Siglec-8

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Siglecs (sialic acid-binding immunoglobulin-like lectins) are single-pass cell surface receptors that have inhibitory activities on immune cells. Among these, Siglec-8 is a CD33-related family member selectively expressed on human mast cells and eosinophils, and at low levels on basophils.

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1. Introduction

Siglecs (sialic acid-binding immunoglobulin-like lectins) are single-pass cell surface receptors that contain sialic acid-binding N-terminal V-set domains[1][2]. These receptors are predominately found on immune cells and most have immunoreceptor tyrosine-based motifs (ITIMs) that are involved in inhibitory cell signaling. The Siglec family of cell surface receptors have emerged as attractive therapeutic targets due to their restricted expression profile on immune cells and their immunomodulatory activities[3][4][5][6].

Mast cells and eosinophils are effector cells in the pathogenesis of many allergic and non-allergic diseases[7][8]. Of all the Siglecs, Siglec-8 is the only one found selectively on mast cells, eosinophils, and to a lesser extent, basophils[9][10]. Siglec-8 was discovered more than 20 years ago in a cDNA library generated from a subject with hypereosinophilic syndrome (HES)[9][10].

2. Siglecs and the Discovery of Siglec-8

Siglecs, formerly called sialoadhesins or sialoadhesin factors (SAF) among other names, is a term that was adopted in 1998 to describe a subset of I-type lectins within the immunoglobulin gene superfamily that bind sialylated glycans and share certain structural motifs within their N-terminal and C-2 set domains[11]. At the time this nomenclature was adopted, only a few Siglecs were known. Since then, the list has expanded to include 10 human and 5 mouse Siglecs, belonging to Siglec-3 (CD33)-related subgroups, and 4 Siglecs were conserved between humans and mice (Siglec-1, Siglec-2 (CD22), Siglec-4, and Siglec-15). Besides the shared extracellular structural characteristics that define them as Siglecs, most possess tyrosine-based signaling motifs in the form of immunoreceptor tyrosine-based inhibitory or switch motifs (ITIMs and ITSMs, respectively). A small number of Siglecs lack such signaling domains because they have very short cytoplasmic tails. Examples include Siglec-14 and Siglec-16 in humans, Siglec-H in mice, and Siglec-15 in mice and humans. Each of these Siglecs lacking ITIM or ITSM domains contain a charged amino acid in their transmembrane domain that allows them to co-associate in the cell membrane with adapter molecules, such as DAP-12, to mediate SYK-dependent signaling.

Siglec-8 was discovered as a result of a joint effort between the Bochner and Schleimer laboratories at The Johns Hopkins University School of Medicine, along with scientists in the Department of Immunology, SmithKline Beecham Pharmaceuticals, and at Human Genome Sciences, Inc. Based on random, high-throughput EST (expressed sequence tag) sequencing, a clone from a cDNA library generated from a donor in the Bochner lab with a very high eosinophil blood count due to a form of HES was identified that possessed novel sequences with high homology to Siglec-7, Siglec-6, Siglec-5 and CD33. Simultaneously, the Crocker lab, via a separate collaboration with scientists at Human Genome Sciences, designed to identify novel Siglecs, discovered the same human eosinophil clone and sequence. Parallel investigations in both labs resulted in the publication of two papers in 2000 describing Siglec-8 (or sialoadhesin factor-2, SAF-2) as highly and selectively expressed on eosinophils [9], but also on human mast cells and very weakly on basophils [10]. As initially discovered, Siglec-8 contained a short cytoplasmic tail devoid of signaling motifs. This so-called short form was subsequently shown to result from a premature stop codon; the full-length mRNA encoded a form of Siglec-8 with a membrane-proximal ITIM and a membrane-distal ITSM that was subsequently shown to be the more predominant form of Siglec-8[12][13].

Soon after Siglec-8 was discovered, mouse mAbs were generated[9][10] and the 2E2 Siglec-8 mAb clone was licensed in 2012 to Allakos, Inc. These have been the foundation of efforts at Allakos, Inc. to develop mAbs against Siglec-8 for clinical use.

