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Title	Laminins affect T cell trafficking and allograft fate
Author(s)	Warren, Kristi J.; Iwami, Daiki; Harris, Donald G. et al.
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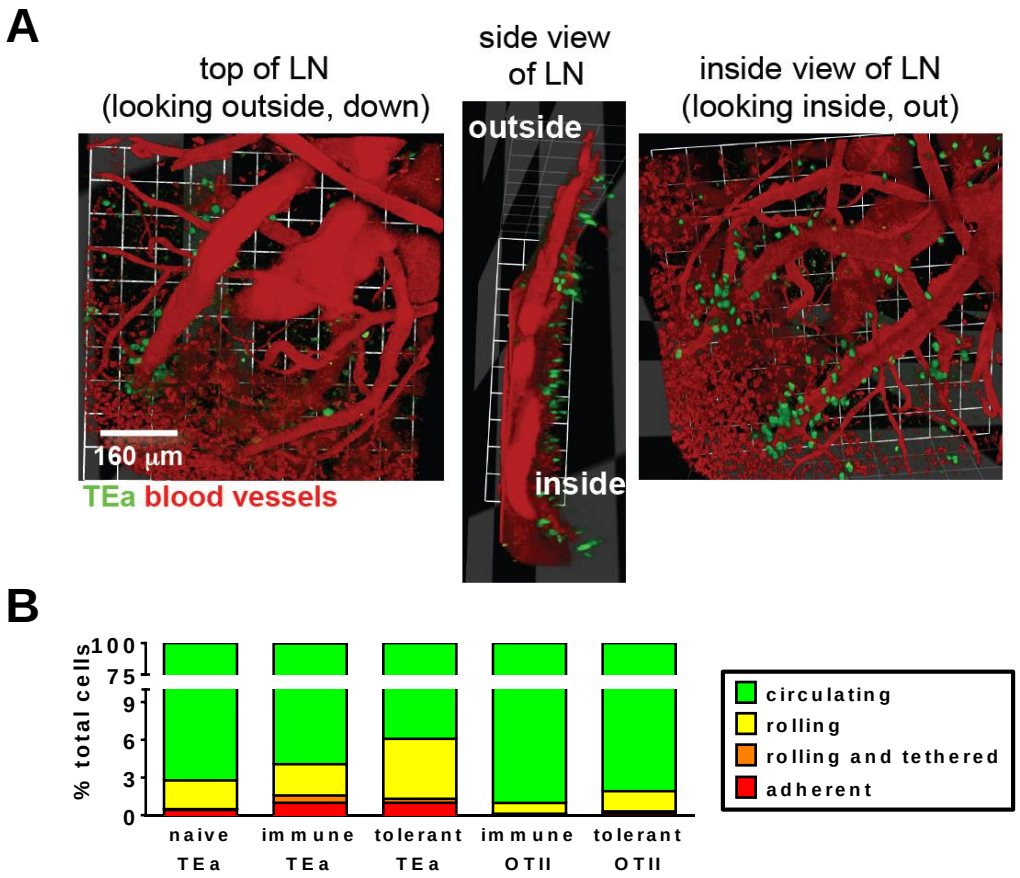
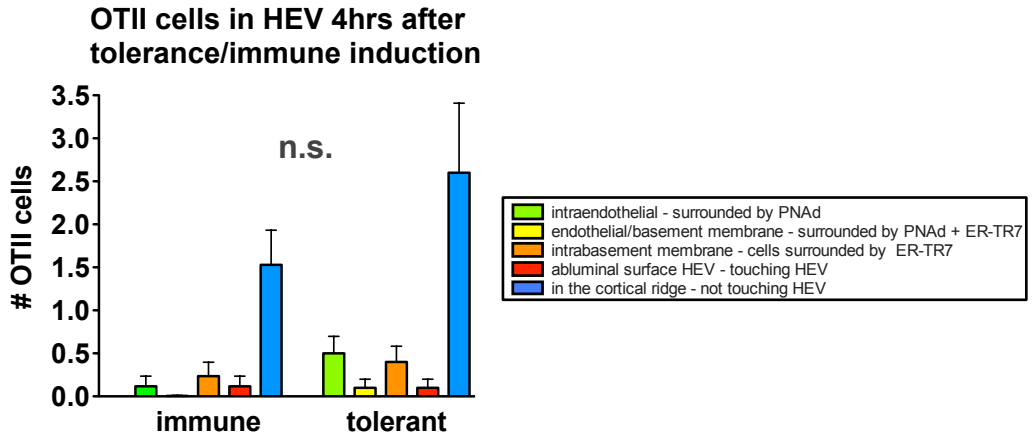
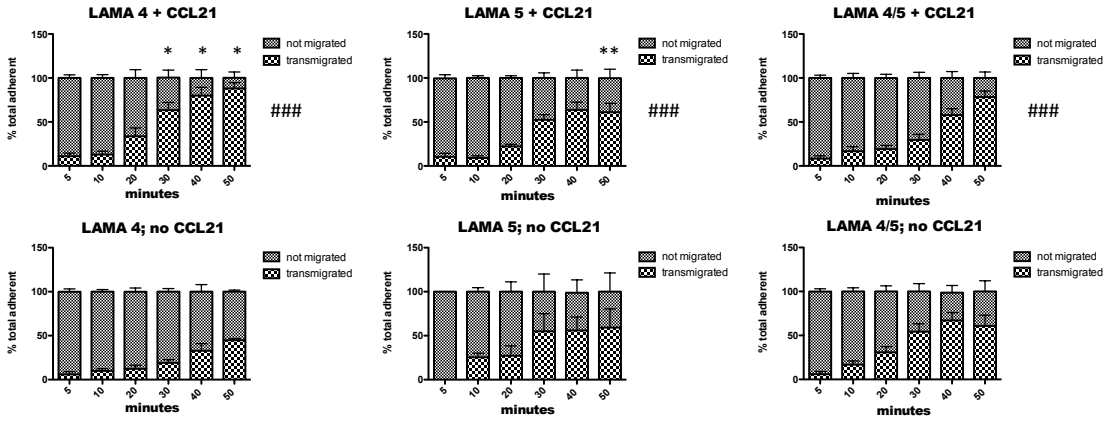
Supplemental Movies: Distinct patterns of CD4+ T cells trafficking through immune and tolerant LN are antigen dependent. Naïve mice received 20 x 10^6 alloantigen reactive CD4+ TEa cells (green) and 2 mg TRITC-70 kDa dextran (vasculature label, red, Movie 1 and 4). Immune (Movie 2 and 5) and tolerant (Movie 3 and 6) mice received CD4+ TEa cells (green), dextran (vasculature label, red), and 20 x 10^6 alloantigen irrelevant CD4+ OTII cells (blue). 800 images were captured over ~12 min. Representative movies are depicted 20 minutes (Movie 1, 2 and 3) and 60 minutes (Movie 4, 5 and 6) after cell transfer. 200x.



