

Original article

Alteration and acquisition of Siglecs during *in vitro* maturation of CD34+ progenitors into human mast cells

Using human mast cells (MC) derived by culture of CD34+ peripheral blood precursors, a comprehensive study was performed of expression of 11 known Siglecs. Analysis was initially performed at the mRNA level using gene arrays. Positive results were then validated at the protein level using indirect immunofluorescence and flow cytometry, and for some Siglecs, Western blot analysis was also used. Culture-derived MC expressed mRNA for CD22 (Siglec-2), CD33 (Siglec-3), Siglec-5, Siglec-6, Siglec-8 and Siglec-10. Flow cytometry confirmed surface expression of all these molecules except for CD22 and Siglec-10, where levels were low or undetectable. However, Western blotting was able to detect MC expression of CD22 and Siglec-10, suggesting that these proteins were mostly cytoplasmic. CD34+ precursor cells from peripheral blood constitutively expressed surface CD33, Siglec-5 and Siglec-10. As they matured into MC, their constitutive levels of CD33 changed little, Siglec-5 and Siglec-10 declined, and Siglec-6 and Siglec-8 appeared *de novo*, all in parallel with accumulation of histamine and other MC markers, such as surface expression of FcεRIα and CD51. Phenotypic analysis of LAD-2 MC yielded a similar pattern of Siglec expression except that CD22 expression was particularly prominent. Finally, immunohistochemistry confirmed expression of these same Siglecs by mature tryptase-positive MC in human lung tissues. These data demonstrate an extensive and previously unappreciated pattern of Siglec expression on human MC. Whether engagement and signaling through these inhibitory Siglecs can impact MC biology will require further investigation.

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Mast cells (MC) are pro-inflammatory, multifunctional immune cells that contribute to a variety of allergic and innate immune responses (1). For example, in allergy, apart from their classical role in eliciting acute responses including anaphylaxis, MC also participate in chronic forms of allergic inflammation by releasing mediators that alter vascular permeability and inflammatory cell recruitment, activation and survival.

Studies on human MC have been hampered in part because of the difficulty in obtaining large numbers in high purity. Recently, however, new methodologies for generating human MC have facilitated advancements in our understanding of their biology. Among these is a greater understanding of the MC transcriptome (2, 3) and surface phenotype (4, 5). It is now well-accepted that besides FcεRI, these cells also express a broad array of molecules involved in cell adhesion, migration, and signal transduction (6).

Sialic acid-binding immunoglobulin-like lectins (Siglecs) are a family of transmembrane I-type lectins characterized by the presence of an N-terminal V-set immunoglobulin domain that binds sialic acid (7). A unique characteristic of many Siglecs is the presence of conserved cytoplasmic sequences containing tyrosine motifs [e.g. immunoreceptor tyrosine-based inhibitory motifs (ITIMs)], suggesting that these molecules possess inhibitory functions (7). Therefore, it is possible that depending on which Siglecs MC express, there may be a number of potential opportunities for inhibiting MC function based on activation or engagement of these receptors. We have reported that Siglec-8 is expressed on human MC, as well as eosinophils and basophils (8). There are other previous reports of mature tissue-derived MC expressing CD33 (Siglec-3) (9) and the HMC-1 MC line expressing Siglec-5 (CD170) (10), but there has never been a systematic study of all Siglecs expressed by human MC.

This manuscript reports the results of a comprehensive analysis of expression of all known Siglecs using culture-derived human MC, initially at the mRNA level (using gene arrays) and then validating positive results by

Abbreviations: MC, mast cells; Siglec, sialic acid-binding immunoglobulin-like lectin; G-CSF, granulocyte-colony stimulating factor; IL, interleukin; SCF, stem cell factor; PBS, phosphate-buffered saline; ITIM, immunoreceptor tyrosine-based inhibitory motif.

examining protein expression with immunofluorescence and flow cytometry as well as Western blot analysis. MC were found to express a wide range of Siglecs, the most prominent of which is Siglec-6. These results set the stage for future functional studies in which the consequences of Siglec ligation on various MC responses can be studied, including degranulation, cytokine production, and survival.

