

## Journal Highlight: Leveraging Siglec-8 endocytic mechanisms to kill human eosinophils and malignant mast cells<sup>1</sup>

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Sialic acid-binding immunoglobulin-type lectin (Siglec)-8 is a receptor that is specifically expressed on eosinophils, mast cells, and basophils. These are important effector cells that play a role in host defense, but also have a pathological role in allergic diseases. Siglec-8 receptor is therefore a potential target for drug therapies for diseases involving these cells. In this study, O'Sullivan *et al.* (2018) studied the mechanism through which Siglec-8 is internalized into cells as a potential strategy for specifically loading them with targeted drugs. Using carefully selected inhibitors, they determined that Siglec-8 was not trafficked into the cell by pinocytosis or phagocytosis, but through clathrin- and caveolae/lipid raft-mediated pathways involving actin cytoskeletal rearrangement, as well as activation of tyrosine kinases and protein kinase C. In comparing the components involved in endocytosis and shuttling of Siglec-8, the authors found these processes to be independently regulated, involving actin rearrangement and tyrosine kinases; however, microtubule polymerization and phosphatidylinositol 3-kinase were only required for shuttling processes. Further, the researchers used confocal microscopy to reveal Siglec-8 colocalization with lysosomes, which indicated the movement of Siglec-8 to lysosomal compartments.

To explore the chemotherapeutic effects on Siglec-8, the researchers delivered saporin, a ribosome-inactivating protein, instead of Siglec-8 antibody, which usually requires eosinophils to be preactivated with cytokines for them to successfully undergo apoptosis. An antibody conjugated to saporin resulted in cell death in eosinophils and human mast cells without the release of pro-inflammatory mediators that normally result from nonapoptotic cell death. O'Sullivan *et al.* are also the first to effectively target Siglec-8 for mast cell death. Dr. Marc E. Rothenberg (Cincinnati Children's Hospital Medical Center, Ohio, USA) provided his expert insights: *"The paper is quite interesting. It starts off mapping out the endocytic pathway involved in the trafficking of Siglec-8. The pathway for endocytosis appears fairly typical of other plasma membrane proteins. But, then the authors go on to show that this pathway can be exploited to deliver a toxic "payload" to eosinophils and mast cells, inducing their death, presumably greater than anti-Siglec-8 antibody would do alone. As such, this strategy has considerable therapeutic potential to treat refractory eosinophilia or mastocytosis diseases."*

Question and Answer with senior author Dr. Bruce Bochner:

**Q:** How long have you been researching eosinophils, and what first inspired work with these cells?

**A:** Back in the 1980's during my fellowship, and in the 1990's during my initial years as a faculty member, I became interested in the mechanisms by which inflammation results in different patterns of leukocyte infiltration. Being interested in allergic inflammation, my work focused on how a basophil or eosinophil gets recruited into tissues, compared to a neutrophil or mononuclear leukocyte. This naturally led us to study leukocyte-endothelial interactions, adhesion molecules, and chemoattractants such as chemokines and their receptors. Finally, as a clinician, I was fascinated by how effective glucocorticosteroids were as anti-eosinophil, but not as anti-neutrophil or anti-mast cell agents, and whether that was differing pharmacology was due to effects on cell trafficking or other mechanisms.

**Q:** Specifically, your lab focuses primarily on Siglec-8 receptor. How did you come up with the idea to look at this receptor specifically and what led to this study?

**A:** The discovery of Siglec-8 was a bit fortuitous. It occurred as a result of a collaboration between myself and other colleagues at Johns Hopkins, and two companies: GlaxoSmithKline and Human Genome

Sciences. The project was based on the hypothesis that human eosinophils would possess cell type-specific genes, besides say eosinophil peroxidase, some of which could potentially make good drug targets. Keep in mind that this discovery effort occurred before the human genome had been sequenced. The discovery of Siglec-8 was made based on the detection of novel CD33-like sequences (CD33 is another Siglec) in a human eosinophil cDNA library. We had no idea what its function or true expression pattern would be like, but it quickly became clear that Siglec-8 was selectively expressed not just on human eosinophils, but also on human mast cells and weakly on basophils, and no other leukocytes. Another hurdle was the fact that Siglec-8 was only expressed in humans, chimps, and some great apes, but no lower species.<sup>2</sup> Nevertheless, this did not dissuade us from pursuing NIH-funded research to understand its function, expression, natural ligands and mechanisms of cell signaling.

