Chapter 9
Siglecs that Associate with DAP12

Takashi Angata

Abstract Siglecs are a family of transmembrane receptor-like glycan-recognition proteins expressed primarily on leukocytes. Majority of Siglecs have an intracellular sequence motif called immunoreceptor tyrosine-based inhibitory motif (ITIM) and associate with Src homology region 2 domain-containing tyrosine phosphatase-1 (SHP-1), and negatively regulate tyrosine phosphorylation-mediated intracellular signaling events. On the other hand, some Siglecs have a positively charged amino acid residue in the transmembrane domain and associate with DNAX activation protein of 12 kDa (DAP12), which in turn recruits spleen tyrosine kinase (Syk). These DAP12-associated Siglecs play diverse functions. For example, Siglec-15 is conserved throughout vertebrate evolution and plays a role in bone homeostasis by regulating osteoclast development and function. Human Siglec-14 and -16 have inhibitory counterparts (Siglec-5 and -11, respectively), which show extremely high sequence similarity with them at the extracellular domain but interact with SHP-1. The DAP12-associated Siglec in such "paired receptor" configuration counteracts the pathogens that exploit the inhibitory counterpart. Polymorphisms (mutations) that render DAP12-associated inactive Siglecs are found in humans, and some of these appear to be associated with sensitivity or resistance of human hosts to bacterially induced conditions. Studies of mouse Siglec-H have revealed complex and intriguing functions it plays in regulating adaptive immunity. Many questions remain unanswered, and further molecular and genetic studies of DAP12-associated Siglecs will yield valuable insights with translational relevance.

Keywords Siglec · DAP12 · ITAM · Syk · Paired receptors

T. Angata (✉)
Institute of Biological Chemistry, Academia Sinica, 128, Section 2, Academia Road, Nangang District, Taipei, Taiwan
e-mail: angata@gate.sinica.edu.tw

S.-L. Hsieh (ed.), *Lectin in Host Defense Against Microbial Infections*,
Advances in Experimental Medicine and Biology 1204,
https://doi.org/10.1007/978-981-15-1580-4_9

9.1 Introduction

Glycan recognition proteins in vertebrates are often expressed by leukocytes, and are often involved in the discrimination of self versus non-self. For example, some of the C-type lectins recognize pathogen-associated molecular patterns (PAMPs) and alert immune system, as described in other chapters. On the other hand, sialic acids, a group of acidic sugars with nine-carbon backbone (Angata and Varki 2002), are rarely found in microbes. Thus, sialic acids can be a good marker of "self" (self-associated molecular patterns, i.e., SAMPs) for vertebrate immune system (Varki 2011). In fact, complement factor H, a protein that regulates the complement cascade by binding to and inhibiting complement component C3b in association with complement factor I, has been known to bind to sialic acids and protect mammalian cells from complement-mediated attack (Meri and Pangburn 1990; Blaum et al. 2015; Blaum 2017). Investigations in the past few decades have revealed that not only soluble protein (complement factor H) but also membrane-bound receptor-like proteins utilize sialic acids as SAMPs. At least some members of Siglec family appear to fall into this category.

As the properties and functions of Siglec family have been extensively reviewed elsewhere (Varki and Angata 2006; Pillai et al. 2012; Macauley et al. 2014), this chapter only reiterates some basic information here. The word "Siglec" is a composite word from "sialic acid (Sia)", "immunoglobulin (Ig) superfamily", and "lectin" (Crocker et al. 1998). As the name suggests, most of them recognize glycans that contain sialic acids. Siglecs share a basic architecture, which consists of an N-terminal V-set Ig-like domain, followed by multiple C2-set Ig-like domains (the number of which varies from 1 to 16), a single-pass transmembrane domain, and a short cytoplasmic tail. The N-terminal Ig-like domain is the most important for sialic acid recognition. A subgroup of Siglecs (i.e., sialoadhesin/Siglec-1, CD22/Siglec-2, myelin-associated glycoprotein (MAG)/Siglec-4, and Siglec-15) is conserved among multiple lineages of vertebrates, whereas others, collectively known as CD33/Siglec-3-related Siglecs (CD33rSiglecs), are less conserved (Fig. 9.1). CD33rSiglecs are encoded in a gene cluster, implying that this subfamily has expanded by repeated gene duplications; sequences of CD33rSiglec genes were further diversified through genetic recombination and exon shuffling events, in addition to the gradual accumulation of nucleotide substitutions (Angata et al. 2004).

The Siglec family can also be classified based on the partner molecule involved in the signal transduction. Majority of Siglecs have a sequence motif called immunoreceptor tyrosine-based inhibitory motif (ITIM) and another ITIM-like motif in the cytoplasmic tail, and recruit Src homology region 2 domain-containing tyrosine phosphatase-1 (SHP-1), a protein tyrosine phosphatase that plays a major role in suppressing autoimmunity (Shultz et al. 1997). This configuration implies that the majority of Siglecs play a negative regulatory role in the cells that express them. This fact fits the current view of the basic role of Siglecs, which is to sense the sialic acids on own cells as SAMPs and to prevent immune responses against them (Lajaunias et al. 2005; Duong et al. 2010; Varki 2011). In contrast, a small number