Materials and methods

Human MC cultures

The study was reviewed and approved by local human subjects research committees. Written informed consent for blood donation was obtained before enrollment in the study. CD34⁺ cells were obtained from normal or allergic volunteers, or volunteers receiving granulocyte-colony stimulating factor (G-CSF) prior to leukapheresis, using positive immunomagnetic selection with a CD34 class II monoclonal antibody kit (QBEND/10; Miltenyi Biotec, Auburn, CA, USA) as previously described (11). MC were then derived in culture using media supplemented either with or without methylcellulose as previously described (12). Media used included recombinant human interleukin (IL)-3 (R & D Systems, Minneapolis, MN, USA), IL-6 (Biosource, Camarillo, CA, USA) and stem cell factor (SCF; Biosource). Briefly, cells were cultured at a starting concentration of 5×10^5 cells/ml in serum-free medium (StemPro-34 SFM; Life Technologies, Grand Island, NY, USA) supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 50 µg/ml streptomycin, 100 ng/ml recombinant human SCF, 100 ng/ml recombinant human IL-6, and 30 ng/ml recombinant human IL-3 (the latter for the first week only). Hemi-depletions of media were performed weekly, replacing this with fresh media containing 100 ng/ml SCF and 100 ng/ml IL-6. For other experiments, CD34⁺ cells were cultured in serum-free Iscove's methylcellulose medium (Stem Cell Technologies, Vancouver, British Columbia, Canada) and Iscove's modified Dulbecco medium containing SCF at 200 ng/ml, IL-6 at 50 ng/ml, and IL-3 at 1 ng/ml, as previously described (3). On day 42 of culture, methylcellulose was dissolved in phosphate buffered saline (PBS), and the cells were resuspended and cultured in StemPro media containing 100 ng/ml SCF and 100 ng/ml IL-6. Hemi-depletions of media were performed weekly, adding fresh media as mentioned above. Duration of all cultures did not exceed 15 weeks.

In additional experiments, the LAD-2 human MC line (generously provided by Drs Arnold Kirshenbaum and Dean Metcalfe) was maintained as previously described (13) and used in phenotyping experiments.

Siglec microarray expression analysis

Total RNA was extracted by RNeasy[®] (Qiagen, Hilden, Germany) according to the manufacturer's instructions as previously described (14). Total RNAs were pooled from four to five separate experiments. Five micrograms of total RNA from each pool were used to prepare cRNA. Gene expression was analyzed with the GeneChip[®] Human Genome U133 plus 2.0 probe array (Affymetrix, Santa Clara, CA, USA), which contains the oligonucleotide probe sets for approximately 54 675 genes, according to the manufacturer's protocol (Expression Analysis Technical Manual). Data analysis was performed with a GeneChip[®] Operating Software version 1.0 (Affymetrix) and a GeneSpring software version

Table 1. Monoclonal antibodies used in this study (all IgG1)

Antigen	Clone name	Source
CD51	AMF7	Immunotech, Miami, FL
FcεRIα	22E7	Dr Richard Chizzonite, Hoffmann-La Roche, Nutley, NJ
CD22 (Siglec-2)	HIB22	BD Biosciences, San Diego, CA
CD33 (Siglec-3)	1C7	(30)
Siglec-5	8H2	(20)
Siglec-6	E20-1232	BD Biosciences
Siglec-8	2E2 and 2C4	(8)
Siglec-10	5G6	(26)

7.2 (Silicon Genetics, San Carlos, CA, USA). To normalize the staining intensity variations among chips, the average difference values for all genes on a given chip were divided by the median of all measurements on that chip.

Histamine analysis

Histamine content was determined from aliquots of cell lysates. Briefly, 1×10^4 cells were lysed in a final volume of 1 ml using 8% perchloric acid. Samples were centrifuged to remove debris and supernatants were analyzed for histamine content using an automated fluorometric technique as previously described (15).

MC surface phenotyping

Beginning with CD34⁺ cells and then every 2 weeks, aliquots of cells were removed and a differential cell count was performed using May-Grunwald-Giemsa staining and light microscopy. Aliquots of cells were centrifuged onto slides using a cytocentrifuge (Shandon, Pittsburgh, PA, USA).

Indirect immunofluorescence and flow cytometric analyses were performed every 2 weeks for a period of 14 weeks as previously described (16). The monoclonal antibodies used are shown in Table 1. All are IgG1 isotype and include those that recognize numerous Siglecs as well as FcεRI and CD51, the latter used to distinguish MC (positive) from basophils (negative). Cells were incubated for 30 min on ice in PBS containing 0.2% BSA and 4mg/ml human IgG to block nonspecific binding (both from Sigma Chemical Company, St Louis, MO, USA). Saturating concentrations of primary antibody or an equivalent concentration of isotype-matched irrelevant control IgG antibody was used (BD Biosciences, San Diego, CA, USA). Cells were then washed and incubated with a 1 : 100 dilution of R-phycoerythrin-conjugated F(ab)₂ goat anti-mouse IgG (Biosource) for an additional 30 min on ice. After fixation in 1% paraformaldehyde, a minimum of 5000 cells was evaluated using a flow cytometer (Becton Dickinson FACSCalibur, Mountain View, CA, USA). Net mean fluorescence intensity was determined after subtracting values for matched IgG controls, which averaged 2 ± 0.3 .