3. Expression Pattern of Siglec-8

Siglec-8 is selectively expressed on human eosinophils, mast cells, and to a lesser extent, basophils, with ~18,000–22,000 Siglec-8 receptors/cell on eosinophils and mast cells and ~500 receptors/cell on basophils[14]. While it appears that the transcription factor Olig2 (Oligodendrocyte transcription factor 2) may participate in the control of Siglec-8 expression[15], more needs to be learned about how Siglec-8 expression is regulated. Unlike other receptors expressed on eosinophils, such as IL-5Rα, the expression of Siglec-8 is stable between blood and tissue compartments, suggesting Siglec-8 remains targetable by antibodies on both blood and tissue eosinophils[14]. Several groups have recently examined the expression pattern of Siglec-8 on eosinophils and mast cells during inflammatory disease states, including HES, asthma, eosinophilic esophagitis (EoE), eosinophilic gastritis (EG), and systemic mastocytosis (SM). The expression of Siglec-8 on blood eosinophils is similarly prominent in patients with eosinophilic diseases, including multiple variants of HES and EoE[16]. In addition, the levels of Siglec-8 remain stable on blood eosinophils after patients received treatment with prednisone or imatinib, despite significant decreases in absolute eosinophil counts [16]. Airway tissue eosinophils from bronchoalveolar lavage and sputum from subjects with asthma have similar levels of surface Siglec-8 as their blood eosinophils[17][18]. Likewise, Siglec-8 expression is comparable on gastrointestinal tissue eosinophils and mast cells from patients with EoE or EG, compared to tissues from control subjects without these diseases, despite elevated numbers and an activated phenotype of these cells in these diseases[19]. Similarly, mature bone marrow mast cells from patients with SM expressed Siglec-8 at levels similar to those observed in other tissues[20][21]. The expression of Siglec-8 remained low on basophils from patients with and without EoE or EG. Interestingly, a soluble form of Siglec-8 can be detected in the serum of some individuals using an ELISA, but the clinical significance and origin of this form of Siglec-8 remains unknown[16]. While flow cytometry is a powerful method for evaluating the expression of Siglec-8, as described above, we have found specificity differences between commercially available Siglec-8 antibodies due to the high sequence identity between some of the CD33-related family members. Using a Siglec-based cross-reactive ELISA, the Siglec-8 mAb clones FAB7975 (R&D Systems, Minneapolis, MN, USA) and 347104 (Biolegend, San Diego, CA, USA) were found to bind specifically to Siglec-8, whereas HPA012556 (Sigma and Atlas) was found to cross-react with multiple Siglecs, including Siglec-9, -7, and -12 (unpublished observations). These studies suggest that Siglec-8 expression is robust and stable on eosinophils and mast cells independent of disease state and tissue of origin.

Siglec-8 is not expressed by hematopoietic stem cells, or eosinophil or mast cell precursors. Based on in vitro studies in which cells were grown from precursors, Siglec-8 is only expressed during late stages of mast cell and eosinophil maturation[21][22]. Siglec-8 was not expressed by any eosinophil cell line tested and was expressed at low levels by the LAD2, LUVA, and HMC 1.2 mast cell lines[10][21][22] (unpublished observations). Like many CD33-related Siglecs, Siglec-8 is only expressed at the human and ape level, and is not detected on rhesus or cynomolgus monkey eosinophils[21]. Furthermore, Siglec-8 does not have a true mouse ortholog—the closest functional paralog in mice is Siglec-F, which is expressed on eosinophils (and other cells such as alveolar macrophages, tuft cells, granulocyte-macrophage progenitors and sometimes on certain tissue neutrophils that co-express Siglec-E) but not mast cells[23][24][25][26][27][28][29][30] (see Table 1 for Siglec-F and Siglec-8 comparison). Researchers in the Bochner laboratory and at Allakos, Inc. have developed strains of mice that selectively express Siglec-8 in the eosinophil compartment (SIGLEC8Eo), in the mast cell compartment (Mcpt5-Siglec8 and Cpa3-Siglec8), or on eosinophils, mast cells, and basophils (Siglec-8 transgenic)[19][31][32]. Strains that express Siglec-8 on specific immune cells rely on cell-specific or cell-selective Cre expression to remove a STOP cassette and allow for the discrimination between the effects of Siglec-8 on each cell population. Siglec-8 transgenic mice that express the human SIGLEC8 gene, including the putative promoter and regulatory elements, most accurately mimic the expression of Siglec-8 in humans. The SIGLEC8Eo strain has been crossed with the Siglec-F null strain, to create mice that express Siglec-8 but not Siglec-F[33]. These mice are useful for testing antibodies (see below) and glycomimetics that preferentially bind to Siglec-8, and for determining their specificity of targetin[34]. In addition, mice with humanized immune systems (engrafted with human thymus, liver, or hematopoietic stem cells) that express SCF, GM-CSF, and IL-3 (NSG-SGM3) generate human mast cells and eosinophils that express functional Siglec-8[35].