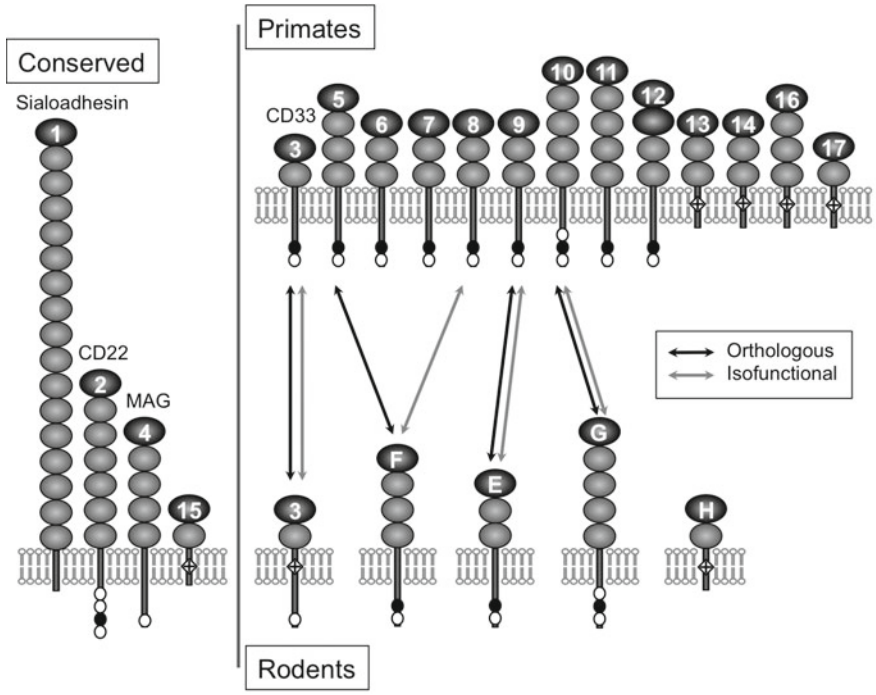

Fig. 9.1 Schematic representation of Siglecs in primates and rodents. Analyses of genomic DNA sequences of mammals so far have revealed that primate genomes encode up to 17 functional Siglecs, while the rodent genomes have nine functional Siglecs. Other lineages of mammals may have different set of Siglecs (Angata 2006). Note that not all primate Siglecs are present in all primates. For example, Siglec-13 is absent in humans, and Siglec-17 is non-functional or absent in many Old World monkeys and apes (including humans). Putative orthologous and isofunctional relationships between primate and rodent Siglecs are indicated with solid and gray lines, respectively. V-set Ig-like domains are represented by larger ovals with darker shade, whereas C2-set Ig-like domains are represented by smaller ovals with lighter shade. Other symbols used are: filled circle: ITIM; open circle: ITIM-like sequence motif; diamond with "+" symbol: positively charged amino acid residue in the transmembrane domain, which is involved in the interaction with DAP12. Modified from Angata (2017)

of Siglecs lack ITIM, and instead have a positively charged amino acid residue in the transmembrane domain, which is involved in the interaction with DNAX activation protein of 12 kDa (DAP12), a small adapter protein that has an immunoreceptor tyrosine-based activating motif (ITAM). Phosphorylated ITAM of DAP12 recruits spleen tyrosine kinase (Syk), a protein tyrosine kinase that plays a major role in the recognition and elimination of foreign agents by the immune system (Kerrigan and Brown 2011; Lowell 2011). Thus, a subset of Siglecs may recognize SAMPs and activate the immune system. This situation appears risky, as it could lead to autoimmunity. How could this risk be mitigated?

In this chapter, the Siglecs that associate with DAP12 are introduced. Siglec-15 is a DAP12-associated Siglec that appears to be conserved in vertebrates. Two human Siglecs (Siglec-14 and -16) and two primate Siglecs (Siglec-13 and -17) absent in humans have been shown to interact with DAP12. In rodents, Siglec-H is known to associate with DAP12. Functions of these Siglecs and the potential mechanisms that prevent the triggering of autoimmunity by these Siglecs will be discussed.

9.2 Primate and Rodent Siglecs that Associate with DAP12

DAP12, also known as killer-activating receptor-associated protein (KARAP), is encoded by *TYROBP* gene in humans. DAP12 is a type I transmembrane protein expressed on natural killer cells and myeloid cells, and has an aspartic acid residue in the transmembrane domain, by which it interacts with numerous membrane proteins that have a positively charged amino acid residue in the transmembrane domain (Turnbull and Colonna 2007). One of these DAP12-associated receptors is triggering receptor expressed on myeloid cells 2 (TREM2, encoded by *TREM2* gene in humans). Mutation in either *TYROBP* or *TREM2* gene causes a hereditary disease called Nasu-Hakola disease (also known as polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy) (Paloneva et al. 2000, 2002, 2003), implying that TREM2 is one of the major receptors that use DAP12 as signaling module. The mechanism that enables various receptors that use DAP12 as a common signaling module to play diverse receptor-specific functions is not fully understood, but the specific recognition of ligands by these receptors is likely of critical importance.

None of the DAP12-associated human Siglecs has been as extensively studied as TREM2, partly because only Siglec-15 among them is conserved between human and mouse, allowing functional investigations using mouse model. Nevertheless, valuable insights have been gained by evolutionary studies, cell-based functional studies, and genetic association studies in humans.

9.2.1 Siglec-15

Siglec-15 is conserved from fish to mammals (Angata et al. 2007). In contrast to most Siglecs that have three cysteine residues in each of the two N-terminal Ig-like domains, which form not only intra-domain disulfide bonds but also an inter-domain disulfide bond that tether these two domains, Siglec-15 has even number of cysteine residues in these domains (4 and 2 cysteines in the first and second Ig-like domain, respectively), that is more typical for Ig superfamily. These facts may possibly imply that Siglec-15 was the ancestor of the Siglec family. Siglec-15 interacts with DAP12, and also with another adapter protein, DNAX activation protein of 10 kDa (DAP10), at least when these proteins are over-expressed (Angata et al. 2007).

Multiple laboratories have demonstrated that Siglec-15 is expressed on osteoclasts and is involved in osteoclast differentiation (Hiruma et al. 2011; Ishida-Kitagawa et al. 2012; Stuible et al. 2014). Siglec-15 deficient mice develop mild osteopetrosis (i.e., an increase of bone density) (Hiruma et al. 2013; Kameda et al. 2013), particularly in the trabecular bone, due to the deficiency in osteoclast differentiation. This property prompted some groups to propose Siglec-15 as a potential therapeutic target for osteoporosis (Hiruma et al. 2013; Stuible et al. 2014; Kameda et al. 2015; Shimizu et al. 2015). Importantly, antibody-mediated cross-linking of osteoclast cell-surface Siglec-15 facilitates internalization and degradation of Siglec-15 (Stuible et al. 2014), mimicking Siglec-15 deficiency.

We have recently demonstrated that CD44 is a biologically relevant ligand of Siglec-15 on an osteoclast model cell line (Chang et al. 2017). Our previous findings that Siglec-15 recognizes tumor-associated glycan structure (sialyl-Tn) and is expressed on tumor-associated macrophages and regulates the production of growth factor (Angata et al. 2007; Takamiya et al. 2013), combined with the high expression of sialyl-Tn and CD44 on various solid tumors (Ponta et al. 2003; Zoller 2011), imply that Siglec-15 may also play some roles in tumor microenvironment through interaction with the ligands displayed on tumor cells.