Western blot analysis for CD22 and Siglec-10

Mast cells were harvested and lysed with lysis buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 5 mM EDTA, 10% glycerol, 1% Triton X-100, 1 mM sodium orthovanadate, and protease inhibitor tablets). Protein concentrations were determined using the bicinchoninic acid assay (Pierce, Rockford, IL, USA). Samples were boiled in 4× SDS buffer (0.5 M Tris, pH 6.8, 16% glycerol, 3%

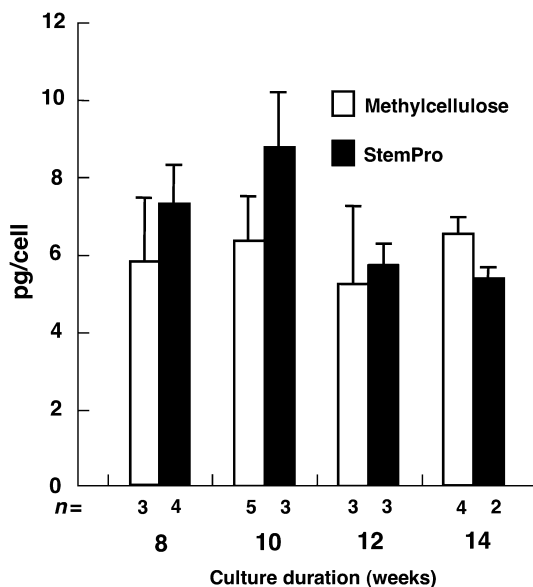


Figure 1. Comparison of the histamine content of CD34+ cell-derived human MC using methylcellulose (open bars) and StemPro alone (black bars). The number of experiments (*n*) is shown. Values represent mean $\pm$ SEM.

SDS, 8% 2-ME, 2 mg bromophenol blue) and 10 μ g of protein was loaded onto a 10% Tris-glycine SDS polyacrylamide gel (Ambrex BioScience Rockland, Rockland, ME, USA). Proteins were transferred to a polyvinylidene difluoride membrane, washed in phosphate-buffered saline (PBS) with Tween buffer (20 mM Tris, 137 mM NaCl, 0.2% Tween 20), and nonspecific binding was blocked overnight with 5% BSA (Fisher Scientific, Pittsburgh, PA, USA) in PBS with Tween buffer. The membranes were then incubated for 1 h with 1 μ g/ml CD22 rabbit polyclonal Ab (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or Siglec-10 goat polyclonal Ab (R & D Systems). Membranes were then washed with PBS/0.1% Tween 20 and incubated with horseradish peroxidase-conjugated anti-rabbit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) or anti-goat antibody (Santa Cruz Biotechnology). Immunoreactive bands were visualized using ECL (Amersham, Arlington Heights, IL, USA).

Immunohistochemical analysis of human tissue mast cell expression of Siglecs

Lungs from human organ donors (age 16–26 years) were procured and shipped on ice by The National Disease Research Institute (Philadelphia, PA, USA) and received 12–24 h after organ removal. Once received, 1 cm blocks of lung containing airways (2–5 mm diameter) were immediately fixed in 4% formaldehyde in PBS (4°C, 18 h) and then rinsed with PBS. Tissue blocks were cryoprotected overnight in 18% sucrose in PBS (4°C), covered with mounting medium and frozen. Cryostat sections (10 μ m) were collected on lysine-coated slides and air-dried at room temperature for 30 min. The sections were treated with avidin/biotin blocking solutions (Vector Laboratories, Burlingame, CA, USA), rinsed, covered with blocking solution containing 1% bovine serum albumin, 10% normal goat serum and 0.1% Tween 20 in PBS for 1 h at room temperature and then incubated (18 h, 4°C) with monoclonal antibody recognizing different Siglec subtypes (see Materials and methods; dilutions used were the same as those used for flow cytometry). Slides were then rinsed in PBS and incubated with a Cy5-labeled goat anti-mouse secondary antibody (Alexa 647; Molecular Probes, Inc., Eugene, OR, USA) for 2 h at room temperature. After rinsing, the sections were covered with biotinylated monoclonal antibody recognizing human MC tryptase (Promega, Madison, WI, USA) for 2 h at room temperature, rinsed, and covered with streptavidin-fluorescein (Vector Laboratories) for 2 h at room temperature. Separate slides were processed similarly but the primary antibodies were replaced with irrelevant IgG1 monoclonal antibody to evaluate nonspecific staining. Rinsed slides were coverslipped with buffered glycerol (pH 8.6) and evaluated and photographed with an epifluorescence microscope (Olympus BX50; Olympus America, Inc., Melville, NY, USA) equipped with appropriate filter sets to allow separate visualization of fluorescein or the Cy5 probe. Mast cells were counted in the mucosa and submucosal regions surrounding the entire airway and the percentage of Siglec-positive cells that were tryptase-positive was determined in two sections of each bronchus.