Table 1. Comparison of Siglec-8 and Siglec-F.

	Siglec-8	Siglec-F

Surface Expression		
Eosinophils	Yes	Yes
Mast cells	Yes	No
Basophils	Yes; weak	No
Alveolar macrophages	No	Yes
Neutrophils	No	Sometimes
T cells	No	No or minimal
Monocytes	No	No
Intestinal tuft/M cells	No	Yes
Expression is at least in part regulated by the transcription factor Olig2	Yes	Unknown
Ligands		
6'-*S*-Sialyl-LacNac	Yes	Yes
6'-*S*-Sialyl-Lewis X	Yes	Yes
Tri and tetra-antennary bisected glycans containing α2,3-linked terminal sialic acid	No	Yes
Sialylated keratan sulfate chains on human aggrecan	Yes	Unknown
Sialylated keratan sulfate chains on human DMBT1	Yes	Unknown
Mouse Muc5b glycans	No	Yes
9-*N*-(2-naphthyl-sulfonyl)-Neu5Acα2-3-[6-*O*-sulfo]-Galβ1-4GlcNAc (6'-*O*-sulfo (NSA)Neu5Ac)	Yes	Yes
6′-sulfo-sialyl Lewis X mimetic retaining the neuraminic acid core, but with a carbocyclic mimetic of the Gal moiety and a sulfonamide substituent in the 9-position	Yes	Unknown
Function		
Eosinophils in vitro*: Non-cytokine primed*		

Crosslinking with antibody induces eosinophil death in non-cytokine-primed cells	Yes; modest	Yes; weak
Death that is caspase-dependent	Yes	Yes
Death that is integrin- and ROS-dependent	No	No
Death that is NADPH oxidase-dependent	No	No
Death is associated with mitochondrial membrane damage	Yes	Yes
Receptor internalized after ligation	Yes	Yes
Eosinophils in vitro*: Cytokine primed*		
Crosslinking with antibody or multivalent ligand induces eosinophil death in cytokine-primed cells	Yes; marked	Yes; weak
Death that is caspase-dependent	No	Yes
Death that is beta-2 integrin- and ROS-dependent	Yes	No
Death that is NADPH oxidase-dependent	Yes	No
Death that is associated with mitochondrial membrane-damage	Yes	Yes
Role for SHP-1 phosphatase in cell death	No	No
Role for MAP kinases in cell death	Yes	Unknown
Mast cells in vitro		
Crosslinking induces cell death	No	Not applicable
Inhibition of IgE receptor-mediated degranulation	Yes	Not applicable
Inhibition of IL-33-stimulated responses	Yes	Not applicable
Receptor internalized after ligation	Yes	Not applicable
Internalization of a toxic payload after ligation causes cell death	Yes	Not applicable

Siglec, sialic acid-binding immunoglobulin-like lectins; DMBT1, deleted in malignant brain tumors 1; Gal, galactose; ROS, reactive oxygen species; MAP, mitogen-activated protein.

