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Possible Influences of Endogenous and Exogenous Ligands on the Evolution of Human Siglecs

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Sialic acids, a group of acidic sugars abundantly expressed in the tissues of deuterostome animals but rarely found in microbes, serve as a "signature of self" for these animals. Cognate sensors for sialic acids include Siglecs, a family of transmembrane lectins of vertebrate immune systems that recognize glycans containing sialic acids. A type of sialic acid called *N*-glycolylneuraminic acid (Neu5Gc) is abundant in many mammalian lineages including great apes, the closest extant relatives of modern human, but was lost in the lineage leading to modern human via the pseudogenization of the *CMAH* gene encoding the enzyme that converts *N*-acetylneuraminic acid (Neu5Ac) to Neu5Gc. Loss of Neu5Gc appears to have influenced the evolution of human Siglecs, such as the adjustment of sialic acid binding preferences and the inactivation of at least one Siglec. In addition, various mechanistic studies using model systems and genetic association studies have revealed that some human Siglecs interact with pathogens and influence the outcome of infections, and these pathogens in turn likely influence the evolution of these Siglecs. By understanding the evolutionary forces affecting Siglecs, we shall achieve a better appreciation of Siglec functions, and by understanding Siglec functions, we can obtain deeper insight into the evolutionary processes driving Siglec evolution.

Keywords: Siglec, sialic acid, Neu5Ac, Neu5Gc, immunity, microbes

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INTRODUCTION

The role of immunity is to distinguish self vs. non-self (or what is not dangerous vs. dangerous) and to eliminate or contain the latter. Various biomolecules (nucleotides, peptides, lipids, polysaccharides, and their combinations) can be a signature of non-self (i.e., pathogen-associated molecular patterns; PAMPs), as exemplified by the diversity of ligands for Toll-like receptors, C-type lectin-like receptors, RIG-I-like receptors, and NOD-like receptors, all of which work as "pattern-recognition receptors" (1–4). Meanwhile, the signature of self (i.e., self-associated molecular patterns; SAMPs) is less well-understood, but some glycoconjugates would qualify as such (5, 6). Sialic acids are commonly synthesized by deuterostome animals and displayed on the cell surface in abundance but are rare in microbes (7), making them an ideal SAMP for distinguishing self- vs. non-self (5, 6).

For a chemical entity to be a molecular signature of self or non-self for the immune system, there must be a sensor that recognizes it. For sialic acids, Siglecs appear to be the primary pattern-recognition receptors (8–11). Siglec is a composite word from "sialic acid," "immunoglobulin (Ig) superfamily," and "lectins" (12). The Siglec family appears to be present only in vertebrates (13, 14). Siglecs are type 1 transmembrane proteins, with an extracellular domain consisting of multiple Ig-like domains (of which the N-terminal Ig-like domain is primarily responsible for the recognition of sialoglycans), followed by a single-pass transmembrane domain and cytoplasmic tail (**Figure 1**). Most of the known mammalian Siglecs are expressed on leukocytes and have an intracellular sequence motif called the immunoreceptor tyrosine-based inhibitory motif (ITIM) that recruits tyrosine phosphatase SHP-1 and thus transduces inhibitory signals. Thus, they are considered to function as sensors for sialic acids as a molecular signature of self. [However, there are some examples that imply this generalization may be somewhat too simplistic (17, 18).]. Although rodents are essential model animals for mechanistic studies in immunology, differences in primate and rodent CD33-related Siglecs (15) impose a significant challenge in the extrapolation of findings in rodents to human immunology. This situation parallels that of other immunoglobulin-like receptor families, leukocyte immunoglobulin-like receptors (LILR) and killer cell immunoglobulin-like receptors (KIR), that are encoded in a gene cluster on the same human chromosomal region as CD33-related Siglecs (chromosome 19q13.4) and are involved in self-recognition through interaction with MHC class I (19–21).

"Sialic acids" is a collective term for various naturally occurring acidic sugars with a common nine-carbon backbone (22). *N*-acetylneuraminic acid (Neu5Ac) is the most common type of sialic acid, and its C5-hydroxylated derivative *N*-glycolylneuraminic acid (Neu5Gc), along with the derivatives of Neu5Ac and Neu5Gc (mostly modified at C4 and/or C7-C9 hydroxyl groups), are generally present in mammalian tissues (22). Neu5Gc is abundant in many mammalian species, whereas humans have lost Neu5Gc, owing to the mutation (exon deletion) of the *CMAH* gene encoding CMP-Neu5Ac hydroxylase that is solely responsible for the *de novo* biosynthesis of Neu5Gc from Neu5Ac (23–26). Although some bacteria have developed ways to synthesize Neu5Ac, so far no study has demonstrated the presence of Neu5Gc on microbes (27) [A recent genomic survey (28) reported the presence of *CMAH*-like sequences in several microbial genomes, including those of several *Helicobacter* species that may express sialic acids. However, their enzymatic function has not yet been investigated]. Thus, Neu5Gc appears to be a quintessential signature of self, which is only present on deuterostome cells and missing on microbes. Indeed, some rodent Siglecs show a strong preference toward glycans containing Neu5Gc (29–32), whereas some others show a strong preference toward Neu5Ac (29, 33, 34). This imposes a conundrum: if one loses the best signature of self, the immune system may become more prone to attack its own cells (i.e., autoimmunity). How did the immune system of the human ancestor cope with the consequences of the dramatic change in the sialic acid landscape (i.e., shift of "sialome" from Neu5Gc to Neu5Ac) on the cell surface?

