

Siglec-6: a potential new biomarker for clonal mast cell diseases

Andrea R. Teufelberger^{*1}, Jingya Wang², Catherine Overed-Sayer³, Maria Ekoff¹, Barbro Dahlén⁴, Theo Gülen^{1,4}, Gunnar P. Nilsson^{1,5}

¹Immunology and Allergy division, Department of Medicine Solna, Karolinska Institutet, and Karolinska University Hospital, Solna, Sweden

²Translational Science and Experimental Medicine, Research and Early Development, Respiratory and Immunology, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, Maryland, USA

³Regeneration, Research and Early Development, Respiratory and Immunology, BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK

⁴Department of Respiratory Medicine and Allergy, Karolinska University Hospital, Huddinge, Stockholm, Sweden

⁵Department of Medical Sciences, Uppsala University, Uppsala, Sweden

Summary

- We identified Sialic acid-binding Ig-like lectin 6 (Siglec-6) as a specifically elevated blood plasma protein in **clonal mast cell disease (CMD)** patients, using the SomaScan proteomics screen.
- Plasma Siglec-6 correlates with urinary mast cell activation markers and serum tryptase.
- Mast cell activation *in vitro* leads to release of Siglec-6.

Background

- CMDs include a group of rare haematological diseases, such as systemic mastocytosis and monoclonal mast cell activation syndrome, in which patients commonly carry a clonal D816V point mutation in the tyrosine kinase receptor KIT.
- Elevated serum tryptase levels are used as a diagnostic criterion for CMDs and as a biomarker for mast cell burden.
- Siglec-6 is a membrane-bound receptor, that is strongly expressed on the surface of mast cells and in the placenta.
- While Siglec-6 is known as an inhibitory receptor for FcεR1, the regulation of Siglec-6 during mast cell activation has not yet been described.

Conclusion

Siglec-6, measured in blood plasma, could be used as a biomarker for mast cell burden and activation in CMDs.

Methods and workflow

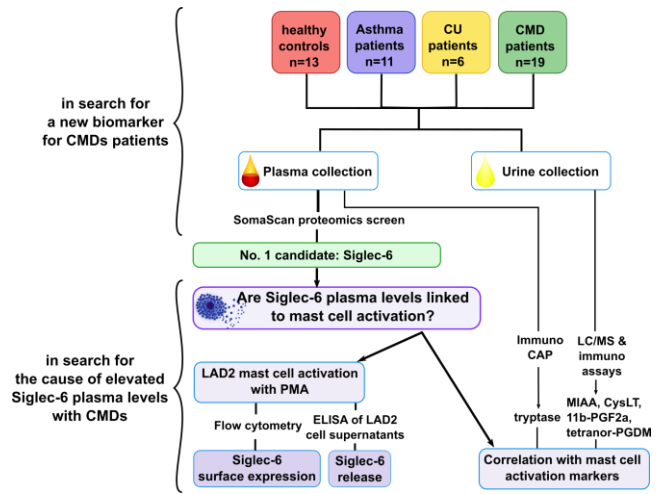


Figure 1: Workflow overview scheme of the study. This study is built up in two phases: screening for new CMD biomarkers and describing the role of the biomarker in CMDs. In the screening phase, plasma and urine samples were collected from healthy controls, asthma patients, chronic urticaria (CU) patients and CMD patients at Karolinska Hospital Huddinge. The SomaScan proteomics screen was performed in plasma samples and revealed Siglec-6 as the most promising candidate. In the second phase, an *in vitro* mast cell activation assay with the LAD2 mast cell line was performed to evaluate Siglec-6 surface expression, mast cell activation and Siglec-6 release to the cell supernatants. Further, known mast cell activation markers in blood and urine were measured and analysed for their correlation with Siglec-6 levels. Methylimidazoleacetic acid (MIAA); Cysteine Leukotriene (CysLT); 11-epi-prostaglandin F2alpha (11b-PGF2a); 11,15-Dioxo-9α-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid (tetranor-PGDM).