Results

Initial experiments were performed using culture conditions with or without methylcellulose to compare cell yields, histamine content, and Siglec-8 surface expression.

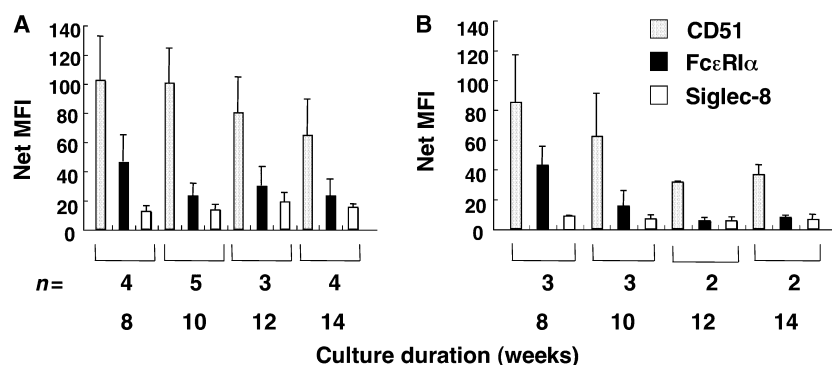


Figure 2. Comparison of the induction or alteration of Siglec-8, CD51, and FcεRIα surface expression by flow cytometry on CD34+ cell-derived human MC using methylcellulose (A) or StemPro alone (B). The number of experiments (*n*) is shown. Values represent mean $\pm$ SEM.

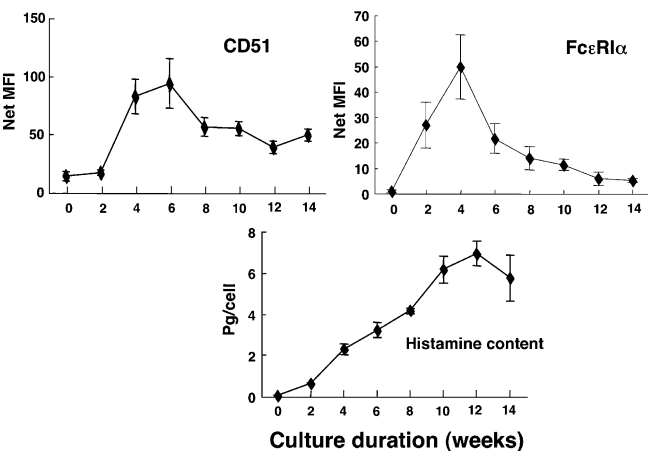


Figure 3. Kinetics of surface expression of CD51, FcεRIα, and histamine content, for CD34+ cell StemPro-derived human MC. Values represent mean ± SEM. N = 3–6.

Although cells cultured using methylcellulose initially expanded faster, the final MC yields at 8–14 weeks were not significantly different between the two culture conditions (data not shown). Using Wright–Giemsa staining, cells from both culture methods appeared similar by light microscopy. Maturation toward MC was clearly evident by 6–8 weeks, as cells took on the cytologic phenotype of mononuclear metachromatic cells. For example, at week 6, the purity of the culture cells as assessed by metachromatic staining was $92 \pm 3\%$ and after week 8 and beyond, $>99\%$ of the cells were MC. By 8 weeks, the histamine content in mature cells reached a plateau at approximately 6 pg/cell regardless of culture condition (Fig. 1). This was accompanied by the unimodal appearance of similar levels of surface expression of FcεRIα, CD51 and Siglec-8 under both culture conditions (Fig. 2 and 3). Because there were no significant differences between the two types of culture methods, both were employed in subsequent studies.