References

1. Macauley, M.S.; Crocker, P.R.; Paulson, J.C. Siglec-mediated regulation of immune cell function in disease. Nat. Rev. Immunol. 2014, 14, 653–666.

2. Varki, A.; Angata, T. Siglecs—the major subfamily of I-type lectins. Glycobiology 2006, 16, 1R–27R.

3. O'Sullivan, J.A.; Chang, A.T.; Youngblood, B.A.; Bochner, B.S. Eosinophil and mast cell Siglecs: From biology to drug target. J. Leukoc. Biol. 2020, 108, 73–81.

4. Jandus, C.; Simon, H.U.; von Gunten, S. Targeting siglecs—A novel pharmacological strategy for immuno-and glycotherapy. Biochem. Pharmacol. 2011, 82, 323–332.

5. O'Reilly, M.K.; Paulson, J.C. Siglecs as targets for therapy in immune-cell-mediated disease. Trends Pharmacol. Sci. 2009, 30, 240–248.

6. Barenwaldt, A.; Laubli, H. The sialoglycan-Siglec glyco-immune checkpoint—a target for improving innate and adaptive anti-cancer immunity. Expert Opin. Ther. Targets 2019, 23, 839–853.

7. Klion, A.D.; Ackerman, S.J.; Bochner, B.S. Contributions of Eosinophils to Human Health and Disease. Annu. Rev. Pathol. 2020, 15, 179–209.

8. Robida, P.A.; Puzzovio, P.G.; Pahima, H.; Levi-Schaffer, F.; Bochner, B.S. Human eosinophils and mast cells: Birds of a feather flock together. Immunol. Rev. 2018, 282, 151–167.

9. Floyd, H.; Ni, J.; Cornish, A.L.; Zeng, Z.; Liu, D.; Carter, K.C.; Steel, J.; Crocker, P.R. Siglec-8. A novel eosinophil-specific member of the immunoglobulin superfamily. J. Biol. Chem. 2000, 275, 861–866.

10. Kikly, K.K.; Bochner, B.S.; Freeman, S.D.; Tan, K.B.; Gallagher, K.T.; D'Alessio, K.J.; Holmes, S.D.; Abrahamson, J.A.; Erickson-Miller, C.L.; Murdock, P.R.; et al. Identification of SAF-2, a novel siglec expressed on eosinophils, mast cells, and basophils. J. Allergy Clin. Immunol. 2000, 105, 1093–1100.

11. Crocker, P.R.; Clark, E.A.; Filbin, M.; Gordon, S.; Jones, Y.; Kehrl, J.H.; Kelm, S.; Le Douarin, N.; Powell, L.; Roder, J.; et al. Siglecs: A family of sialic-acid binding lectins. *Glycobiology* **1998**, *8*, 5–6, 10.1093/oxfordjournals.glycob.a018832.

12. Foussias, G.; Yousef, G.M.; Diamandis, E.P. Molecular characterization of a Siglec-8 variant containing cytoplasmic tyrosine-based motifs, and mapping of the Siglec-8 gene. Biochem. Biophys. Res. Commun. 2000, 278, 775–781.

13. Aizawa, H.; Plitt, J.; Bochner, B.S. Human eosinophils express two Siglec-8 splice variants. J. Allergy Clin. Immunol. 2002, 109, 176.

14. Bradford A. Youngblood; Emily C. Brock; John Leung; Rustom Falahati; Paul J. Bryce; Jessica Bright; Jason Williams; Leonard D. Shultz; Dale L. Greiner; Michael A. Brehm; et al. AK002, a Humanized Sialic Acid-Binding Immunoglobulin-Like Lectin-8 Antibody that Induces Antibody-Dependent Cell-Mediated Cytotoxicity against Human Eosinophils and Inhibits Mast Cell-Mediated Anaphylaxis in Mice. *International Archives of Allergy and Immunology* **2019**, *180*, 91-102, 10.1159/000501637.

