

Deciphering the interplay of lymph node profiles with outcomes in vascularized composite allotransplantation

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Background: As crucial secondary lymphoid organs located at the junction between allograft and host, the complex cellular and molecular processes within lymph nodes (LNs) following vascularized composite allotransplantation (VCA) are poorly understood. Additionally, the connection between these processes and the eventual outcome of the allograft remains to be clarified.

Materials and Methods: The lymph nodes were collected from within the hindlimb-derived osteomyocutaneous VCA and the draining lymph nodes in recipients confirmed to either undergo rejecting the transplants or develop donor-specific tolerance. Flow cytometry was used to assess lymphocyte compositions, while RNA sequencing (RNAseq) was employed to analyze gene expression.

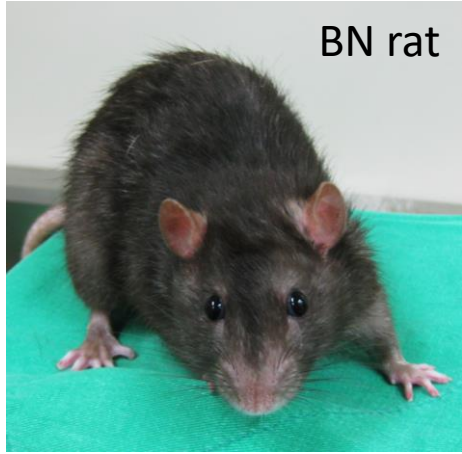
Results and Discussions: Flow cytometric analysis revealed significant different B cells levels and dendritic cell population in LNs associated with the VCA outcome and the LN origins. Biased donor chimerism levels were also observed. The RNAseq analysis unveiled a wealth of alterations of gene expression within donor-versus recipient-derived LNs linked to rejection, comprising approximately 1000 up-regulated and 370 down-regulated genes. Notable changes in cytokines, chemokine, receptors, major histocompatibility complexes (MHC) genes, as well as genes implicated in cytoskeleton remodeling and metabolism control were observed. The ongoing investigation aims to explore the mechanistic roles played by these genes, seeking to unravel their intricate interplay with cellular dynamics and studying their potential as therapeutic targets to enhance VCA outcomes.

Conclusion: Our investigation unveiled LN cellular and molecular changes linked to rejection of VCA and development of donor-specific tolerance. Further detailed exploration holds promise for developing targeted interventions to enhance VCA survival and establish tolerance.

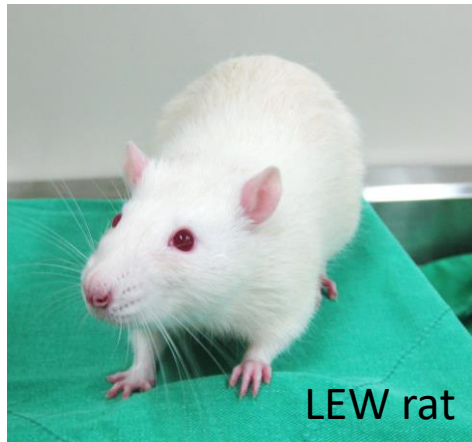
Keywords:

[1] VCA [2] graft rejection [3] donor-specific tolerance [4] RNA sequencing [5] lymph node

Experimental Design



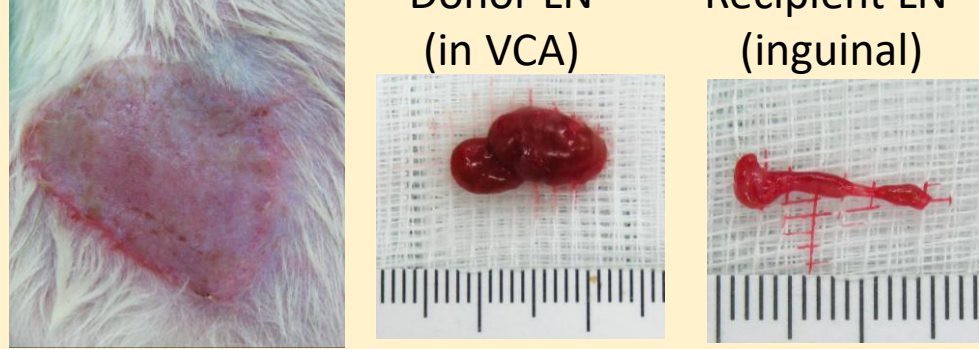
osteomyo-
cutaneous
VCA



Rejection

Donor LN (in VCA)