There may be several safeguarding mechanisms to prevent Siglec-15 from promoting autoimmunity by recognizing sialic acid-containing ligands. For example, the expression of Siglec-15 is restricted to osteoclasts (as mentioned above) and a subset of lymph node macrophages in normal tissues (Angata et al. 2007); its expression on lymph node macrophages appears to be predominantly intracellular (Angata et al. 2007); the glycan structure it preferentially recognizes (sialyl-Tn) is also expressed in a spatio-temporarily restricted manner in normal tissues (Ogata et al. 1995; Julien et al. 2012).

9.2.2 Siglec-14

The gene encoding Siglec-14 (*SIGLEC14*) was initially identified as a genetic segment that shows extreme sequence identity with *SIGLEC5*, the gene encoding Siglec-5 (Angata et al. 2004). A follow-up study revealed that this segment encodes a functional protein, which was designated Siglec-14 (Angata et al. 2006). Siglec-14 is conserved among primates and some other lineages of mammals, but is absent in rodents (Angata et al. 2004, 2006; Angata 2006). Siglec-14 is expressed on myeloid cells and enhances pro-inflammatory responses by interacting with DAP12 (Yamanaka et al. 2009). Given that the glycan binding preference of Siglec-14 is somewhat promiscuous (Angata et al. 2006), it is possible that Siglec-14 may recognize endogenous sialylated glycans and induce pro-inflammatory responses.

One possible mechanism by which the immune system circumvents this problem is to couple it with an inhibitory counterpart. The gene encoding Siglec-14 (*SIGLEC14*) is located right next to *SIGLEC5* encoding Siglec-5, a SHP-1-associated inhibitory Siglec (Cornish et al. 1998), in a tandem (head-to-tail) orientation. The expression

patterns of Siglec-14 and Siglec-5 overlap at least partially (Yamanaka et al. 2009). [A caution is required for the interpretation of the earlier reports on the expression pattern of Siglec-5, as most antibodies against Siglec-5 cross-react with Siglec-14 (Angata et al. 2006).] As mentioned above, the segment of *SIGLEC14* gene that encodes the N terminus of the protein (signal peptide and first two Ig-like domains) shows extreme sequence similarity with the corresponding segment in *SIGLEC5* gene, and thus Siglec-14 and Siglec-5 show similar glycan binding preferences (Angata et al. 2006). This extreme sequence similarity between *SIGLEC14* and *SIGLEC5* is observed in multiple species, likely implying that the sequence similarities between these two genes have been maintained through repeated gene conversion events in multiple lineages and selected for under selective pressure (Angata et al. 2006). Based on the findings that Siglec-5 is exploited by some bacterial pathogens (Carlin et al. 2009; Angata et al. 2013; Ali et al. 2014) and that Siglec-14 appears to counteract this exploitation by binding to the bacteria and triggering cell-activating signals (Angata et al. 2013; Ali et al. 2014), it was proposed that Siglec-14 may have arisen to counter the pathogens that exploit Siglec-5.

This hypothesis gained support from human genetic studies. An allele in which *SIGLEC14* and *SIGLEC5* are fused (resulting in *SIGLEC14–SIGLEC5* fusion gene) is common (allele frequency > 0.05) in all human populations tested, and is even a dominant allele in some parts of East Asia (Yamanaka et al. 2009). This fusion gene lacks the segment that is unique to Siglec-14, and encodes a protein identical to Siglec-5 in amino acid sequence; thus, it is functionally a *SIGLEC14*-null allele. By focused genetic association studies, this *SIGLEC14*-null allele was found to be associated with the increased risk of pre-term delivery in the presence of group B *Streptococcus* infection (Ali et al. 2014), whereas the same allele is associated with the reduced risk of exacerbation among the patients of chronic obstructive pulmonary disease (Angata et al. 2013) and the development of meningitis among the individuals infected by *Mycobacterium tuberculosis* (Graustein et al. 2017). While the first case implies that Siglec-14 counters the pathogens that exploit Siglec-5 (thus protect the host), the latter two imply that strong pro-inflammatory responses caused by bacterial engagement of Siglec-14 may be detrimental to the host under some circumstances. This fact may explain why *SIGLEC14*-null allele is dominant in some geographical areas; in the area where some environmental condition that causes chronic lung inflammation is widespread and the inflammation can be exacerbated by some bacteria (e.g., non-typeable *Haemophilus influenzae*, a commensal bacteria present in the airways of a majority of healthy individuals), carriers of *SIGLEC14*-null allele may have had some selective advantage. However, definitive proof to support this hypothesis is still lacking.

9.2.3 *Primate Siglec-16*

Siglec-16 is another Siglec that is coupled with inhibitory counterpart (in this case Siglec-11). The gene encoding human Siglec-16 (*SIGLEC16*) was initially considered a pseudogene (designated *SIGLECP16* or *SIGLEC16P*) because of a 4-nucleotide deletion in exon 2 (Angata et al. 2002), but an independent study correctly pointed out that this deletion in human *SIGLEC16* is polymorphic and a functional allele also exists (Cao et al. 2008). The latter study also demonstrated that Siglec-16 interacts with DAP12 and transduces cell-activating signal. Thus, Siglec-16 associates with DAP12, whereas Siglec-11 associates with SHP-1 and transduces inhibitory signal (Angata et al. 2002). These proteins show high sequence similarity, which is particularly high at the N-terminal three Ig-like domains (Hayakawa et al. 2005). A putative ortholog of *SIGLEC11* gene is found in multiple mammalian lineages, whereas *SIGLEC16* appears to be confined to primate lineage (Cao et al. 2008).

Similar to *SIGLEC14* and *SIGLEC5*, *SIGLEC16* and *SIGLEC11* genes are also located next to each other, but in this case the two genes are situated in an inverted (head-to-head) orientation (Angata et al. 2002). In the lineage leading to modern humans, these two genes have undergone several rounds of gene conversion events (Wang et al. 2012a). In addition, our recent study has revealed that *SIGLEC16* and *SIGLEC11* have also undergone concerted evolution in other lineages of primates (Hayakawa et al. 2017). The frequency of gene conversion events appears to be lower than that of *SIGLEC14*/*SIGLEC5* pair, likely because of the inverted orientation of the genes (Hayakawa et al. 2017). Siglec-11 was reported to preferentially recognize α2–8-linked oligosialic acids (Angata et al. 2002), and the gene conversions appear to have maintained this binding preference of Siglec-11 and Siglec-16 proteins (Hayakawa et al. 2017). These findings imply that the Siglec-11 and Siglec-16 have also been maintained as paired receptors under some selective pressure.