**Q: Could you please elaborate on the implications of these novel findings in the clinical setting (as there is an anti-Siglec-8 antibody in clinical trials)?**

**A:** The key finding in Dr. O'Sullivan's work was based on the knowledge that most, if not all, Siglecs get internalized once engaged by ligands.<sup>1</sup> This biology opens up the possibility of using this phenomenon to deliver drug payloads. Siglec-8 antibody conjugated with a ribosomal toxin not only got internalized into eosinophils (and mast cells), but resulted in effective killing that was not seen in situations where the antibody alone did not cause killing. Based on experiments in which HEK293T cells were transduced with the full-length form of Siglec-8 or versions in which the tyrosine residues in the cytoplasmic signaling motifs were mutated to phenylalanine, he found that the ITIM and not the ITSM was necessary for ligation-induced Siglec-8 endocytosis. The therapeutic strategy being advanced by Allakos (AK002) focuses on having humanized a mouse anti-Siglec-8 antibody into a non-fucosylated IgG1 antibody, which should possess the ability to also deplete Siglec-8 expressing cells like eosinophils via antibody-dependent cellular cytotoxicity (ADCC). While phase 1 studies in normal volunteers showed rapid depletion of eosinophils after IV infusion of AK002, ongoing studies in clinical trials for four different eosinophil and mast cell-associated conditions are looking at both anti-eosinophil and anti-mast cell effects.

**Q: What are your next steps with this research?**

**A:** We are currently funded to work with collaborators at Scripps (Drs. James Paulson and Corwin Nycholat) because they have the expertise to develop liposomes and other nanoparticles for the exact kind of targeting we want to do. For example, they can coat them with either Siglec-8 antibodies or Siglec-8-specific glycomimetic ligands for targeting, and within the particle can load things such as toxins, glucocorticosteroids, or small molecule inhibitors of signaling. We will then use these materials in vitro and in vivo (using Siglec-8 knock-in mice developed by Dr. O'Sullivan et al<sup>3</sup>) to selectively inhibit or eliminate eosinophils and mast cells.

**Q: How would you explain your findings to a non-specialist audience?**

**A:** Because eosinophils and mast cells, cells involved in allergic diseases, need to be inactivated or eliminated to effectively treat allergies, we are taking advantage of the fact that they have a very unique protein on their surface called Siglec-8 that we want to use as a drug bulls-eye. The approach is to develop a treatment that allows a drug to be selectively delivered into these cells, kind of like a Trojan horse strategy, whereby the drug will only get inside these cells and not others, causing them to either die off or have their functions inactivated.

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1. O'Sullivan JA, Carroll DJ, Cao Y, Salicru AN, Bochner BS. Leveraging Siglec-8 endocytic mechanisms to kill human eosinophils and malignant mast cells. *J Allergy Clin Immunol.* 141:1774-85, 2018.

2. Hudson SA, Herrmann H, Du J, Cox P, Haddad el B, Butler B, *et al.* Developmental, malignancy-related, and cross-species analysis of eosinophil, mast cell, and basophil Siglec-8 expression. *J Clin Immunol.* 31:1045-53, 2011).

3. O'Sullivan JA, Wei Y, Carroll DJ, Moreno-Vinasco L, Cao Y, Zhang F, *et al.* Frontline Science: Characterization of a novel mouse strain expressing human Siglec-8 only on eosinophils. *J Leukoc Biol.* 104:11-9, 2018.