One possible consequence of Neu5Gc loss in human (and a possible response to the consequential autoimmune-prone state, in the evolutionary time scale) was a series of changes involving Neu5Gc-specific Siglecs, such as re-adjustment of binding specificity to Neu5Ac and "forced retirement," as explained in the following section.

POSSIBLE INFLUENCES OF NEU5GC LOSS ON HUMAN SIGLECS: ALTERED BINDING SPECIFICITIES

To understand the consequences of a species-specific event, it is natural to compare the phenotypes between the closest relatives that have undergone the event or have not. For human, the obvious choice is great apes including chimpanzee, which is the closest extant relative of modern human. Several earlier studies have shown that at least some great ape Siglecs preferentially recognize Neu5Gc (35–37). More recent data using the sialoglycan microarray also showed that primate CD33-related Siglecs generally tend to prefer Neu5Gc (38). Reported preferences of human and chimpanzee Siglecs toward Neu5Ac and Neu5Gc are summarized in **Table 1**. Thus, the loss of Neu5Gc likely meant attenuation of the interactions between Siglecs and self-associated ligands in the human ancestor.

One Siglec that may have been substantially affected by the loss of Neu5Gc in the human ancestor is Siglec-12 (36). Chimpanzee Siglec-12 and human Siglec-XII are expressed on macrophages and lumenal epithelia (36, 46). Human Siglec-XII has a universal mutation (R122C) that makes the protein unable to recognize sialic acids (36). [Roman numerals are used for primate Siglecs that have a mutation at the essential arginine residue required for sialic acid recognition and thus cannot recognize sialic acid (15)]. Arginine-restored human Siglec-XII, as well as chimpanzee Siglec-12, strongly prefers Neu5Gc over Neu5Ac (36). In addition, some human *SIGLEC12* alleles have acquired additional mutations (stop codon, rs16982743, and frame-shift, rs66949844) that cause premature termination of Siglec-XII protein synthesis (36, 46). These "null" mutations are common in the modern human populations (global frequency of "null" alleles: 0.19 for rs16982743, 0.59 for rs66949844). These results imply a scenario in which a Siglec that lost an endogenous ligand was forced to "retire" and then is further getting eliminated. Given that the R122C mutation is fixed in modern human populations, it is tempting to speculate that the presence of functional "Neu5Gc-recognizing" Siglec-12 may have caused a disadvantage in ancestral humans. For example, zoonotic infection of some Neu5Gc-coated envelope virus from other mammalian species may represent such selective pressure. A possible scenario for the further elimination of "signal transduction-competent but sialic acid recognition-incompetent" Siglec-XII may be that the recruitment of SHP-2 by Siglec-XII (47) on epithelial cells may assist the transformation of the epithelial cell by an oncogenic driver (e.g., receptor tyrosine kinase or RAS mutation/amplification) through activation of