Recipient LN (inguinal)

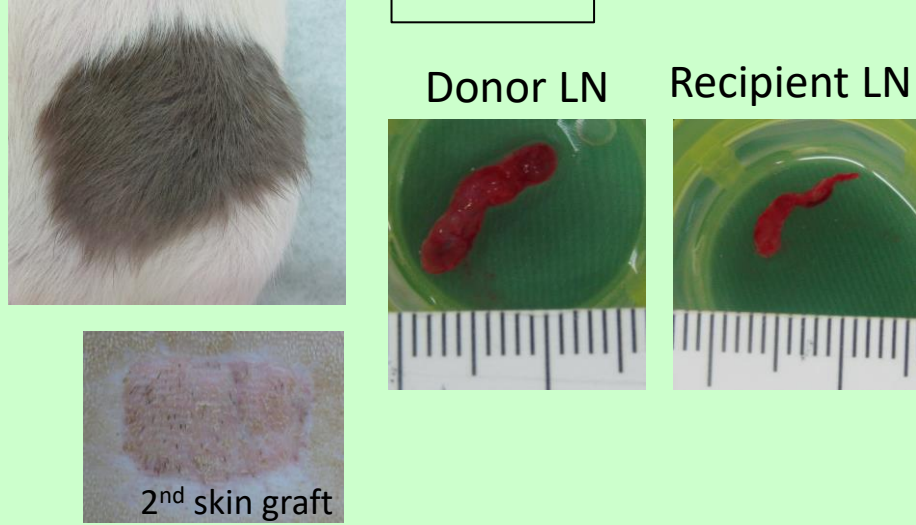


Tolerant

Donor LN

Recipient LN

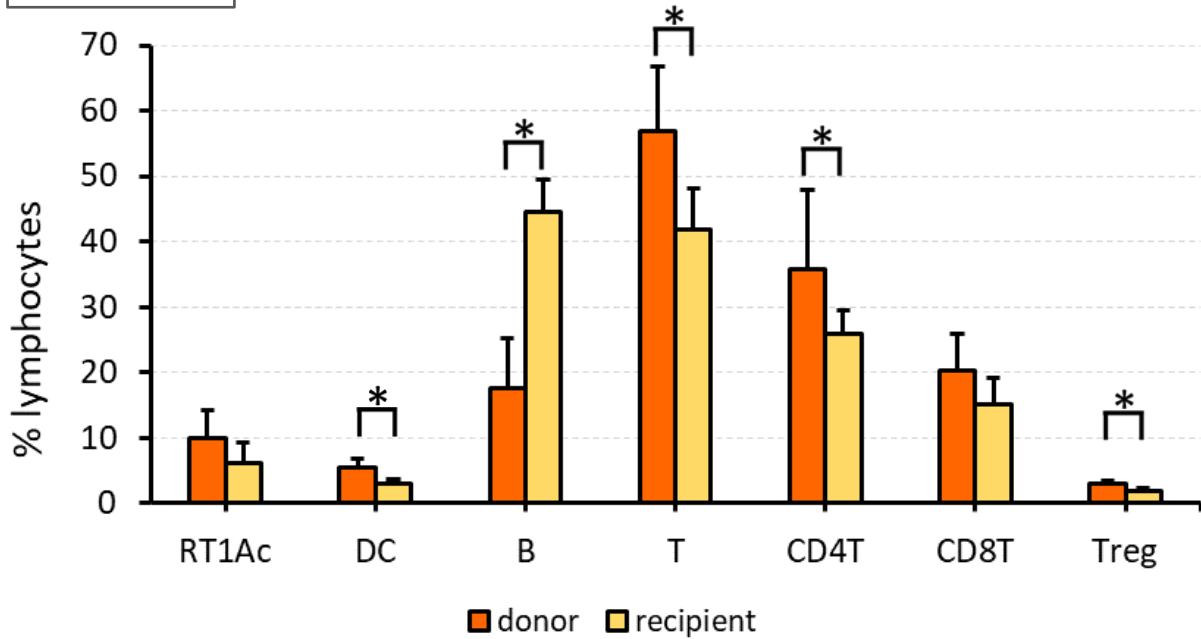
2nd skin graft



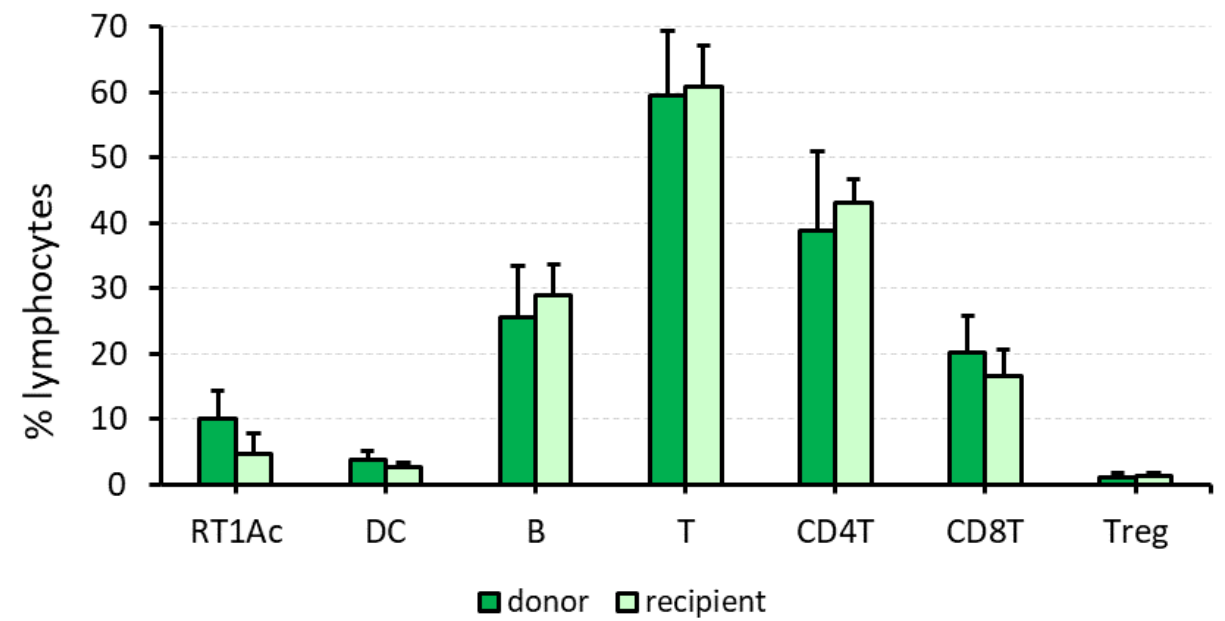
- Lymph nodes for**
- **Cell profiling (flow cytometry)**
 - **Gene profiling (RNAseq)**

Flow cytometric analysis showed uneven cellular distribution between donor- and recipient-originated lymph nodes