Siglec-11 and Siglec-16 are both expressed on myeloid cells, including brain microglia (Hayakawa et al. 2005; Cao et al. 2008). The expression of Siglec-11 (and/or Siglec-16) in brain is considered to be a human-specific event (Hayakawa et al. 2005), which was likely caused by a gene conversion by the promoter region of the non-functional allele of *SIGLEC16* gene (i.e., *SIGLEC16P*) (Wang et al. 2012a). Whereas earlier reports on the expression patterns of Siglec-11 (Hayakawa et al. 2005; Wang et al. 2011) using a monoclonal antibody against Siglec-11 may require re-evaluation due to the cross-reactivity of the antibody with Siglec-16, the expression of Siglec-11 and Siglec-16 on myeloid cells was confirmed with a set of monospecific antibodies that recognize either Siglec-11 or Siglec-16 alone (Schwarz et al. 2017).

Curiously, the non-functional allele of *SIGLEC16* appears to be dominant in modern humans (Cao et al. 2008; Wang et al. 2012a; Hayakawa et al. 2017). A study showing genetic association of this polymorphism with any human disease is yet to be seen. Regardless, a functional study using mouse model, in which Siglec-E (an

inhibitory Siglec widely expressed on myeloid cells) was converted to an activating-type "Siglec-E16" by replacing its native transmembrane-intracellular segment with that of human Siglec-16, revealed that the mice expressing Siglec-E16 is protected against K1 strain of *Escherichia coli*, a bacterial strain covered with capsular polysaccharide consisting of polysialic acids and causes meningitis in humans (Schwarz et al. 2017). Thus, an overall picture that was proposed for Siglec-14–Siglec-5 pair, that is, activating-type Siglec counters the pathogen that exploits inhibitory counterpart, appears to be applicable to Siglec-16–Siglec-11 pair as well. The risk of Siglec-16-mediated autoimmune reaction by recognition of SAMP may be mitigated by the presence of Siglec-11. In addition, low prevalence of oligo/polysialic acids in non-neuronal tissues compared with those of other linkages (i.e., α2-3 and α2-6-linked sialic acids) may also contribute to the avoidance of SAMP-mediated stimulation of innate immune system by Siglec-16 engagement [although the distribution of oligo/polysialic acids in mammalian tissues is much broader than it was once assumed in the past (Sato and Kitajima 2013; Colley et al. 2014)].

Another study using transgenic mice that express Siglec-11 in mononuclear phagocytes demonstrated that Siglec-11 has anti-inflammatory function by recognizing polysialic acids (Karlstetter et al. 2017). As inflammation in nervous system can lead to detrimental consequences (e.g., neurodegenerative disorders), the prevalence of non-functional *SIGLEC16* allele in modern humans may imply that the carrier of the allele had some selective advantage (Hayakawa et al. 2017). However, again, definitive proof to support this hypothesis is still lacking.

9.2.4 Primate Siglec-13 and Siglec-17

The gene encoding Siglec-13 (*SIGLEC13*) was eliminated from human genome, while it is present in the genomes of other primates (Angata et al. 2004). The deletion of this gene is uniform in all humans examined (Wang et al. 2012b). The same study demonstrated that chimpanzee Siglec-13 is expressed in myeloid cells, recognizes sialylated glycans, interacts with DAP12, and enhances inflammatory cytokine responses upon lipopolysaccharide (LPS; a ligand for Toll-like receptor (TLR) 4) stimulation of the myeloid cells that express it (Wang et al. 2012b). Paradoxically, stimulation of the same myeloid cells (RAW264.7 mouse macrophage cell line) that express chimpanzee Siglec-13 with sialylated bacterial pathogen resulted in the reduced production of pro-inflammatory cytokine (tumor necrosis factor α; TNFα), which is opposite to what was observed by the stimulation with LPS.

The same study revealed that the gene for primate Siglec-17 (gene symbol *SIGLEC17P*, originally designated *SIGLECP3*, in humans) is a pseudogene in human (due to 1 bp deletion in coding sequence) and many other Old World monkeys (due to independent events that rendered it non-functional or non-existent), but it is a functional gene in marmoset, a New World monkey (Wang et al. 2012b). The *SIGLEC17P* transcript is selectively expressed in natural killer cells in humans. The authors "resurrected" human Siglec-17 (i.e., re-created functional human Siglec-17 cDNA), which

recognizes sialylated glycans, interacts with DAP12, and enhances inflammatory cytokine responses upon LPS stimulation of the myeloid cells that express it (Wang et al. 2012b).

Given that the glycan binding patterns of these Siglecs appear to be rather promiscuous (Wang et al. 2012b), the mechanisms that prevented these activating-type Siglecs from causing inflammatory responses against own cells is not clear. As mentioned above, chimpanzee Siglec-13 expression on myeloid cells enhanced TNFα production in response to LPS stimulation but suppressed the TNFα response to sialylated bacteria. A similar suppressive signal via DAP12-associated Siglec was reported for Siglec-H (as described below), implying that Siglec-13 may have acquired a new signaling modality to transduce suppressive signaling in a sialylated ligand-dependent manner. In either case, the pseudogenization of *SIGLEC13* and *SIGLEC17* appears to be selected for, possibly suggesting that the loss of these genes were favorable at some stage of primate evolution (Wang et al. 2012b).

9.2.5 Rodent Siglec-H

Mouse Siglec-H was the first Siglec to be reported as a DAP12-associated Siglec (Blasius et al. 2006). Siglec-H is a rodent-specific Siglec (Zhang et al. 2006) and shows unique expression on plasmacytoid dendritic cells (pDCs) (Blasius et al. 2004, 2006; Zhang et al. 2006), a subset of macrophages in spleen and in lymph nodes (Zhang et al. 2006), and microglia (Konishi et al. 2017). DAP12 is required for the cell-surface expression of Siglec-H (Blasius et al. 2006), which is similar to many other DAP12-associated proteins (Turnbull and Colonna 2007).