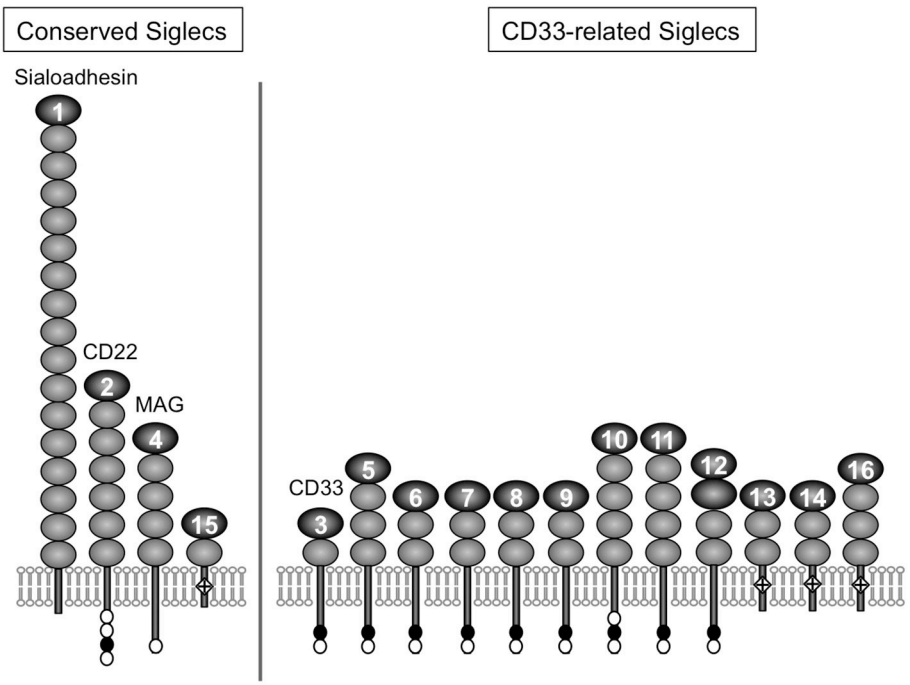

FIGURE 1 | Illustrative representation of ape Siglecs. Mammalian Siglecs can be classified as relatively conserved members (Sialoadhesin/Siglec-1, CD22/Siglec-2, MAG/Siglec-4, and Siglec-15) and less conserved members (CD33-related Siglecs). CD33-related Siglecs are encoded in the gene cluster (15). Siglecs can be also classified based on the partner molecule involved in downstream signal transduction (i.e., those that have ITIM and interact with tyrosine phosphatase SHP-1, and those that have positively charged amino acid residue in the transmembrane domain and interact with adapter molecule DAP12, which has the "immunoreceptor tyrosine-based activating motif" (ITAM) and recruits tyrosine kinase Syk). Siglec-13 is missing in humans but present in apes and old-world monkeys (15). Modified from (16). Closed circle, open circle, and diamond with + mark in the figure represent ITIM, ITIM-like motif, and positively charged amino acid residue in the transmembrane domain (that is required for the interaction with DAP12), respectively.

MAPK pathway (48–52), which may have been disadvantageous for the overall fitness of the carriers of the functional allele. However, at present there is no solid experimental evidence to support these speculations.

Primate Siglec-9 (from chimpanzee, gorilla, and baboon) also prefers Neu5Gc, whereas human Siglec-9 appears to have acquired affinity toward Neu5Ac (37, 38). Human CD33/Siglec-3 and Siglec-5 also show a similar acquired affinity to Neu5Ac compared with their counterparts in baboon, which show a strong preference for Neu5Gc (38). Given that Siglec-9 has an ortholog in rodents (Siglec-E), it may play an important role in regulating innate immunity and be indispensable (although expression patterns and functions of primate Siglec-9 and rodent Siglec-E may not completely overlap (53–55)). Human Siglec-9 may have had to undergo rapid evolution to catch up with the change in the human sialome, to resume its original functionality. It is of note that the N-terminal Ig-like domain (Ig1) of great ape Siglec-9 shows much greater inter-species sequence differences than does the adjacent C2-set Ig-like domain (Ig2) (37), which is consistent with the idea that human Siglec-9 had to evolve rapidly to respond to the loss of Neu5Gc.

In fact, the CD33-related Siglec gene cluster is among the most rapidly diversifying gene families between human and chimpanzee (56), and the N-terminal Ig-like domain of CD33-related ape Siglecs is evolving faster than the other parts of the molecule (15, 37, 57). It is of interest whether the loss of Neu5Gc contributed to the accelerated evolution of human Siglecs. Assuming this is the case, we would expect that more amino acid changes have accumulated in the first Ig-like domain (Ig1) of human Siglecs than in Ig1 of chimpanzee Siglecs. In reality, the data (**Table 1**) do not appear to support this prediction. Although it is true that Ig1 is undergoing faster evolution than Ig2 (total human-specific changes in Ig1 and Ig2: 33.5 and 15, respectively; total chimpanzee-specific changes in Ig1 and Ig2: 40.5 and 14, respectively; average amino acid length of Ig1 and Ig2: 126 and 96, respectively), the Ig1 of Siglecs in the lineage leading to human has accumulated less sequence changes than that leading to chimpanzee. Some of the sequence changes in human Siglecs probably represent a genuine sign of selection due to the loss of Neu5Gc, whereas the majority of them may not be. Ig1 of primate Siglecs (and likely those in other species) is evolving rapidly under selective pressure that may include, but not be limited to, the changes in the landscape of endogenous sialoglycans (38).

It is of note that, in contrast to the adaptation of some human Siglecs to Neu5Ac-dominant sialome, many Siglecs still appear to prefer Neu5Gc (**Table 1**). Possible explanations for this fact may include: (1) the adaptation of human Siglecs

TABLE 1 | Binding preferences (Neu5Ac vs. Neu5Gc) and lineage-specific mutations in human and chimpanzee Siglecs.