Rejection



Tolerance



N=6, Mean + stdev

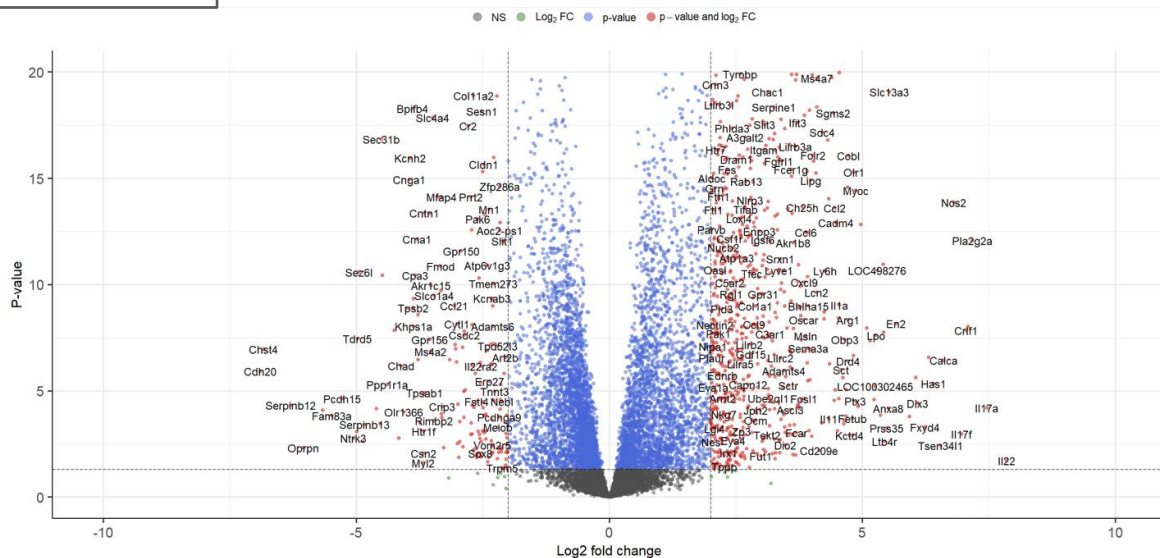
*: $p < 0.05$ by Student's t test

RNAseq revealed different degrees of gene expression alterations in donor vs recipient lymph nodes depending on VCA outcome

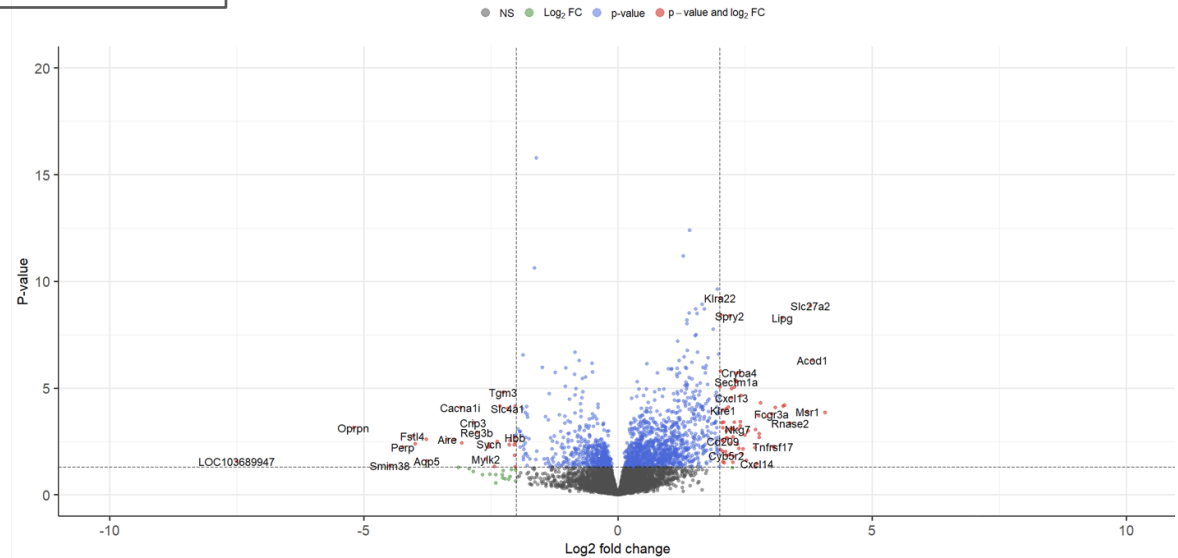
VCA outcome	characterized genes	donor vs recipient $p < 0.05$		donor vs recipient $p < 0.001$	
		up regulation	down regulation	up regulation	down regulation
Rejection	12019	1888	1028	1392	706
Tolerant	11105	216	35		