Functional studies using antibody have shown that Siglec-H is involved in the regulation of type I interferon (IFN) production in response to TLR9 and TLR7 engagement. However, unexpectedly, Siglec-H does not enhance the production of type I IFN but rather suppresses it (Blasius et al. 2004, 2006). This antibody-mediated suppression of type I IFN response appears selective, in that an independent study revealed that the pDC treatment with TLR9 ligand in the presence of anti-Siglec-H antibody did not alter the production of several other cytokines (TNF-α, interleukin (IL)-10, and IL-6) (Zhang et al. 2006).

Type I IFN is a key coordinator of the host defense against viral infection, and pDC is the primary source of type I IFN. Therefore, some studies have addressed whether Siglec-H plays any significant role in the immune reaction against viral infection. A study, with intention to investigate the role of pDCs in immunity, developed a mouse line that turned out to be deficient in Siglec-H (Takagi et al. 2011). This study revealed that Siglec-H deficiency leads to exaggerated activation of NF-κB pathway in pDCs, enhanced production of type I IFN and IL-12p40 in response to TLR9 stimulation in vivo, and stronger antigen-specific $CD4^+$ T cell responses and weaker antigen-specific $CD8^+$ T cell responses in vivo, as compared with control mice. The Siglec-H-deficient mice were less efficient in clearing herpes simplex virus (Takagi et al. 2011). Another study, using an independent line of Siglec-H deficient

mice, revealed that the type I IFN production in response to mouse cytomegalovirus (mCMV) infection was indeed enhanced, but it did not result in enhanced clearance of the virus (Puttur et al. 2013). Interestingly, the Siglec-H-deficient mice develop autoimmune disease similar to lupus several weeks after the mCMV infection, in a type I IFN-dependent manner (Schmitt et al. 2016). Aging Siglec-H-deficient mice are also reported to develop mild autoimmunity (Schmitt et al. 2016).

Siglec-H has endocytic function (Zhang et al. 2006) that is dependent on DAP12 (Kopatz et al. 2013). This property was exploited to efficiently deliver antigen to pDCs for cross-presentation, facilitating the development of antigen-specific $CD8^+$ (cytotoxic) T cells (Zhang et al. 2006). On the other hand, another study found that the delivery of antigen to pDC via Siglec-H inhibits the development of $CD4^+$ (helper) T cell-dependent immunity (Loschko et al. 2011). Thus, the delivery of antigen to pDCs via Siglec-H appears to have opposite effects on $CD4^+$ T versus $CD8^+$ T cell-mediated immunity. These results appear to be in line with the phenotype of Siglec-H-deficient mice, which showed stronger antigen-specific $CD4^+$ T cell responses and weaker antigen-specific $CD8^+$ T cell responses compared with control mice (Takagi et al. 2011). The molecular mechanism behind this biased support for T cell subsets by Siglec-H is not fully understood. It appears possible that the antibody-mediated internalization of Siglec-H (mimicking Siglec-H deficiency), rather than the pDC-targeted antigen delivery, induced the phenotype observed.

Although mouse Siglec-H has canonical sequence features of Siglecs, including conserved arginine residue on the β-strand F of the first Ig-like domain that makes contact with the carboxyl group of sialic acid, glycan ligand for Siglec-H has not been found so far (Zhang et al. 2006). Given that rat Siglec-H lacks the conserved arginine residue essential for sialic acid recognition, it appears possible that rodent Siglec-H does not interact with glycans that contain sialic acid, but rather with some other ligand (Zhang et al. 2006). Whereas a study found that Siglec-H binds to glioma cell lines but not to normal astrocytes or other normal cells, the molecular entity of the ligand was not identified (Kopatz et al. 2013). If Siglec-H does not recognize common sialylated ligands (i.e., SAMPs), it is not likely to trigger autoimmunity against own tissues. Siglec-H may be also sequestered in an intracellular compartment in certain cell types (Zhang et al. 2006), which may prevent the contact between Siglec-H and its ligand.

The signal transduction mechanism by which DAP12-mediated signal from Siglec-H leads to suppression of type I IFN production is not fully understood. Based on previous findings that DAP12-deficient myeloid cells show enhanced responses to TLR agonists (Hamerman and Lanier 2006; Hamerman et al. 2005), it was proposed that low-degree Siglec-H engagement by antibody or endogenous ligand leads to sequestration of Siglec-H or recruitment of inhibitory mediator, whereas high-degree engagement by pathogen may lead to cell activation and enhanced type I IFN production (Blasius and Colonna 2006). In an alternative model proposed by the same authors, co-engagement of TLR and Siglec-H by pathogen may be required for strong type I IFN response (Blasius and Colonna 2006). Definitive proof for either of these models is yet to be published.

9.2.6 Other Siglecs that Potentially Associate with DAP12

Some other primate and rodent Siglecs have been implied to interact with DAP12. For example, mouse CD33/Siglec-3 was predicted to interact with DAP12, based on the sequence similarity in the transmembrane domain with that of mouse Siglec-H (Blasius et al. 2006). Mouse CD33/Siglec-3 is a putative ortholog of primate CD33/Siglec-3, but unlike primate CD33/Siglec-3 that has canonical ITIM and associates with SHP-1 (Paul et al. 2000; Taylor et al. 1999; Ulyanova et al. 1999), it lacks canonical ITIM (although it retains a membrane-distal ITIM-like motif). Several other genes/pseudogenes for CD33-related Siglecs in mammals were also predicted to have a conserved lysine residue in the transmembrane domain, which may be engaged in the interaction with DAP12 (Cao et al. 2008). Experimental confirmation of these predictions is awaited.

Sialoadhesin/Siglec-1 was also reported to be associated with DAP12 (Wu et al. 2016; Zheng et al. 2015), although the transmembrane domain of sialoadhesin/Siglec-1 lacks positively charged amino acid residue, and thus it is unlikely to directly interact with DAP12. Further investigation would be required to find out how sialoadhesin/Siglec-1 interacts with DAP12 (e.g., by way of another adapter protein that bridges between sialoadhesin/Siglec-1 and DAP12).

