A novel Th17-prone CD146CCR5 T cell population as an early marker of intestinal graft-versus-host disease

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Proteomics Workflow

Pre-GI GVHD
~ D14 post-HSCT
(2w before GVHD signs)
Plasma pool 300 µl

No GVHD
~ D14 post-HSCT
Plasma pool 300 µl

Heavy isotope labeling ↔ Light isotope labeling

Tandem mass spectrometry

Data mining (Ratio of heavy/light > 1.5, system biology analysis)

2 unique and novel proteins: CD146 and CCL14 (CCR5 ligand)

Paczesny, Hanash, Zhang
CD146 and CCR5

**CD146**
Known endothelial receptor overexpressed during inflammation (Bardin et al., Inflamm Bowel Dis 2006)

Expression increased on activated T cells (Pickl et al., J. Immunol. 1997; Dagur et al., J Autoimmun 2011; Larochelle et al., Brain. 2012)

Endothelial CD146 allows entry of CD146 T cells in the gut (Xing et al. Am J Pathol 2014)

**CCR5**

CCR5 blockade inhibits lymphocyte trafficking in GVHD patients (Reshef et al., N Engl J Med. 2012)
Hypothesis

Increased blood CD146 or CCR5 or the double positive CD146CCR5 T cell frequencies are intestinal GVHD markers
CD146CCR5 T cell frequency is increased at onset of GI GVHD

- HD
- Auto
- GI GVHD
- No GVHD
- Non-GVHD Enteritis

% CD146CCR5 T cells

- p < 0.0001
- p < 0.0001

Table:

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Median day onset/Sample post-HSCT</th>
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<tbody>
<tr>
<td>HD</td>
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<td>29</td>
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<tr>
<td>Auto</td>
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<td>29</td>
</tr>
<tr>
<td>GI GVHD</td>
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<td>29</td>
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<tr>
<td>No GVHD</td>
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<tr>
<td>Non-GVHD Enteritis</td>
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<td>28</td>
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Gomez, Braun
CD146CCR5 T cell frequency is not correlated to other GI GVHD markers

R² = 0.07

% CD146CCR5 T cells vs Log REG3α

R² = 0.13

% CD146CCR5 T cells vs Log ST2
CD146CCR5 T cell frequency is increased prior to the clinical signs

GI GVHD

<table>
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<th>Median day post-HSCT</th>
<th>Pre</th>
<th>Onset</th>
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Non-GVHD Enteritis

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<th>Pre</th>
<th>Onset</th>
<th>p</th>
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p = 0.16

p = 0.83
CD146CCR5 T cells are Th17

Transcriptome (nanostring)

Intracellular Proteins (Flow Cytometry)

CXCR6
RORC
CCR6
IL23R
PDCD1
OX40
TBX21
TRAF4
CCR5

Fold change

% RORC

p = 0.04

% IL17

p < 0.001

N 35 35

N 41 41

Zhang, Gomez, Mumaw
Th17 cells express more CD146 than Th1 cells
ICOS stimulation is critical for the expression of CD146CCR5 on T cells
T cell transmigration model in GI GVHD

- CD146
- CCR5
- CCL14

Endothelium

Epithelium
T cell transmigration model in GI GVHD

Endothelium

Conditioning Cytokines

CD146
CCR5
CCL14

Epithelium
T cell transmigration model in GI GVHD

Endothelium

CD146

CCR5

CCL14

Epithelium

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Endothelium

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T cell transmigration model in GI GVHD

Endothelium

CD146
CCR5
CCL14

Epithelium

Conditioning Cytokines
T cell transmigration model in GI GVHD

Endothelium

Conditioning Cytokines

Epithelium

CD146

CCR5

CCL14
Endothelial CD146 is increased in GI GVHD colon biopsies

CD146

Non-GVHD enteritis

GI GVHD

CCL14

Intensity of epithelial CCL14

CD146+ vessels count, 10X

Hammer, Greenson
CD146 Th17 transmigration is reduced by CD146 shRNA knockdown on T cells

Knockdown of CD146 on endothelial cells did not reduce T-cell transmigration (not shown)

p = 0.0005

p = 0.0143
Donor human T cells knockdown with CD146 shRNA in xenogeneic GVHD model
Conclusions

The CD146CCR5 T cell population is a biomarker of GI GVHD with diagnostic and prognostic value

This population is Th17-prone

It traffics more efficiently through the endothelium which expresses more CD146 during GI GVHD

ICOS, CD146, CCR5 and RORC are potential therapeutic targets

Early measurement of this population in the blood may allow identification of patients at risk of GI GVHD and preemptive intervention
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