15. Sae Mi Hwang; Tae Gi Uhm; Seol Kyung Lee; Su-Kang Kong; Il Yup Chung; Kyung Hwa Jung; Bert Binas; Young Gyu Chai; Sung Woo Park; Olig2 is expressed late in human eosinophil development and controls Siglec-8 expression. *Journal of Leukocyte Biology* **2016**, *100*, 711-723, 10.1189/jlb.1a0715-314rrr.

16. Fanny Legrand; Yun Cao; Joshua B. Wechsler; Xiang Zhu; Nives Zimmermann; Shakuntala Rampertaap; Joseph Monsale; Kimberly Romito; Bradford A. Youngblood; Emily C. Brock; et al. Sialic acid–binding immunoglobulin-like lectin (Siglec) 8 in patients with eosinophilic disorders: Receptor expression and targeting using chimeric antibodies. *Journal of Allergy and Clinical Immunology* **2019**, *143*, 2227-2237.e10, 10.1016/j.jaci.2018.10.066.

17. Johansson, M.W.; Kelly, E.A.; Nguyen, C.L.; Jarjour, N.N.; Bochner, B.S. Characterization of Siglec-8 Expression on Lavage Cells after Segmental Lung Allergen Challenge. Int. Arch. Allergy Immunol. 2018, 177, 16–28.

18. Kerr, S.C.; Gonzalez, J.R.; Schanin, J.; Peters, M.C.; Lambrecht, B.N.; Brock, E.C.; Charbit, A.; Mark Ansel, K.; Youngblood, B.A.; Fahy, J.V. An anti-siglec-8 antibody depletes sputum eosinophils from asthmatic subjects and inhibits lung mast cells. Clin. Exp. Allergy 2020.

19. Youngblood, B.A.; Brock, E.C.; Leung, J.; Falahati, R.; Bochner, B.S.; Rasmussen, H.S.; Peterson, K.; Bebbington, C.; Tomasevic, N. Siglec-8 antibody reduces eosinophils and mast cells in a transgenic mouse model of eosinophilic gastroenteritis. JCI Insight 2019, 4.

20. Youngblood, B.A.; Brock, E.C.; Leung, J.; Chang, A.T.; Bebbington, C.; Tomasevic, N. AK002, a novel humanized monoclonal antibody to siglec-8, inhibits mast cell activity and depletes eosinophils in ex vivo bone marrow tissue from patients with systemic mastocytosis. Blood 2018, 132, 1104.

21. Hudson, S.A.; Herrmann, H.; Du, J.; Cox, P.; El Haddad, B.; Butler, B.; Crocker, P.R.; Ackerman, S.J.; Valent, P.; Bochner, B.S. Developmental, malignancy-related, and cross-species analysis of eosinophil, mast cell, and basophil siglec-8 expression. J. Clin. Immunol. 2011, 31, 1045–1053.

22. H. Yokoi; A. Myers; K. Matsumoto; Paul R Crocker; Hirohisa Saito; B. S. Bochner; Alteration and acquisition of Siglecs during in vitro maturation of CD34+ progenitors into human mast cells. *Allergy* **2006**, *61*, 769-776, 10.1111/j.1398-999

5.2006.01133.x.