9.3 Summary

Siglecs that associate with DAP12 have diverse functions, ranging from bone homeostasis, pathogen recognition to the regulation of adaptive immune responses, as described in the previous section. Evolutionary processes appear to have employed several mechanisms to prevent self-inflicted injury by the immune system (i.e., autoimmunity) triggered by the engagement of DAP12-associated Siglecs by sialylated glycans (SAMP), such as the sequestration of Siglec into intracellular compartment (Siglec-15 and Siglec-H), restricted distribution of sialylated ligands (Siglec-15), or loss of binding to common sialylated ligands (Siglec-H), coupling with inhibitory counterpart (Siglec-14 and Siglec-16), acquisition of alternative signaling modality (Siglec-13 and Siglec-H), and ultimately, pseudogenization or loss of the gene encoding DAP12-associated Siglec (Siglec-13, -14, -16, -17) (Fig. 9.2).

Despite the efforts by several groups in the past decade, many unanswered questions remain regarding the functions of DAP12-associated Siglecs and the molecular mechanisms they use for immune regulations. For example, biologically relevant ligands for these Siglecs are not clearly identified in many cases. Such information may shed new light on the function of these Siglecs. Further studies on the genetic association of polymorphisms of human Siglecs would be necessary to further reveal appropriate biological contexts in which these Siglecs play significant roles. Identification of intracellular molecular partner(s) in addition to DAP12 may also allow us to better understand how these Siglecs operate (e.g., the mechanism by which

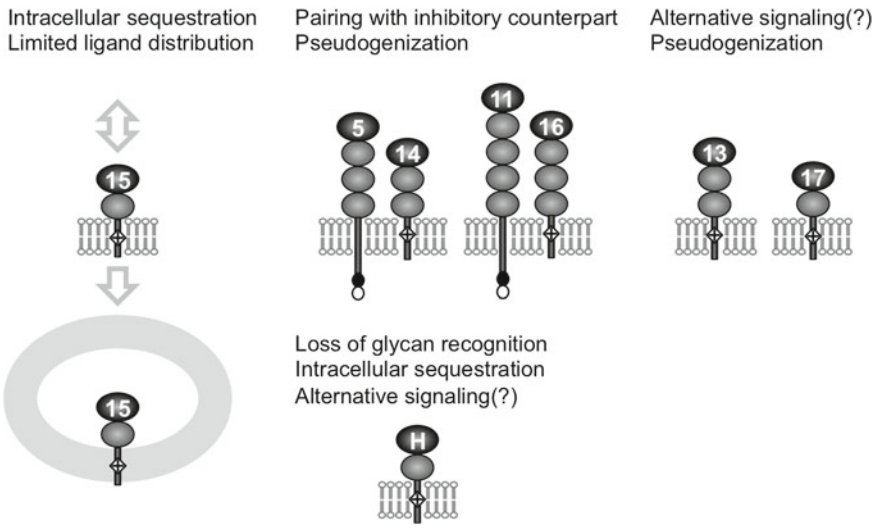

Fig. 9.2 Possible mechanisms that prevent the development of autoimmunity through the recognition of sialylated glycans by DAP12-associated Siglecs. Some potential mechanisms that may prevent self-oriented attacks (autoimmunity) by the immune cells triggered by the interaction between sialylated glycans (self-associated molecular patterns) and DAP12-associated Siglecs are illustrated. Such mechanisms include sequestration into intracellular compartments (Siglec-15 and Siglec-H), restricted distribution of sialylated ligands (Siglec-15) or loss of binding toward sialylated ligands (Siglec-H), coupling with inhibitory counterpart (Siglec-14 and Siglec-16), altered signaling modality (Siglec-13 and Siglec-H), and pseudogenization or gene deletion (Siglec-13, -14, -16, -17)

primate Siglec-13 and rodent Siglec-H transduce suppressive regulatory signaling). Further investigations of DAP12-associated Siglecs will lead to deeper understanding of Siglec family as a whole, and potentially lead to clinical translations.

Acknowledgements The work in the author's laboratory has been supported by intramural funding from Academia Sinica and extramural funding from the Ministry of Science and Technology, Taiwan [MOST 104-2311-B-001-017-MY3, 105-2627-M-007-001, and 106-2321-B-001-032].

References

Ali S, Fong J, Carlin A, Busch T, Linden R, Angata T, Areschoug T, Parast M, Varki N, Murray J, Nizet V, Varki A (2014) Siglec-5 and Siglec-14 are polymorphic paired receptors that modulate neutrophil and amnion signaling responses to group B Streptococcus. J Exp Med 211:1231–1242

Angata T (2006) Molecular diversity and evolution of the Siglec family of cell-surface lectins. Mol Divers 10:555–566

Angata T (2017) Polymorphisms and mutations in SIGLEC genes and their associations with diseases. J Jpn Biochem Soc 89:652–659

Angata T, Varki A (2002) Chemical diversity in the sialic acids and related alpha-keto acids: an evolutionary perspective. Chem Rev 102:439–470

Angata T, Kerr S, Greaves D, Varki N, Crocker P, Varki A (2002) Cloning and characterization of human Siglec-11. A recently evolved signaling molecule that can interact with SHP-1 and SHP-2 and is expressed by tissue macrophages, including brain microglia. J Biol Chem 277:24466–24474

Angata T, Margulies E, Green E, Varki A (2004) Large-scale sequencing of the CD33-related Siglec gene cluster in five mammalian species reveals rapid evolution by multiple mechanisms. Proc Natl Acad Sci U S A 101:13251–13256

Angata T, Hayakawa T, Yamanaka M, Varki A, Nakamura M (2006) Discovery of Siglec-14, a novel sialic acid receptor undergoing concerted evolution with Siglec-5 in primates. FASEB J 20:1964–1973

Angata T, Tabuchi Y, Nakamura K, Nakamura M (2007) Siglec-15: an immune system Siglec conserved throughout vertebrate evolution. Glycobiology 17:838–846

Angata T, Ishii T, Motegi T, Oka R, Taylor R, Soto P, Chang Y, Secundino I, Gao C, Ohtsubo K, Kitazume S, Nizet V, Varki A, Gemma A, Kida K, Taniguchi N (2013) Loss of Siglec-14 reduces the risk of chronic obstructive pulmonary disease exacerbation. Cell Mol Life Sci 70:3199–3210