Using MC of 14–15 weeks of age generated with the methylcellulose culture method, gene array analysis was performed in replicate experiments and data on Siglec mRNA was evaluated. As expected, mRNA was easily detectable for CD33, Siglec-5, Siglec-8, and positive controls GAPDH and β-actin, and little or no mRNA was detected for Siglec-1 (sialoadhesin, CD169), Siglec-4, Siglec-7, Siglec-9 or Siglec-11 (Table 2). Surprisingly, modest-to-high levels of CD22, Siglec-6 and Siglec-10 mRNA were detected (Table 2). To evaluate and verify the positive mRNA data, surface phenotyping was performed using immunofluorescence and flow cytometry. Surface expression of each marker was consistently unimodal at week 6 and beyond (Fig. 4A), suggesting a pure population of MC with uniform surface expression of each marker. As shown in Fig. 3 and 4B, CD34+ progenitors used to initiate these cultures did not express FcεRIα, nor did they express Siglec-8, although they did

Table 2. Siglec gene expression in 14–15-week-old culture-derived human MC

Transcript (abbreviation or CD)	Experiment 1		Experiment 2	
	Raw AD*	Flag**	Raw AD	Flag
Siglec-1 (Sialoadhesin, CD169)	181.1	P	44.7	A
CD22 (Siglec-2)	1932.9	P	614.6	P
CD33 (Siglec-3)	973	P	411.2	P
Siglec-4 (MAG)	68.9	A	20.8	A
Siglec-5 (CD170)	513.8	P	417.3	P
Siglec-6 (OB-BP1)	4938	P	2290.5	P
Siglec-7 (p75/AIRM-1)	260.8	P	75.5	A
Siglec-8	948.3	P	229.3	P
Siglec-9	11.5	A	1.9	A
Siglec-10	3775.1	P	1002.9	P
Siglec-11	17.4	A	3.4	A
GAPDH	10 574.7	P	5188.6	P
Beta actin	14 941.8	P	8182.7	P

Each experiment was performed using two pools of total RNA from four to five separate experiments. Two measures of gene expression were used: average difference (AD*) and absolute call (Flag**). Average difference is a quantitative measure of the level of gene expression, calculated by taking the difference between mismatch and perfect match of every probe pair and averaging the differences over the entire probe set. Absolute call is a qualitative measure generated using the algorithm in the GeneChip® Operating Software (Affymetrix) in which each gene is assigned a call of present (P), marginal, or absent (A), based on the hybridization of the RNA to the probe set. Raw AD levels of two housekeeping genes (GAPDH and β-actin) are also shown as a control. Abbreviations: MAG, myelin-associated glycoprotein; OB-BP1, leptin-binding protein 1; p75/AIRM1, 75 kDa adhesion inhibitory receptor molecule 1.

express modest levels of CD51. CD34+ progenitors also constitutively expressed surface CD33, Siglec-5 and Siglec-10 along with very low levels of Siglec-6 (Fig. 4A,B). During maturation into MC, surface levels of FcεRIα appeared *de novo* and peaked at 4–6 weeks, gradually falling to low but persistent levels by 8 weeks, consistent with the fact that there was no IgE present in the culture to maintain the higher levels of FcεRIα. As FcεRIα appeared on the cells, there was a marked, parallel increase in Siglec-6 and Siglec-8 expression, which then reached a plateau; this was also the case for CD51. As CD34+ cells matured into MC, both Siglec-5 and Siglec-10 surface expression declined, but still remained detectable. Siglec-6 expression also declined, but its surface expression remained the brightest on mature MC among all the Siglecs studied (Fig. 4A,B). CD33 levels remained relatively unchanged as CD34+ cells matured into MC. In sharp contrast, despite high levels of mRNA for CD22 and Siglec-10, their surface expression was barely detectable, if detectable at all, by flow cytometry (Fig. 4A,B). However, using Western blot analyses, CD22 and Siglec-10 were readily detectable, suggesting the presence of intracellular pools of these molecules in culture-derived MC (Fig. 5). Finally, LAD-2 cells also underwent surface phenotyping (Fig. 4A) and were found to express an identical, unimodal pattern of Siglecs as did the culture-derived MC, except that LAD-2 cells expressed a much higher level of CD22.