23. Aizawa, H.; Zimmermann, N.; Carrigan, P.E.; Lee, J.J.; Rothenberg, M.E.; Bochner, B.S. Molecular analysis of human Siglec-8 orthologs relevant to mouse eosinophils: Identification of mouse orthologs of Siglec-5 (mSiglec-F) and Siglec-10 (mSiglec-G). Genomics 2003, 82, 521–530.
24. Zhang, J.Q.; Biedermann, B.; Nitschke, L.; Crocker, P.R. The murine inhibitory receptor mSiglec-E is expressed broadly on cells of the innate immune system whereas mSiglec-F is restricted to eosinophils. Eur. J. Immunol. 2004, 34, 1175–1184.
25. Tateno, H.; Crocker, P.R.; Paulson, J.C. Mouse Siglec-F and human Siglec-8 are functionally convergent paralogs that are selectively expressed on eosinophils and recognize 6’-sulfo-sialyl Lewis X as a preferred glycan ligand. Glycobiology 2005, 15, 1125–1135.
26. Dyer, K.D.; Moser, J.M.; Czapiga, M.; Siegel, S.J.; Percopo, C.M.; Rosenberg, H.F. Functionally competent eosinophils differentiated ex vivo in high purity from normal mouse bone marrow. J. Immunol. 2008, 181, 4004–4009.
27. Gerbe, F.; Sidot, E.; Smyth, D.J.; Ohmoto, M.; Matsumoto, I.; Dardalhon, V.; Cesses, P.; Garnier, L.; Pouzolles, M.; Brulin, B.; et al. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. Nature 2016, 529, 226–230.
28. Gicheva, N.; Macauley, M.S.; Arlian, B.M.; Paulson, J.C.; Kawasaki, N. Siglec-F is a novel intestinal M cell marker. Biochem. Biophys. Res. Commun. 2016, 479, 1–4.
29. Bolden, J.E.; Lucas, E.C.; Zhou, G.; O’Sullivan, J.A.; de Graaf, C.A.; McKenzie, M.D.; Di Rago, L.; Baldwin, T.M.; Shortt, J.; Alexander, W.S.; et al. Identification of a Siglec-F+ granulocyte-macrophage progenitor. J. Leukoc. Biol. 2018, 104, 123–133.
30. Pfirschke, C.; Engblom, C.; Gungabeesoon, J.; Lin, Y.; Rickelt, S.; Zilionis, R.; Messemaker, M.; Siwicki, M.; Gerhard, G.M.; Kohl, A.; et al. Tumor-Promoting Ly-6G(+) SiglecF(high) Cells Are Mature and Long-Lived Neutrophils. Cell Rep. 2020, 32, 108164.
31. O’Sullivan, J.A.; Wei, Y.; Carroll, D.J.; Moreno-Vinasco, L.; Cao, Y.; Zhang, F.; Lee, J.J.; Zhu, Z.; Bochner, B.S. Frontline Science: Characterization of a novel mouse strain expressing human Siglec-8 only on eosinophils. J. Leukoc. Biol. 2018, 104, 11–19.
32. Wei, Y.; Chhiba, K.D.; Zhang, F.; Ye, X.; Wang, L.; Zhang, L.; Robida, P.A.; Moreno-Vinasco, L.; Schnaar, R.L.; Roers, A.; et al. Mast Cell-Specific Expression of Human Siglec-8 in Conditional Knock-in Mice. Int. J. Mol. Sci. 2018, 20, 19.
33. Eva Knuplez; Rebecca Krier-Burris; Yun Cao; Gunther Marsche; Jeremy O'sullivan; Bruce S. Bochner; Frontline Science: Superior mouse eosinophil depletion in vivo targeting transgenic Siglec-8 instead of endogenous Siglec-F: Mechanisms and pitfalls. *Journal of Leukocyte Biology* **2020**, *108*, 43-58, 10.1002/jlb.3hi0120-381r.
34. Corwin M. Nycholat; Shiteng Duan; Eva Knuplez; Charli Worth; Mila Elich; Anzhi Yao; Jeremy O’Sullivan; Ryan McBride; Yadong Wei; Steve M. Fernandes; et al. A Sulfonamide Sialoside Analogue for Targeting Siglec-8 and -F on Immune Cells. *Journal of the American Chemical Society* **2019**, *141*, 14032-14037, 10.1021/jacs.9b05769.
35. Paul J. Bryce; Rustom Falahati; Laurie L. Kenney; John Leung; Christopher Bebbington; Nenad Tomasevic; Rebecca A. Krier; Chia-Lin Hsu; Leonard D. Shultz; Dale L. Greiner; et al. Humanized mouse model of mast cell–mediated passive cutaneous anaphylaxis and passive systemic anaphylaxis. *Journal of Allergy and Clinical Immunology* **2016**, *138*, 769-779, 10.1016/j.jaci.2016.01.049.