Siglec	Human preference	Chimpanzee preference	Human-specific changes in Ig1 and Ig2*	Chimpanzee-specific changes in Ig1 and Ig2*	References**
Sialoadhesin/Siglec-1	**Ac** >> Gc	ND	1, 1	1, 0	(39)
CD22/Siglec-2	Ac ≈ Gc	Ac ≈ Gc	2, 0	2, 0	(35, 39)
CD33/Siglec-3	Ac < **Gc** (weak preference)	Ac < **Gc** (weak preference)	2.5, 2	5.5, 0	(35, 38, 39)
MAG/Siglec-4	ND	ND	0, 1	0, 0	[for the glycan preference of rodent MAG, see (33, 34, 40, 41)]
Siglec-5	Ac < **Gc** (weak preference)	X (Arg mut)	7.5, 2	5.5, 1	(35, 38)
Siglec-6	Ac < **Gc**	ND	0, 1	3, 0	(35)
Siglec-7	Ac ≈ Gc	Ac < **Gc**	1.5, 0	3.5, 0	(37)
Siglec-8	ND	ND	0.5, 2	1.5, 3	[for the glycan preference of human Siglec-8, see (42)]
Siglec-9	**Ac** > Gc (weak preference)	Ac < **Gc** (weak preference)	4, 0	3, 1	(37, 38)
Siglec-10	Ac < **Gc**	ND	0, 1	2, 1	(Consortium for Functional Glycomics data)
Siglec-11	Ac < **Gc**	Ac < **Gc**	2.5, 1	2.5, 0	(43, 44)
Siglec-12	X (Arg mut)	Ac << **Gc**	2, 2	1, 3	(36)
Siglec-13	X (absent)	Ac ≈ Gc	(Cannot be determined)	(Cannot be determined)	(45)
Siglec-14	ND (likely Ac < **Gc**, from Siglec-5 data)	X (Arg mut)	6.5, 1	4.5, 1	(38)
Siglec-15	**Ac** > Gc	ND (**Ac** > Gc)	2, 1	0, 1	Unpublished
Siglec-16	**Ac** > Gc	Ac < **Gc**	1.5, 0	5.5, 3	(43, 44)

ND, not determined; X, cannot be determined (either the protein is absent in the species or is present but does not recognize sialic acid owing to the mutation of essential Arg residue); >> or <<, strong preference; > or <, preference; ≈, no preference; Arg mut: mutation of arginine residue that is essential for sialic acid recognition. Sialic acid (Ac, Neu5Ac; Gc, Neu5Gc) preferentially recognized by each Siglec is highlighted with underline and bold typeface.

**The numbers of human- and chimpanzee-specific amino acid changes were deduced by aligning the amino acid sequences of Siglec orthologs from human, chimpanzee, and orangutan. In case the lineage specificity of the amino acid change cannot be unambiguously determined (i.e., when the amino acid at one position was different in all three species), "0.5 difference" was assigned to both human and chimpanzee. For Siglec-12 with two V-set domains, amino acid changes in the N-terminal V-set domain (Ig1) were counted as those in "Ig1," and those in the first C2-set domain (Ig3) were counted as those in "Ig2." Note that the "species-specific changes" were counted based on a reference sequence of human Siglecs and "best hit" putative protein sequences in chimpanzee and orangutan by BLASTP search, without considering the polymorphisms in each species.*

***The majority of the references in this table are reports that directly compare human and chimpanzee Siglec binding preferences. Note that different methods for analyzing Siglec–glycan interactions, such as glycan microarray vs. polymer-based probe binding, or even between different formats of glycan microarrays, may yield results that are not fully consistent in some cases.*

to Neu5Ac-dominant sialome is still incomplete, and over the time (in the scale of millions of years) most human Siglecs will eventually acquire Neu5Ac preference; (2) some Siglecs did not have strong preference toward Neu5Gc over Neu5Ac prior to the loss of Neu5Gc in human ancestor, or have already accumulated mutations to make them sufficiently suitable for Neu5Ac recognition, thus it is not necessary for them to adapt further to Neu5Ac-dominant sialome; (3) the interaction of Siglecs with exogenous ligands (e.g., bacterial nonulosonic acids) prevent complete switch from Neu5Gc to Neu5Ac preference. Although these explanations are purely speculative, some of these scenarios may be tested experimentally. For example, an independent event has eliminated Neu5Gc in the lineage leading to New World monkeys approximately 30 million years ago (58). In contrast, the timing of Neu5Gc loss in human is far more recent, which is estimated to be 3 million years ago (26). It would be interesting to see whether the Siglecs in New World monkeys prefer Neu5Ac, or some of them still prefer Neu5Gc, to test the validity of the explanation (1) above.

POSSIBLE INFLUENCES OF NEU5GC LOSS ON HUMAN SIGLECS: ALTERED EXPRESSION PATTERNS

It is of interest to know whether there is any change in the expression patterns of Siglecs between human and chimpanzee, which might also represent a consequence of Neu5Gc loss in human. Antibody-based comparative analyses of Siglec expression patterns in human and chimpanzee (and gorilla) have revealed several examples of altered expression of Siglecs in human, as summarized in **Table 2**. Naturally, it is more difficult to establish the influence of the loss of Neu5Gc on the expression patterns of Siglecs than its effect on the binding preferences of Siglecs, as it is indirect. Nevertheless, it appears to be implied in some cases.

The first reported example of altered expression of Siglec in human compared with chimpanzee was the wider distribution of Sialoadhesin/Siglec-1^{+} macrophages in chimpanzee spleen as compared with those in human spleen (39). Although

TABLE 2 | Expression patterns of human and chimpanzee Siglecs.