up regulation: ≥ 2 fold; down regulation: ≤ 0.5 fold

Rejection



Tolerance



Differentially expressed genes in donor LNs of rejecting VCA participate in various processes

Donor LN-upregulated genes: 1392

Category	Term	Gene count
Molecular function	Hydrolase	173
	Oxidoreductase	80
	Protease	74
	Cytokine	31
Cellular component	Secreted	225
	Lysosome	64
	Cell membrane	268
	Extracellular matrix	41
Biological process	Innate immunity	60
	Immunity	89
	Inflammatory response	32
	Chemotaxis	20
KEGG pathway	Lysosome	42
	Cytokine-cytokine receptor interaction	58
	Phagosome	47
	Rheumatoid arthritis	30

Donor LN-downregulated genes: 706

Category	Term	Gene count
Molecular function	DNA-binding	62
	Kinase	41
	Transferase	81
	Chromatin regulator	13
Cellular component	Nucleus	172
	Synapse	28
	Cell projection	39
	Extracellular matrix	13
Biological process	Transcription regulation	67
	Transcription	68
	Exocytosis	7
	Endocytosis	8
KEGG pathway	Phospholipase D signaling	13
	Phosphatidylinositol signaling	10
	Herpes simplex virus 1 infection	24
	Nicotinate and nicotinamide metabolism	6

Analyzed the $p < 0.001$ genes by *DAVID bioinformatics*, NIH

Differentially expressed genes in donor LNs of rejecting VCA participate in various processes

Donor LN-upregulated genes: 216

Donor LN-downregulated genes: 35

Category	Term	Gene count
Molecular function	Receptor	59
	Cytokine	8
	Tyrosine-protein kinase	5
	Serine protease	6
Cellular component	Cell membrane	55
	Membrane	123
	Secreted	30
	Cell junction	7
Biological process	Chemotaxis	10
	Innate immunity	16
	Immunity	22
KEGG pathway	Complement pathway	7
	Complement and coagulation cascades	14
	Staphylococcus aureus infection	12
	Viral protein interaction with cytokine and cytokine receptor	11
	Cytokine-cytokine receptor interaction	15

Category	Term	Gene count
Molecular function	Ion Channel	4
	Calmodulin-binding	3
	Voltage-gated channel	3
	Calcium channel	2
Cellular component	Cell membrane	8
Biological process	Ion transport	6
	Calcium transport	3
	Potassium transport	3
KEGG pathway	Transport	8
	Calcium signaling pathway	4
	African trypanosomiasis	2
	Malaria	2
	Circadian entrainment	2

Analyzed the $p < 0.05$ genes by *DAVID bioinformatics*, NIH

Conclusion

- Following VCA, the donor-derived LNs within the graft and the recipient's draining LNs exhibited different cellular and gene expression patterns depending on the fate of the VCA, whether it was rejection or the induced donor-specific tolerance.
- During VCA rejection, donor-derived LNs exhibited significantly higher levels of dendritic cells, CD4⁺ T cells, and Tregs, along with a lower level of B cells. There were also notable changes in gene expression, including those involved in activating lysosomes, immune responses, and chemotaxis, while suppressing the expression of certain nuclear proteins.
- When donor-specific tolerance was induced, the donor- and the recipient-derived LNs showed similar levels of B cells, DCs, and T cells, although donor LNs had significantly higher level of donor-originated cells. The gene expression patterns were more similar between the two, with fewer genes exhibiting significantly altered expression levels.

Acknowledgements

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