miRNA-181 Promotes Graft Prolongation by Plasmacytoid Dendritic Cells by increasing T Regulatory cells and Decreasing B cells as Revealed by Mass Cytometry (CyTOF)

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I have no financial relationships with commercial interests to disclose

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Introduction

Dendritic Cells (DC)

Major subsets are the conventional (c)DC and plasmacytoid (p)DC

Adapted from HX Zheng et al, Cytokine & Growth Factor Reviews 19 (2008): 333-337
**Plasmacytoid (p)DC**

- Important in innate and adaptive T cell responses to viral infections
- Type 1 IFN-producing cells
- Regulate immune reactivity
  * shown to be involved in Treg generation
- Hepatic pDC express lower MHCII and CD86 as well as have poor allostimulatory capacity compared to splenic pDC

**Methods – Freshly-Isolated DC**

- Liver
- Digestion
- Density gradient
- T cells
- B cells
- Flow Sort
- CD11c magnetic bead purification ~95% purity
- Bulk CD11c+ cells

**Adoptive Transfer and Transplant**

- Day -7: Bead-purified CD11c+ DC or Flow Sorted pDC (C57BL/6)
- i.v. injection (allogeneic BALB/c recipient)

- Day 0:
  - Allogeneic heterotopic cardiac allograft transplant (C57BL/6) graft
Liver transplant patients are more likely to attain tolerance to their graft

**What properties are inherent to hepatic pDC which promote tolerance?**

**microRNA**

~22 nucleotides long single-stranded RNA

Regulate one or more target genes by binding and degrading mRNA

A Harris et al, American J Transplant 10 (2010) 713-719
Affymetrix version 4 microRNA gene chip Profiles all known microRNA 113 differentially regulated microRNA (FDR 10%)

Differential microRNA profile of pDC

Differential mRNA expression in miR-181a1b1-/- KO pDC

Affymetrix Mu430-2 gene chip SAM analysis with FDR<10%
miR-181a1b1 modulates ~380 genes in pDC Genes involved in immune regulation and associated processes

miR-181a1b1 targets activation of pathways

- GSEA of >3000 gene sets identified 14 immune sets with FDR<30% and p<0.06
- Majority represented DC immune processes
CD86 and MHCII are more highly upregulated on miR-181αβ1−/− KO pDC

* p<0.05 by Wilcoxon sign rank test
n = 5-9

Loss of miR-181αβ1 abrogates the tolerogenic properties of WT pDC

Mantel-Cox log rank p<0.005

Analysis of Alloactivation by Cytometry Time-of-Flight (CyTOF, mass cytometry)

Examining the Alloimmune Response

Injection of pDC C57BL/6J
Heart transplant C57BL/6J
Spleen
BALB/cJ

Day -7  Day 0  Day +7

Analysis of Mass Cytometry Data: Citrus

• a method for unsupervised identification of significant cellular populations, clustered on the basis of the expression of markers studied

Each node is a specific cell population
Color scale
Node size

CyTOF: Differing alloimmune response in mice pre-treated with miR-181a1b1-/- pDC
Increased CD3+CD4+CD127lo T cells are detected in graft recipients that receive hepatic pDC

Increased B cells are detected in graft recipients that receive miR-181a1b1-/- pDC

FACS: Marginal Zone B cells and Plasmablasts are decreased in miR-181a1b1-expressing pDC treated mice

p < 0.05

WT n=5
KO n=3
CONCLUSIONS

miR-181a/b1 is a critical regulator of the tolerogenic properties of plasmacytoid dendritic cells

miRNA-181 promotes graft prolongation by pDC by increasing Treg cells and decreasing B cells

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