Plasma Siglec-6 is elevated in CMD patients

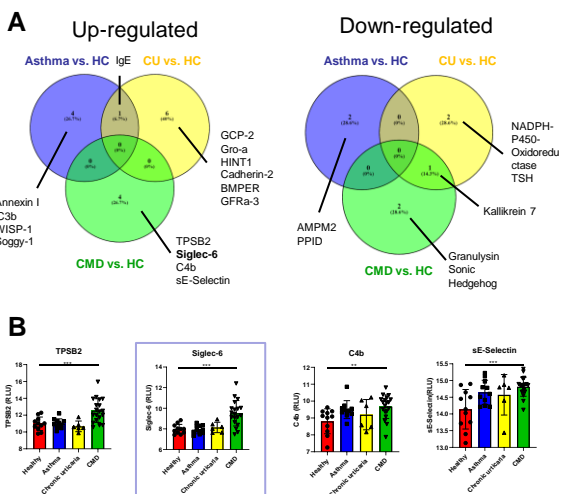


Figure 2: Proteomics screen reveals plasma Siglec-6 as a promising CMD-specific candidate protein. A) Venn-Diagram of up- and down-regulated proteins in asthma, chronic urticaria (CU) and/or CMD patients compared to the healthy control (HC) group. Cut off for p-value is <0.01, and fold change >1.5. B) Levels of up-regulated proteins (Tryptase (TPSB2), Siglec-6, Complement component 4b (C4b) and soluble E-Selectin) in CMD in comparison to all other groups.

Plasma Siglec-6 correlates with mast cell activation markers

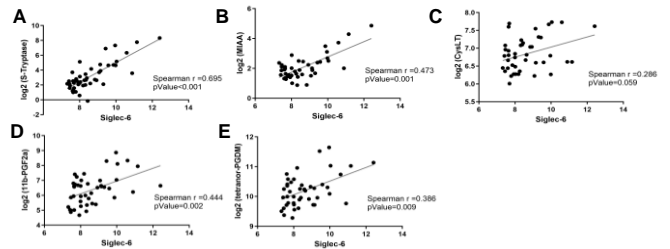


Figure 3: Plasma Siglec-6 correlates with mast cell activation markers. Siglec-6 significantly correlates with serum tryptase levels in the blood (A); urinary histamine metabolite MIAA (B); urinary 11b-PGF2a, a metabolite of PGD2 (D); and urinary tetranor-PGDM, another metabolite of PGD2 (E). Siglec-6 correlates with urinary CysLT by trend but not significantly (C).

Siglec-6 is released by activated LAD2 mast cells

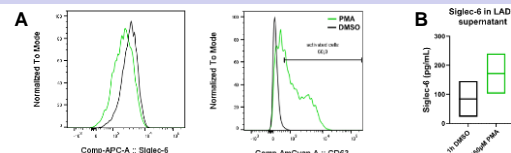


Figure 4: LAD2 cells express Siglec-6 and release it to the supernatant upon activation. A) Siglec-6 and the mast cell activation marker CD63 are expressed on LAD2 cells. LAD2 cells were stimulated for one hour with Phorbol 12-myristate 13-acetate (PMA, green line). Di-methyl-sulfoxide (DMSO, black line) treated LAD2 cells served as negative control condition. The upregulation of CD63 in PMA treated LAD2 cells coincides with a decreased Siglec-6 surface expression. B) Released Siglec-6, measured by ELISA, is higher in PMA-treated LAD2 supernatants compared to the DMSO negative control. Data is shown from three independent experiments.

Karolinska Institutet

Allergy and Immunology Division,
Department of Medicine Solna
Research group Gunnar Nilsson

E-mail: andrea.teufelberger@ki.se

CANCERFONDEN



AstraZeneca



Karolinska
Institutet