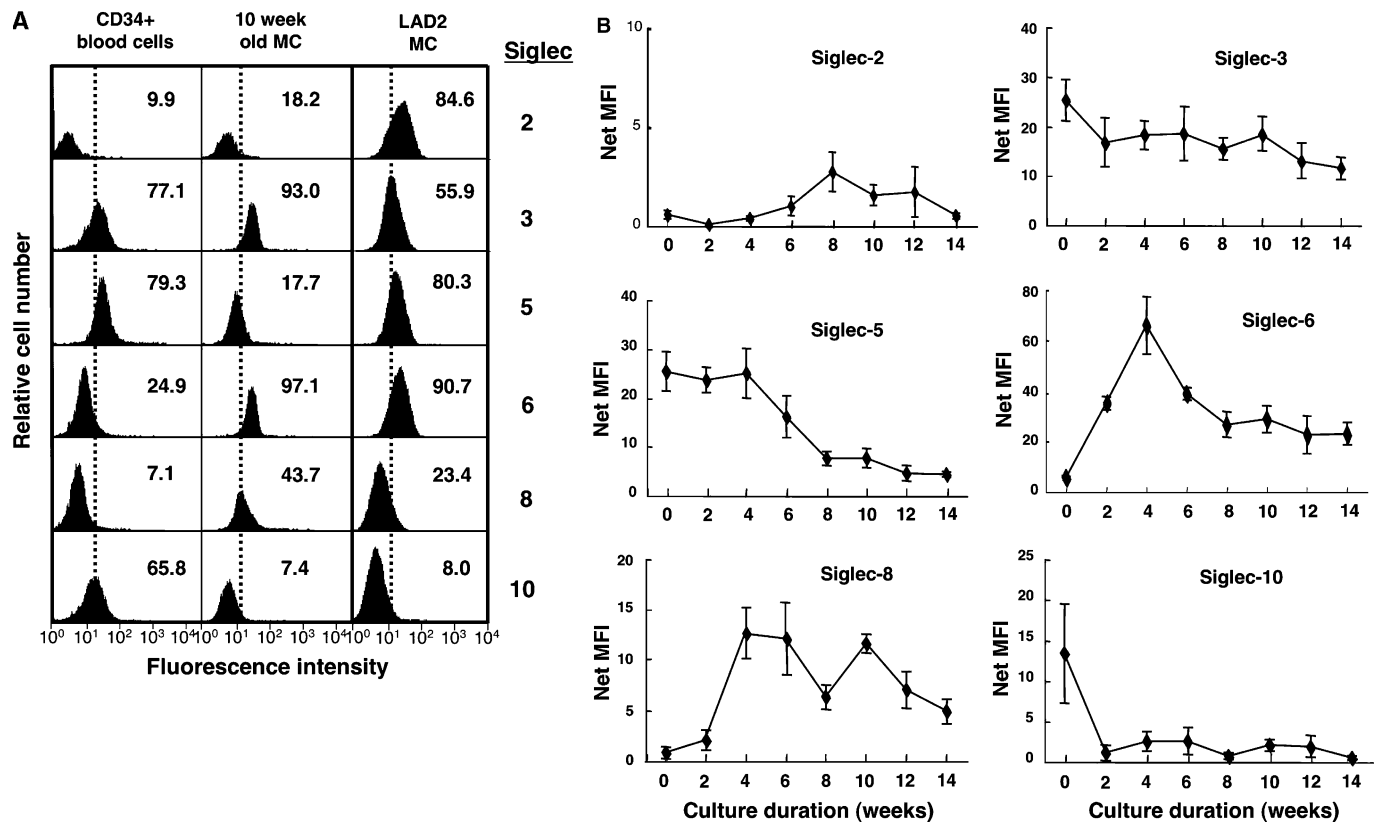


Figure 4. (A) Surface expression of CD22 (Siglec-2), CD33 (Siglec-3), Siglec-5, Siglec-6, Siglec-8 and Siglec-10 on peripheral blood CD34+ cells, CD34+ cell-derived cultured MC at 10 weeks of culture, and the LAD-2 cell line. Histograms shown are representative of three top six experiments using indirect immunofluorescence and flow cytometry. The vertical dotted line represents the cutoff used for the IgG1 isotype-matched negative control. (B) Kinetics of surface expression of the same Siglecs as in panel A on CD34+ cell StemPro-derived human MC. Values represent mean $\pm$ SEM. $N = 3-6$.

To confirm that mature tissue MC resembled cultured MC in their pattern of Siglec expression, human lung tissue was examined. Using the same monoclonal antibodies that were used for flow cytometry, immunochemical evaluation of MC in human bronchial sections was performed. Cryostat sections of bronchi that were known to contain MC from four different organ donors

were examined using double immunofluorescent staining of human MC tryptase with different Siglec antibodies (see Materials and methods). Mast cells were observed in the mucosa, submucosa and adventitial areas in three of the four different specimens; one specimen had relatively few MC only in the mucosal area. All Siglec antibodies tested demonstrated co-localization in tryptase-positive cells (e.g. Fig. 6, shown in the panel for Siglec-6, arrows), although some single tryptase-only and Siglec-only positive cells were also seen (Fig. 6, shown in the panel for Siglec-6, arrow heads). The Siglec-only positive cells were not further characterized but probably represent those known to express these Siglecs (e.g. B cells for Siglec-2 and eosinophils for Siglec-8).