Supplemental Figure 1. (A) 3D rendering of LN from tolerized group, 4 hours after transfer, treated as in Figure 1. Images depict transferred CD4+ antigen specific TEa T cells moving from the HEV down into the medulla of the LN. Top view looking from the outside and top of the LN, down; side view looking at cross-section of the LN; and inside view looking from the inside out of the LN. 200x. (B) Changes in patterns of CD4+ T cell HEV LN trafficking are antigen dependent. CD4+ OTII cells labeled with Qtracker 655 (right two bars) and observed circulating through LN 20 min. after co-transfer with CD4+ TEa cells (left three bars). Data is depicted as the percent of total cells detected in circulation (green), rolling (yellow), rolling and tethered (orange) and adherent (red) as defined in Figure 1. Representative data from one representative time point and experiment of 3 is shown. n.s., not significant.



Supplemental Figure 2. T cell interaction with HEV is antigen specific. Antigen irrelevant CD4+ OTII cells transferred to immune or tolerized mice. Recipients euthanized 4 hours after cell transfer. LN harvested, 5 μm cryosections analyzed for cell migration and location by fluorescent immunohistochemistry of structures identified by ER-TR7+ stromal fibers and PNAd+ HEV. Cells categorized as intraendothelial, within endothelium/basement membrane, within basement membrane, outside HEV, and within cortical ridge as defined in Figure 3. n.s., not significant.