Blasius A, Colonna M (2006) Sampling and signaling in plasmacytoid dendritic cells: the potential roles of Siglec-H. Trends Immunol 27:255–260

Blasius A, Vermi W, Krug A, Facchetti F, Cella M, Colonna M (2004) A cell-surface molecule selectively expressed on murine natural interferon-producing cells that blocks secretion of interferon-alpha. Blood 103:4201–4206

Blasius A, Cella M, Maldonado J, Takai T, Colonna M (2006) Siglec-H is an IPC-specific receptor that modulates type I IFN secretion through DAP12. Blood 107:2474–2476

Blaum BS (2017) The lectin self of complement factor H. Curr Opin Struct Biol 44:111–118

Blaum BS, Hannan JP, Herbert AP, Kavanagh D, Uhrin D, Stehle T (2015) Structural basis for sialic acid-mediated self-recognition by complement factor H. Nat Chem Biol 11:77–82

Cao H, Lakner U, de Bono B, Traherne J, Trowsdale J, Barrow A (2008) SIGLEC16 encodes a DAP12-associated receptor expressed in macrophages that evolved from its inhibitory counterpart SIGLEC11 and has functional and non-functional alleles in humans. Eur J Immunol 38:2303–2315

Carlin A, Chang Y, Areschoug T, Lindahl G, Hurtado-Ziola N, King C, Varki A, Nizet V (2009) Group B Streptococcus suppression of phagocyte functions by protein-mediated engagement of human Siglec-5. J Exp Med 206:1691–1699

Chang L, Chen YJ, Fan CY, Tang CJ, Chen YH, Low PY, Ventura A, Lin CC, Chen YJ, Angata T (2017) Identification of Siglec ligands using a proximity labeling method. J Proteome Res 16:3929–3941

Colley KJ, Kitajima K, Sato C (2014) Polysialic acid: biosynthesis, novel functions and applications. Crit Rev Biochem Mol Biol 49:498–532

Cornish A, Freeman S, Forbes G, Ni J, Zhang M, Cepeda M, Gentz R, Augustus M, Carter K, Crocker P (1998) Characterization of siglec-5, a novel glycoprotein expressed on myeloid cells related to CD33. Blood 92:2123–2132

Crocker P, Clark E, Filbin M, Gordon S, Jones Y, Kehrl J, Kelm S, le Douarin N, Powell L, Roder J, Schnaar R, Sgroi D, Stamenkovic K, Schauer R, Schachner M, van den Berg T, van der Merwe P, Watt S, Varki A (1998) Siglecs: a family of sialic-acid binding lectins [letter]. Glycobiology 8:v

Duong BH, Tian H, Ota T, Completo G, Han S, Vela JL, Ota M, Kubitz M, Bovin N, Paulson JC, Nemazee D (2010) Decoration of T-independent antigen with ligands for CD22 and Siglec-G can suppress immunity and induce B cell tolerance in vivo. J Exp Med 207:173–187

Graustein AD, Horne DJ, Fong JJ, Schwarz F, Mefford HC, Peterson GJ, Wells RD, Musvosvi M, Shey M, Hanekom WA, Hatherill M, Scriba TJ, Thuong NTT, Mai NTH, Caws M, Bang ND, Dunstan SJ, Thwaites GE, Varki A, Angata T, Hawn TR (2017) The SIGLEC14 null allele is associated with *Mycobacterium tuberculosis*- and BCG-induced clinical and immunologic outcomes. Tuberculosis (Edinb) 104:38–45

Hamerman JA, Lanier LL (2006) Inhibition of immune responses by ITAM-bearing receptors. Sci STKE re1

Hamerman JA, Tchao NK, Lowell CA, Lanier LL (2005) Enhanced Toll-like receptor responses in the absence of signaling adaptor DAP12. Nat Immunol 6:579–586

Hayakawa T, Angata T, Lewis A, Mikkelsen T, Varki N, Varki A (2005) A human-specific gene in microglia. Science 309:1693

Hayakawa T, Khedri Z, Schwarz F, Landig C, Liang SY, Yu H, Chen X, Fujito NT, Satta Y, Varki A, Angata T (2017) Coevolution of Siglec-11 and Siglec-16 via gene conversion in primates. BMC Evol Biol 17:228

Hiruma Y, Hirai T, Tsuda E (2011) Siglec-15, a member of the sialic acid-binding lectin, is a novel regulator for osteoclast differentiation. Biochem Biophys Res Commun 409:424–429

Hiruma Y, Tsuda E, Maeda N, Okada A, Kabasawa N, Miyamoto M, Hattori H, Fukuda C (2013) Impaired osteoclast differentiation and function and mild osteopetrosis development in Siglec-15-deficient mice. Bone 53:87–93

Ishida-Kitagawa N, Tanaka K, Bao X, Kimura T, Miura T, Kitaoka Y, Hayashi K, Sato M, Maruoka M, Ogawa T, Miyoshi J, Takeya T (2012) Siglec-15 protein regulates formation of functional osteoclasts in concert with DNAX-activating protein of 12 kDa (DAP12). J Biol Chem 287:17493–17502

Julien S, Videira PA, Delannoy P (2012) Sialyl-tn in cancer: (how) did we miss the target? Biomolecules 2:435–466

Kameda Y, Takahata M, Komatsu M, Mikuni S, Hatakeyama S, Shimizu T, Angata T, Kinjo M, Minami A, Iwasaki N (2013) Siglec-15 regulates osteoclast differentiation by modulating RANKL-induced phosphatidylinositol 3-kinase/Akt and Erk pathways in association with signaling Adaptor DAP12. J Bone Miner Res 28:2463–2475

Kameda Y, Takahata M, Mikuni S, Shimizu T, Hamano H, Angata T, Hatakeyama S, Kinjo M, Iwasaki N (2015) Siglec-15 is a potential therapeutic target for postmenopausal osteoporosis. Bone 71:217–226

Karlstetter M, Kopatz J, Aslanidis A, Shahraz A, Caramoy A, Linnartz-Gerlach B, Lin Y, Luckoff A, Fauser S, Duker K, Claude J, Wang Y, Ackermann J, Schmidt T, Hornung V, Skerka C, Langmann T, Neumann H (2017) Polysialic acid blocks mononuclear phagocyte reactivity, inhibits complement activation, and protects from vascular damage in the retina. EMBO Mol Med 9:154–166