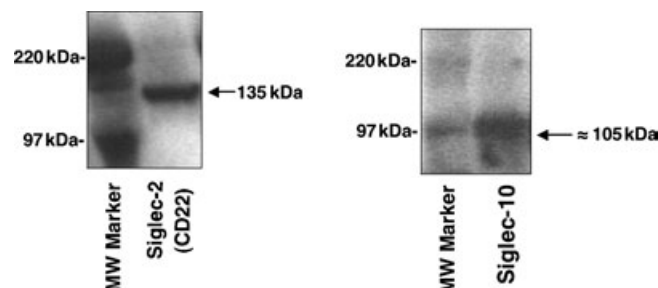


Figure 5. Western blot analyses of 14-week-old StemPro-derived MC for the presence of CD22 (Siglec-2) and Siglec-10 protein in cell lysates. Data are representative of two experiments yielding similar results.

Discussion

This is the first paper to extensively evaluate MC expression of Siglecs, and as part of this analysis expression of selected Siglecs was also evaluated on CD34+ progenitors. Using gene array analysis, as

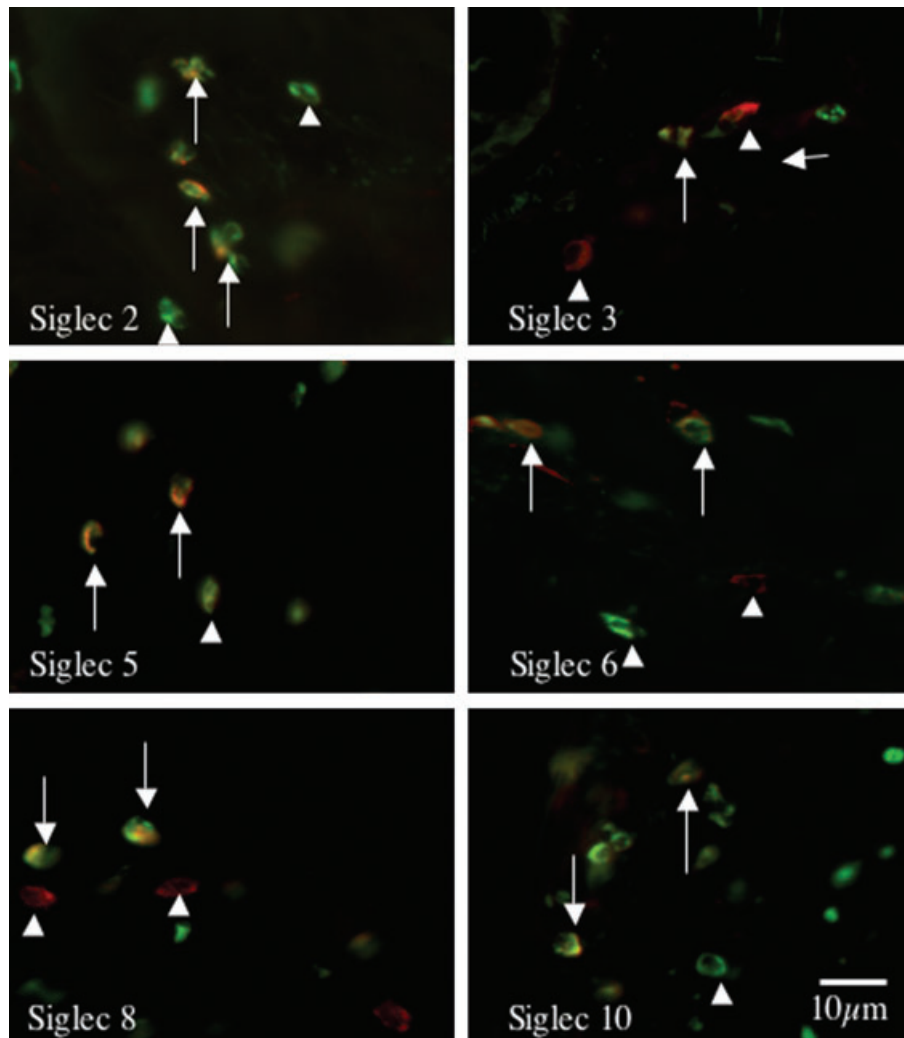


Figure 6. Dual color immunofluorescence staining of human bronchial tissue sections for human mast tryptase (green) and Siglecs (red) as indicated. Cells are seen that are immunofluorescent for both mast cell tryptase and a given Siglec (e.g. orange stain, arrows for Siglec-6). Not all tryptase-positive cells (green stain, arrow head for Siglec-6) were Siglec positive, and Siglec-only positive cells (red stain, arrow heads for Siglec-6) were also present in all sections. Original magnification, 40 $\times$; scale bar = 20 μ m.