Supplemental Figure 3. Percentage of adherent T cells that transmigrated across MS-1 and laminins over a 50 minute imaging period. Laminar flow channels were coated with laminin α4, laminin α5, or laminin α4/α5, and seeded with mouse endothelial cells (MS-1). Channels were treated with CCL21 to induce transmigration (top graphs); no chemokine was used as a control (bottom graphs). Percentage of total adherent cells that migrated across MS-1 cells represented in the figures. Data presented as mean ± SEM. n = 4 replicates per experiment, experiment repeated 3 times. ### $p < 0.0005$ for a significant effect of treatment over time for the CCL21 groups. * $p < 0.05$ for less transmigration in the laminin α4/α5 + CCL21 treated channels at 30, 40 and 50 minutes as compared to laminin α4 + CCL21. $p < 0.005$ for a higher percentage of transmigrated CD4+ T cells in the laminin α4 + CCL21 treated channels compared to laminin α5 + CCL21.

Supplementary Table 1: Anti-laminin antibodies/peptide for in vivo use

in vivo	Source	Isotype	Reactivity	Target Molecule	Route	[Conc.]	per mouse	Company
	Rabbit		Mouse	LAMA 4	f.p.; i.v.	1 mg/mL	1 µg; 100 µg	Novus Biologicals
	Rabbit		Mouse,Human	LAMA 5	f.p.; i.v.	1 mg/mL	1 µg; 100 µg	Novus Biologicals
			Mouse	MMP14-i (peptide)	i.v.	3mg	100 µg	Genscript

Supplementary Table 2: Primary antibodies for immunohistochemistry

in vitro	Source	Isotype	Clone	Reactivity	Target Molecule	Dilution	Tissue	Company
Primary	Rat	IgG2a	ER-TR7	Mouse	ER-TR7	1:400	LN, SPL	BMA Biomedicals
	Rabbit	IgG	***	Mouse, Human	LAMA 4	1:100	LN, SPL	Novus Biologicals
	Rabbit	IgG	***	Mouse, Human	LAMA 5	1:100	LN, SPL	Novus Biologicals
	Goat	IgG	***	Mouse, Human	Collagen III	1:20	LN	Southern Biotech
	Rabbit	IgG	***	Mouse	FoxP3	1:200	LN, SPL	abcam
	Rat	IgM,κ	MECA-79	Mouse	PNAd	1:100	LN, SPL	BD Pharmingen
	Mouse	IgG2b	eBio Y-Ae	Mouse	I-Ab-Ea52-68 (Y-Ae)	1:50	LN, SPL	Ebioscience
	Arm.Hamster	IgG1, lambda2	HL3	Mouse	CD11c	1:100	LN, SPL	BD Pharmingen
	Rat	IgG2b, κ	eBio927	Mouse	PDCA-1	1:200	LN, SPL	Ebioscience

Supplementary Table 3: Secondary antibodies for immunohistochemistry

in vitro	Source	Isotype	Clone	Reactivity	Target Molecule	Flourophore	Dilution	Company
Secondary	Donkey	IgG	***	Rabbit	IgG(H+L)	Cy3	1:200	Jackson ImmunoResearch
	Goat	IgG	***	Arm.Hamster	IgG (H+L)	Cy5	1:400	Jackson ImmunoResearch
	Goat	IgG	***	Rabbit	IgG(H+L)	Cy5	1:200	Jackson ImmunoResearch
	Donkey	IgG	***	Rat	IgG(H+L)	DyLight 488	1:400	Jackson ImmunoResearch
	Goat	IgG	***	Rat	IgM (µ chain)	DyLight 405	1:50	Jackson ImmunoResearch
	Goat	IgG	***	Rat	IgG (H+L)	Cy5	1:200	Jackson ImmunoResearch
	Goat	IgG	***	Rat	IgM (µ chain)	Cy5	1:400	Jackson ImmunoResearch
	Donkey	IgG	***	Rat	IgG(H+L)	DyLight 405	1:50	Jackson ImmunoResearch
	Donkey	IgG	***	Goat	IgG(H+L)	DyLight 488	1:400	Jackson ImmunoResearch
	Goat	IgG	***	Rat	IgM (µ chain)	FITC	1:400	Jackson ImmunoResearch
	Donkey	IgG	***	Rabbit	Fab' fragment		1:100	Jackson ImmunoResearch
					streptavidin	Cy5	1:200	Jackson ImmunoResearch
					streptavidin	DyLight 405	1:50	Jackson ImmunoResearch