Siglec	Human	Chimpanzee	References
Sialoadhesin/Siglec-1	Mac	Mac (broader)	(39)
CD22/Siglec-2	B	B (mRNA)	(39)
CD33/Siglec-3	Mono, Mac (broader), Microglia	Mono, Mac, Microglia	(38)
MAG/Siglec-4	Schwann cells, Oligodendroglia	(Myelin)	(59, 60)
Siglec-5	Neutro, Mac (broader), B (low), **amniotic epithelium**	Neutro, Mac, **T**, B	(38, 61–63)
Siglec-6	B, DC subset, **placenta**	B	(64)
Siglec-7	NK, Mono, Mast, Neutro, Baso, Platelets, T (subset)	ND	(65–70)
Siglec-8	Eosino, Baso, Mast	ND	(71, 72)
Siglec-9	Neutro, Mono, Mac (broader)	Neutro, Mono, Mac	(38)
Siglec-10	B, Mono, DC	ND	(73, 74)
Siglec-11	Mac, **Microglia**, ovarian fibroblasts	Mac, ovarian fibroblasts	(43, 75–77)
Siglec-12	Mac, lumenal epithelia	Mac, lumenal epithelia	(36, 46)
Siglec-13	X (absent)	Mono	(45)
Siglec-14	Neutro, Mono, **amniotic epithelium**	Neutro (& Mono?)	(62, 63)
Siglec-15	OC, Mac subset	ND	(78–81)
Siglec-16	Mac, **Microglia**	Mac	(43, 75, 82)

Mac, macrophage; Mono, monocytes; B, B cells; T, T cells; NK, natural killer cells; Mast, mast cells; Neutro, neutrophils; Eosino, eosinophils; Baso, basophils; DC, dendritic cells; OC, osteoclasts; ND, not determined.

**Tissue/cell type that showed clear difference in Siglec expression between human and chimpanzee are highlighted with underline and bold typeface. Reports that directly compared human and chimpanzee Siglec expression patterns are primarily cited in this table. For human Siglecs, expression in the cell types not listed in the table are also reported, such as: CD22/Siglec-2 on basophils (83); CD33/Siglec-3 on mast cells (84), basophils and neutrophils (low) (85); Siglec-6 on mast cells (86); Siglec-9 on T cell subset (68) and NK cell subset (55). Note that the expression of Siglec-6 on human B cells is restricted to CD27$^+$ memory B cells (87). Tumor-infiltrating T cells express several human Siglecs, including CD33/Siglec-3, Sigec-5, Siglec-7, Siglec-9, and Siglec-10 (88).*

the binding specificity of chimpanzee Sialoadhesin/Siglec-1 has not been analyzed, given that both human and mouse Sialoadhesin/Siglec-1 preferentially recognize Neu5Ac (39) and the sequence differences between human and chimpanzee Sialoadhesin/Siglec-1 are small (**Table 1**), it is likely that chimpanzee Sialoadhesin/Siglec-1 prefers Neu5Ac. Thus, the altered distribution of human Sialoadhesin/Siglec-1$^+$ macrophages may be a consequence of the loss of Neu5Gc in humans (39). It is possible that the altered distribution of Sialoadhesin/Siglec-1$^+$ macrophages may be more relevant to the increased density of Neu5Ac in human tissues that may influence the migration of macrophages, rather than a change in cell types that express Sialoadhesin/Siglec-1. In this regard, it would be interesting to know whether the distribution of Sialoadhesin/Siglec-1$^+$ macrophages in *Cmah* knockout mice is different from that in wild-type mice.

One of the most striking changes in Siglec expression patterns in the human immune system is the near-complete absence of Siglec-5 on human T cells, in contrast to its prominent expression on chimpanzee and gorilla T cells (61, 62). The loss of Siglec-5 from human T cells appears to be correlated with the relative hyper-activation of human T cells in response to various stimuli compared with those from other great apes. [Although Siglec-5 and Siglec-14 show extremely high sequence similarity at the extracellular domain, one study (62) used a combination of antibodies that distinguish Siglec-5 and Siglec-14 to demonstrate that Siglec-5 is expressed on chimpanzee T cells]. However, it is not clear whether the loss of Siglec-5 on human T cells has a causative relationship with the loss of Neu5Gc, as human Siglec-5 does not show strong preference for either Neu5Ac or Neu5Gc (38), and its great ape counterparts have a mutation at the essential arginine residue and lack the ability to recognize sialic acids (15, 89). It is also worth mentioning that a recent work demonstrated that Siglec-5 is inducibly expressed by the activation of human T cells (88).

Siglec-11 and Siglec-16 also have undergone unique changes in their expression patterns in humans. Whereas, human Siglec-11 and Siglec-16 are expressed on brain microglia and tissue macrophages, chimpanzee Siglec-11 and Siglec-16 appear to be absent on microglia (but present on tissue macrophages) (43, 75). The change in expression patterns appears to be a consequence of a partial gene conversion of *SIGLEC11* by *SIGLEC16*. Of note, *SIGLEC16* in humans has functional and non-functional alleles (82), and the non-functional allele appears to be the one that converted *SIGLEC11* (90). *SIGLEC11* and *SIGLEC16* have undergone a complex series of concerted evolution through gene conversions in human lineage (90) and also in other lineages of apes (44). Both human and chimpanzee Siglec-11 and Siglec-16 appear to prefer Neu5Gc over Neu5Ac (43, 44), and thus it is tempting to speculate that the loss of Neu5Gc may have had some influence on the altered expression patterns of these Siglecs. Although it is known that the Neu5Gc level is extremely low in mammalian brains (91), Siglec-11 and Siglec-16 also preferentially recognize α2-8–linked Neu5Ac dimers, which are abundant in the brain and serve as ligands for these Siglecs on human microglia.