expected, mRNA was easily detectable for CD33, Siglec-5 and Siglec-8, and little or no mRNA was detected for Siglec-1, Siglec-4, Siglec-7, Siglec-9, and Siglec-11. Surprisingly, modest-to-high levels of CD22, Siglec-6 and Siglec-10 mRNA were also detected. With antibodies against the various Siglecs, we determined that CD34 $^{+}$ cells from peripheral blood constitutively expressed surface CD33, Siglec-5 and Siglec-10, but did not express Siglec-6 or Siglec-8. The latter two Siglecs were induced *de novo* during MC maturation culture conditions at approximately 4 weeks. This timing corresponds to increases in histamine content and CD51 and Fc ϵ RI α surface expression, reliable markers of MC, even though maximal appearance of histamine and full granulation takes several weeks longer. As the CD34 $^{+}$ cells matured into MC, their constitutive levels of Siglec-5, Siglec-6, and

Siglec-10 declined, while Siglec-6 expression was maintained at the highest levels. CD33 levels changed the least during this maturation process. Ultimately, CD33, Siglec-5, and Siglec-10 expression on MC became much lower than on CD34 $^{+}$ precursors. The fate of all down-regulated Siglecs is unknown. Whether this is due to changes in gene transcription or translation, and whether surface expression was reduced as a result of shedding or internalization, will require further investigation. Nevertheless, immunofluorescent staining of Siglecs and MC tryptase confirmed that mature human tissue MC can express these same six Siglecs (Fig. 6).

Perhaps the most unanticipated findings of the study relate to expression of CD22, Siglec-5, Siglec-6, and Siglec-10. CD22 is thought to be restricted to resting B cells, where it serves to regulate B-cell receptor signaling

(17, 18). Indeed, CD22 surface expression was even more prominent on LAD-2 MC (Fig. 4A). Recently, CD22 has also been detected in basophils, although there were some differences in epitope recognition in basophils and B cells by various antibodies in the CD22 β isoforms (19). High levels of CD22 β were found in the cytoplasm of B cells during maturation, and another isoform, CD22 α , reportedly exists only in the cytoplasm of cells and does not get transported to the cell surface (19). The relative molecular weight seen in Western blots of MC lysates, however, was more consistent with CD22 β rather than CD22 α . Siglec-5 was originally found to be expressed by macrophages, neutrophils, and monocytes (20, 21). One recent screen of human skin and lung MC using antibodies to Siglec-1 (7D2) and Siglec-5 (1A5) failed to detect MC staining, although basophils and the HMC-1 MC line did stain for Siglec-5 (10). Another Siglec-5 study (22) used antibody 1A5 along with antibody 2H8, and did not report staining of MC, but it is unclear whether the methods used would have specifically detected such staining because a second MC-specific antibody is typically needed to co-localize MC staining in tissues (23). Siglec-6, like CD22, is expressed on B cells in blood, but unlike CD22, it is also prominently expressed on placental trophoblasts (24). No expression by MC was mentioned in this report. Siglec-10 has been detected on human dendritic cells, monocytes, and natural killer-like cells, with some weak expression on eosinophils (25–27). At least five splice variants of Siglec-10 exist (28), but we speculate, based on Western blotting, that the isoform expressed by culture-derived MC is the full-length form. Finally, expression of CD51

was unexpected given our past experience finding little or no expression on CD34+ precursors from blood (16). It is possible that the difference might be due in part to the G-CSF pretreatment of subjects prior to leukapheresis.

In summary, CD34+ -derived human MC were found to express CD22, CD33, Siglec-5, Siglec-6, Siglec-8, and Siglec-10. The so-called CD33-related Siglecs (Siglec-3, 5–11) are characterized by ITIM-containing motifs in their cytoplasmic tails. These motifs can recruit protein tyrosine phosphatases such as SHP-1 that then mediate inhibitory signaling (7, 29). Additional experiments will need to be performed to explore the functional consequences of Siglec expression by human MC.

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