Sialic Acid Recognition is Key to Immune Regulation—Role of Siglecs

Bruce S. Bochner, MD
Samuel M. Feinberg Professor of Medicine

Northwestern University Feinberg School of Medicine
Division of Allergy-Immunology
Chicago, Illinois
bruce.bochner@northwestern.edu
Siglec-8

- Inhibitory receptor on eosinophils and mast cells

- Active antibodies to Siglec-8:
  - Are eosinophil-depleting (induces apoptosis)
  - Are mast cell-neutralizing (inhibits histamine and PGD$_2$ release)
  - Inhibit IgE-receptor-mediated human airway smooth muscle contraction
Mouse Siglec-F is the closest functional paralog to human Siglec-8

<table>
<thead>
<tr>
<th></th>
<th>Siglec-8</th>
<th>Siglec-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Mast cells</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Basophils</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Alv Mφ</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>T cells</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Monocytes</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Zhang et al., Blood 109:4280, 2007
Stevens et al., J Immunol Methods 327:63, 2007
In vivo effects of Siglec-F antibody

Decreased blood and tissue eosinophils; decreased GI and lung fibrosis

Zimmermann et al., Allergy 2008
Song et al., Clin Immunol 2009
Song et al., J Immunol 2009
Rubinstein et al., J Pediatr Gastroenterol Nutr 2011
What glycan ligands engage Siglec-8 and Siglec-F?
Consortium for Functional Glycomics glycan array screening for Siglec-8 and Siglec-F ligands

Siglec-8/-F Fc

Biotinylated glycosides

Streptavidin coated plate
Siglec-8 and Siglec-F recognize the exact same glycans

Bochner et al, JBC 2005 and Kiwamoto et al, JACI 2014
What tissue ligands engage Siglec-8 and Siglec-F?
Siglec-F ligands are detected in mouse lung using Siglec-F-Fc and increase during allergic inflammation.

Zhang et al., Blood 109:4280, 2007
See also Cho et al., Respir Research 11:154, 2010
Enzymes required for 6’-sulfo-sialyl Lewis X synthesis

6’-su-sLex

α2,3-sialyltransferases (St3gal1-5)

KSGal6ST (Chst1)

Four in lung

Lung epithelium
Siglec-F-Fc staining is lost in ST3Gal3−/− mouse lung

Guo et al., Am J Respir Cell Molec Biol 44:238, 2011
ST3Gal3−/− mice have exaggerated allergic eosinophilic lung inflammation

Kiwamoto et al, JACI 133:240-7, 2014
ST3Gal3−/− mice have exaggerated allergic eosinophilic lung inflammation

Kiwamoto et al, JACI 133:240-7, 2014
Siglec-F-Fc immunoprecipitates high molecular weight glycoproteins

**Flowchart:**
- **IP:** SiglecF-Fc
- **Blot:** Siglec-F-Fc
- **Lung lysate**
  - Sialidase -
  - Sialidase +
  - Reduction & alkylation
  - Trypsin digestion
  - Excise bands from gel
- **LC-MS/MS (LTQ Orbitrap)**
ST3Gal3⁻/⁻ mice have diminished expression of Siglec-F ligands in lung and airways

Kiwamoto et al, JACI 133:240-7, 2014
ST3Gal3<sup>+-</sup> airway epithelial cells are less effective in inducing eosinophil apoptosis in vitro.

Suzukawa et al, JI 190:5939-48, 2013
Summary

- Mouse airway epithelium and glands are sources of sialylated Siglec-F ligands that are diminished in ST3Gal3−/− mice.
- These lung ligands are increased during Th2-type inflammation.
- ST3Gal3−/− mice display a selectively enhanced lung eosinophilia during Th2-type inflammation.
- Sialidase-sensitive high molecular weight ligands are detected by western blotting of lung cell-derived lysates using Siglec-F-Fc.
Acknowledgments
Siglec-8

• The Johns Hopkins University School of Medicine
  – Ronald L. Schnaar et al (Pharmacology and Molecular Sciences)
• Yale University Medical Center
  – Zhou Zhu, mouse core
• Consortium for Functional Glycomics (Glycan Array Synthesis and Screening Cores)
  – Richard Alvarez et al., Oklahoma Health Sciences Center
  – Nicolai Bovin et al., Russian Academy of Sciences
  – David Smith, Richard Cummings et al., Emory University
• University of Georgia Complex Carbohydrate Research Center
  – Mike Tiemeyer et al
    • Analytical carbohydrate chemistry
• The Scripps Research Institute
  – James Paulson, Corwin Nycholat et al
    • Carbohydrate pharmacology and drug targeting

NIH grants R01AI41472, R01AI72265, P01 HL107151, GM62116 and the Dana Foundation