Siglec-6 was also reported to show different expression patterns between human and chimpanzee. Both human and

chimpanzee Siglec-6 are expressed on B cells, whereas its expression on placental trophoblasts is observed only in humans (64). This altered expression is thought to be associated with the sequence change in the promoter region and transcription factor binding (64).

There are some reports of the presence of Siglec ligands in human tissues that are absent in chimpanzee tissues (64, 76). Although the exact nature of these ligands has not been identified, these findings imply that the difference in Siglec ligand expression patterns beyond the absence/presence of Neu5Gc may exist between human and chimpanzee and may also contribute to the rapid evolution of the Siglec family (particularly at Ig1) and/or their altered expression patterns.

AN ALTERNATIVE DRIVING FORCE BEHIND SIGLEC EVOLUTION: INTERACTION WITH MICROBES

Given that Ig1 of Siglecs (particularly that of CD33-related Siglecs) is undergoing rapid evolution (57), and not all of this may be attributed to the changing endogenous ligand landscape, there is likely an alternative driving force behind their rapid evolution. Obviously, one such force could be microbial pathogens that engage Siglecs. Indeed, recent studies have provided evidence that many Siglecs are involved in the interaction with various pathogenic microbes [for recent reviews, see (92, 93)]. These microbes include viruses, bacteria, and eukaryotic pathogens (**Table 3**). Many of them cover themselves with sialic acids (either by *de novo* biosynthesis or by "salvage" from the human body by various mechanisms), which may be considered examples of "molecular mimicry" by microbes.

The majority of the microbes reported to interact with Siglecs so far are bacteria (**Table 3**). This makes sense, as sialic acids (and sialic acid-like nonulosonic acids) are occasionally found in bacterial extracellular components, such as lipopolysaccharides/lipooligosaccharides (LPS/LOS), capsular polysaccharides (CPS), and flagella. For example, group B streptococcus (GBS) type III interacts with Siglec-9 through sialylated CPS and dampens inflammatory responses by neutrophils (98), whereas GBS type Ia engages Siglec-5 by β-protein and also suppresses inflammatory responses of myeloid cells (100). It should be noted that the latter case does not involve sialic acids. Similarly, non-typeable *Haemophilus influenzae*, an opportunistic airway pathogen, engages Siglec-5 and attenuates pro-inflammatory cytokine production by myeloid cells (102), and *Escherichia coli* K1 strain, a neurotropic pathogen, engages Siglec-11 and escape killing (75). Siglecs are likely under pressure to escape the exploitation by these pathogens, which may partially explain the driving force behind their rapid evolution.

It appears that Siglecs were not just escaping from these pathogens; they appear to have developed "counter-traps" against these pathogens. Some Siglecs (i.e., Siglec-5 and Siglec-14; Siglec-11 and Siglec-16) are found to be "paired receptors," which are two Siglecs with highly homologous extracellular domains recognizing similar ligands, combined with intracellular signaling modules transducing opposing signals (i.e., one of the pair interacts with SHP-1 and transduces the inhibitory signal, whereas the other interacts with adapter protein DAP12 and tyrosine kinase Syk and transduces the activating signal). In fact, whereas the engagement of inhibitory Siglec by pathogenic bacteria suppresses anti-bacterial responses, the engagement of activating Siglec counteracts this effect (63, 75, 102). It is of note that these "paired" Siglecs appear to show more sequence differences between human and chimpanzee than other "stand-alone" Siglecs (**Table 1**), possibly implying that these Siglecs are under higher selective pressure to diversify than are other Siglecs. These paired Siglecs are undergoing concerted evolution through repeated gene conversions (43, 44, 89, 90), which is likely necessary to maintain the effectiveness of activating-type Siglec as "counter-traps." It is also intriguing that, in humans, null alleles for these activating-type Siglecs (Siglec-14 and Siglec-16) are found at very high frequencies (82, 113).

Evidence supporting the relevance of these interactions between Siglecs and bacterial pathogens in infectious diseases is emerging from genetic association studies (**Table 4**). Small-scale case–control studies investigating the possible correlations between the polymorphisms of *SIGLEC* genes and infectious disease susceptibility have revealed some correlations, such as *SIGLEC14* null polymorphism and COPD exacerbation (102), pre-term delivery in the presence of GBS (63), *Mycobacterium tuberculosis* meningitis (138), and *SIGLEC9* polymorphism and COPD exacerbation (133). In addition, large-scale genome-wide association studies (GWAS) have also revealed possible associations between *SIGLEC* polymorphisms and infectious diseases, such as *SIGLEC5* polymorphism and leprosy (129) and severe periodontitis (128), although these GWAS did not demonstrate a direct interaction between the etiological agents and Siglec protein. Some *SIGLEC* genetic polymorphisms appear to influence the leukocyte counts (142); thus it is possible that the influence of *SIGLEC* genetic polymorphisms on antibacterial defense may be indirect. Regardless, the application of GWAS to infectious diseases may further reveal the relevance of Siglecs for immunological defense against bacterial pathogens.

