells at 1 Treg prep cell to 50 MLR responder cells. MLRs were allowed to react for 6 days, then tritiated thymidine was added for 18 hours before harvesting; an MLR without any Treg prep cells added back provided the denominator while the 1:50 Treg prep cell: responder cell readout was the numerator to determine the suppressive percent of MLR of a given preparation.

^eCells were CD25 immunomagnetically selected (median of 1.9%; range 0.4–2.5%) from Day 4 of a bulk mixed leukocyte reaction (MLR) setup between prospective recipient responders and DLA-mismatched third party stimulators. Expansion over 9–10 days with CD86⁺, IL-15-secreting K562 cells ‘painted’ with CD3 produced a median 5.7 fold increase (range 3.0–10.8 fold).