With regard to viral pathogens, recent studies have revealed that Sialoadhesin/Siglec-1 (also known as CD169) may play a major role in retrovirus infection (103). For example, several groups have reported that human immunodeficiency virus (HIV) exploits Sialoadhesin/Siglec-1 to enhance infection of $CD4^+$ T cells (the primary target cells) by trans-infection (i.e., the virus particle is captured by macrophages with Sialoadhesin/Siglec-1, which transfers the virus to $CD4^+$ T cells and facilitates the infection) (104–107). Although a rare "null" mutation in the *SIGLEC1* gene was found not to protect carriers from HIV infection (144), the low frequency of this mutation (allele frequency: ∼1.3% in Europeans) may preclude us from making a definitive conclusion (145). Given that Sialoadhesin/Siglec-1 appears to be involved in retroviral infection in both mouse and human (103), one may expect that it should evolve rapidly to avoid viral infections; however, Sialoadhesin/Siglec-1 does not appear to be evolving rapidly (**Table 1**). This may be because enveloped viruses are coated with a host-derived membrane (a

TABLE 3 | Direct interaction of human Siglecs and microbes.

Microbe	Microbial molecule involved	Human siglec involved	Outcome	References
BACTERIA				
Neisseria meningitidis	Sialic acids on LPS	Sialoadhesin/Siglec-1 Siglec-5	Enhanced binding and phagocytosis	(94)
Campylobacter jejuni	Sialic acids on LPS	Sialoadhesin/Siglec-1 Siglec-7	Modulation of factors affecting helper T-cell differentiation	(95–97)
	Pseudaminic acid on flagellin	Siglec-10	Promote anti-inflammatory response	(74)
Group B Streptococcus type III	Sialic acids on CPS	Siglec-9	Attenuated immune responses	(98, 99)
Group B Streptococcus type Ia	β protein (Sia-independent)	Siglec-5 Siglec-14	Siglec-5: Attenuated responses Siglec-14: Enhanced responses	(63, 100)
		Siglec-13 (chimpanzee)	Attenuated response	(45)
Pseudomonas aeruginosa	Sialic acids on glycoproteins, adsorbed from human body fluid	Siglec-9	Attenuated immune responses	(101)
Non-typeable *Haemophilus influenzae*	Sialic acids on LOS + Sia-independent interaction	Siglec-5 Siglec-14	Siglec-5: Attenuated responses Siglec-14: Enhanced responses	(102)
Escherichia coli K1 strain	CPS (polysialic acids)	Siglec-11 Siglec-16	Siglec-11: Attenuated responses Siglec-16: Enhanced responses	(75)
VIRUSES				
Human immunodeficiency virus (HIV)	Sialic acids on gp120 envelope glycoprotein; host-derived gangliosides on envelope	Sialoadhesin/Siglec-1 Siglec-7	Enhanced infection	(103–108)
Varicella zoster virus (VZV), herpes simplex virus (HSV)	Glycoprotein B (sialic acids required)	MAG/Siglec-4	Enhanced infection	(109, 110)
EUKARYOTES				
Candida albicans	zymosan (?)	Siglec-7	Enhanced immune responses	(111)
Leishmania donovani	Surface sialic acids	Sialoadhesin/Siglec-1 Siglec-5	Enhanced infection	(112)

Updated from Angata and Varki (93).

part of "self"), and thus there is no way Sialoadhesin/Siglec-1 can evolve to completely evade such an interaction (unless the virus develops a protein that binds Sialoadhesin/Siglec-1 in sialic acid-independent manner). It is worth noting that myelin-associated glycoprotein (MAG)/Siglec-4, the other Siglec known to interact with another enveloped virus (109, 110), is also highly conserved among mammals, and in both cases sialic acids are required for the interaction between the virus and Siglecs (**Table 3**).

CONCLUSION AND PERSPECTIVES

Cmah null mouse is a valuable tool for the investigation of the physiological roles of Neu5Gc and the short-term consequences of its loss, although it may not be a perfect model of modern human. Using this mouse model, it was shown that the expression of Neu5Gc itself makes T cells less responsive to stimulus, without any change in Siglec expression (146). Likewise, Neu5Gc appears to have a general suppressive effect on mouse monocyte/macrophage activities, without the apparent involvement of Siglecs (147). In line with these findings, the loss of Neu5Gc has had major influences on human biology that reach far beyond Siglecs (148), explaining some of the differences between human and our close relatives (e.g., chimpanzee) in pathophysiological phenotypes (149). Although Neu5Gc from dietary sources (in the form of meat or milk from the animals that express Neu5Gc) can be incorporated into human tissue glycoproteins and glycolipids (150), the level of Neu5Gc in human tissues tends to be low, accounting for <1% of total sialic acids (151). Given that the current set of human Siglecs lack a strong preference toward Neu5Gc, and the affinity between Siglecs and sialic acids tends to be low (K_d in ~mM range), this level of Neu5Gc in human tissues may not influence human physiology by way of Siglecs. The trace amount of Neu5Gc

TABLE 4 | Polymorphisms in human *SIGLEC* genes and association with disease/phenotype.

Gene	Polymorphism	Associated phenotype	References
SIGLEC1	rs656635, rs609203, rs3859664, rs4813636 (SNPs in intron or 3'UTR)	Lung function	(114)
SIGLEC1	rs6037651 (nonsynonymous SNP)	Serum IgM level	(115)
CD22	rs34826052 (synonymous SNP)	Limited cutaneous systemic sclerosis	(116)
CD22	rs4805119 etc. (intronic SNP)	B-precursor leukemia	(117, 118)
CD33	rs3865444 (promoter SNP) rs12459419 (nonsynonymous SNP, influencing splicing)	Late-onset Alzheimer's disease	(119–122)
CD33	rs35112940, rs12459419 (nonsynonymous SNPs)	Efficiency of antibody therapy in pediatric acute myeloid leukemia	(123, 124)
MAG	rs720309 (intronic SNP) rs7249617 (intronic SNP)	Schizophrenia	(125, 126) (127)
SIGLEC5	rs4284742 (intronic SNP)	Periodontitis	(128)
SIGLEC5	rs10414149 (intronic SNP)	Leprosy	(129)
SIGLEC6	rs2305772 (non-synonymous SNP, influencing splicing)	Systemic lupus erythematosus	(130)
SIGLEC8	rs36498 (promoter SNP) rs10409962 (nonsynonymous SNP)	Allergic asthma	(131)
SIGLEC9	rs16988910 (nonsynonymous SNP)	Short-term survival of lung cancer patients; Emphysema	(132)
SIGLEC9	rs2075803, rs2258983 (nonsynonymous SNP)	COPD exacerbation	(133)
SIGLEC11	rs12165127 (intronic SNP)	Lung cancer in never-smokers	(134)
SIGLEC12	rs16982743 (stop codon generated)	Cardiovascular outcomes in patients with hypertension on antihypertensive therapy	(135)
SIGLEC12	rs3752135 (nonsynonymous SNP)	Stress fracture	(136)
SIGLEC14	rs10412972, rs11084102 (upstream SNPs)	Plasma plasminogen level	(137)
SIGLEC14	*SIGLEC14-SIGLEC5* fusion (*SIGLEC14* deletion)	COPD exacerbation	(102)
SIGLEC14	*SIGLEC14-SIGLEC5* fusion (*SIGLEC14* deletion)	Pre-term delivery in the presence of GBS infection	(63)
SIGLEC14	*SIGLEC14-SIGLEC5* fusion (*SIGLEC14* deletion)	*Mycobacterium tuberculosis* meningitis	(138)
Various	Various	Plasma protein levels	(139, 140)
Various	Various	Cerebrospinal fluid protein levels	(141)
Various	Various	Blood cell counts	(142)

Updated from Angata (16) and Angata (143).
Some of the studies listed above are small-scale case–control studies, whereas some others are large-scale genome-wide association studies (GWAS). Some of the associations listed are not prominently featured in the references cited but found in the GWAS catalog (https://www.ebi.ac.uk/gwas/).

incorporated into human tissues may be more relevant to the bacterial toxins that specifically recognize Neu5Gc (152) and xeno-autoantibodies that recognize Neu5Gc (as discussed in other articles of this series). Regardless, the loss of Neu5Gc appears to have left some footprint on the evolution of human Siglecs, as discussed above.

The evolution of human Siglecs was also likely influenced by the interaction with microbes. A recent population genetics-based study implied that some Siglecs may have been subjected to population-specific hard selective sweeps, as judged by the presence of long-range linkage disequilibrium (153). These *SIGLEC* genes include *SIGLEC8* and *SIGLEC10* among Africans, *SIGLEC5*, *SIGLEC6*, *SIGLEC12*, and *SIGLEC14* among Europeans, and *CD22* and *MAG* among Asians. Although it remains speculative, the population-specific difference in the signatures of selection imply that the evolution of the Siglec family in the human population is an ongoing process, and different pathogen pressures are present in different geographical locations (or through different agricultural constraints, e.g., use of different domestic animals, which may carry different kinds of bacteria/viruses).

Many questions remain with regard to the function and evolution of Siglecs. For example, do viruses really target only conserved Siglecs and are they not relevant to the rapid evolution of Siglecs? What was or is the selective force behind the spread of "null" alleles of *SIGLEC14* and *SIGLEC16* (and perhaps others, such as *SIGLEC1*) in modern human populations? Do the bacteria that express sialic acid-like nonulosonic acids (154) generally engage Siglecs to modulate immune responses and thus play a role in the evolution of Siglecs? Does the interaction between Siglecs and commensal bacteria (e.g., normal gut microbiota) play any role in the modulation of immunity and the evolution of Siglecs? Some of these questions can be addressed experimentally and will deepen our understanding of the biology of Siglecs and sialic acids.

AUTHOR CONTRIBUTIONS

TA analyzed the literature and wrote the manuscript.

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