Kerrigan AM, Brown GD (2011) Syk-coupled C-type lectins in immunity. Trends Immunol 32:151–156

Konishi H, Kobayashi M, Kunisawa T, Imai K, Sayo A, Malissen B, Crocker PR, Sato K, Kiyama H (2017) Siglec-H is a microglia-specific marker that discriminates microglia from CNS-associated macrophages and CNS-infiltrating monocytes. Glia 65:1927–1943

Kopatz J, Beutner C, Welle K, Bodea LG, Reinhardt J, Claude J, Linnartz-Gerlach B, Neumann H (2013) Siglec-h on activated microglia for recognition and engulfment of glioma cells. Glia 61:1122–1133

Lajaunias F, Dayer J, Chizzolini C (2005) Constitutive repressor activity of CD33 on human monocytes requires sialic acid recognition and phosphoinositide 3-kinase-mediated intracellular signaling. Eur J Immunol 35:243–251

Loschko J, Heink S, Hackl D, Dudziak D, Reindl W, Korn T, Krug AB (2011) Antigen targeting to plasmacytoid dendritic cells via Siglec-H inhibits Th cell-dependent autoimmunity. J Immunol 187:6346–6356

Lowell CA (2011) Src-family and Syk kinases in activating and inhibitory pathways in innate immune cells: signaling cross talk. Cold Spring Harb Perspect Biol 3

Macauley M, Crocker P, Paulson J (2014) Siglec-mediated regulation of immune cell function in disease. Nat Rev Immunol 14:653–666

Meri S, Pangburn MK (1990) Discrimination between activators and nonactivators of the alternative pathway of complement: regulation via a sialic acid/polyanion binding site on factor H. Proc Natl Acad Sci U S A 87:3982–3986

Ogata S, Ho I, Chen A, Dubois D, Maklansky J, Singhal A, Hakomori S, Itzkowitz SH (1995) Tumor-associated sialylated antigens are constitutively expressed in normal human colonic mucosa. Cancer Res 55:1869–1874

Paloneva J, Kestila M, Wu J, Salminen A, Bohling T, Ruotsalainen V, Hakola P, Bakker AB, Phillips JH, Pekkarinen P, Lanier LL, Timonen T, Peltonen L (2000) Loss-of-function mutations in TYROBP (DAP12) result in a presenile dementia with bone cysts. Nat Genet 25:357–361

Paloneva J, Manninen T, Christman G, Hovanes K, Mandelin J, Adolfsson R, Bianchin M, Bird T, Miranda R, Salmaggi A, Tranebjaerg L, Konttinen Y, Peltonen L (2002) Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. Am J Hum Genet 71:656–662

Paloneva J, Mandelin J, Kiialainen A, Bohling T, Prudlo J, Hakola P, Haltia M, Konttinen YT, Peltonen L (2003) DAP12/TREM2 deficiency results in impaired osteoclast differentiation and osteoporotic features. J Exp Med 198:669–675

Paul S, Taylor L, Stansbury E, McVicar D (2000) Myeloid specific human CD33 is an inhibitory receptor with differential ITIM function in recruiting the phosphatases SHP-1 and SHP-2. Blood 96:483–490

Pillai S, Netravali I, Cariappa A, Mattoo H (2012) Siglecs and immune regulation. Annu Rev Immunol 30:357–392

Ponta H, Sherman L, Herrlich PA (2003) CD44: from adhesion molecules to signalling regulators. Nat Rev Mol Cell Biol 4:33–45

Puttur F, Arnold-Schrauf C, Lahl K, Solmaz G, Lindenberg M, Mayer CT, Gohmert M, Swallow M, van Helt C, Schmitt H, Nitschke L, Lambrecht BN, Lang R, Messerle M, Sparwasser T (2013) Absence of Siglec-H in MCMV infection elevates interferon alpha production but does not enhance viral clearance. PLoS Pathog 9:e1003648

Sato C, Kitajima K (2013) Disialic, oligosialic and polysialic acids: distribution, functions and related disease. J Biochem 154:115–136

Schmitt H, Sell S, Koch J, Seefried M, Sonnewald S, Daniel C, Winkler TH, Nitschke L (2016) Siglec-H protects from virus-triggered severe systemic autoimmunity. J Exp Med 213:1627–1644

Schwarz F, Landig CS, Siddiqui S, Secundino I, Olson J, Varki N, Nizet V, Varki A (2017) Paired Siglec receptors generate opposite inflammatory responses to a human-specific pathogen. EMBO J 36:751–760

Shimizu T, Takahata M, Kameda Y, Endo T, Hamano H, Hiratsuka S, Ota M, Iwasaki N (2015) Sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) mediates periarticular bone loss, but not joint destruction, in murine antigen-induced arthritis. Bone 79:65–70

Shultz LD, Rajan TV, Greiner DL (1997) Severe defects in immunity and hematopoiesis caused by SHP-1 protein-tyrosine-phosphatase deficiency. Trends Biotechnol 15:302–307

Stuible M, Moraitis A, Fortin A, Saragosa S, Kalbakji A, Filion M, Tremblay G (2014) Mechanism and function of monoclonal antibodies targeting siglec-15 for therapeutic inhibition of osteoclastic bone resorption. J Biol Chem 289:6498–6512

Takagi H, Fukaya T, Eizumi K, Sato Y, Sato K, Shibazaki A, Otsuka H, Hijikata A, Watanabe T, Ohara O, Kaisho T, Malissen B, Sato K (2011) Plasmacytoid dendritic cells are crucial for the initiation of inflammation and T cell immunity in vivo. Immunity 35:958–971

Takamiya R, Ohtsubo K, Takamatsu S, Taniguchi N, Angata T (2013) The interaction between Siglec-15 and tumor-associated sialyl-Tn antigen enhances TGF-beta secretion from monocytes/macrophages through the DAP12-Syk pathway. Glycobiology 23:178–187

Taylor V, Buckley C, Douglas M, Cody A, Simmons D, Freeman S (1999) The myeloid-specific sialic acid-binding receptor, CD33, associates with the protein-tyrosine phosphatases, SHP-1 and SHP-2. J Biol Chem 274:11505–11512

Turnbull I, Colonna M (2007) Activating and inhibitory functions of DAP12. Nat Rev Immunol 7:155–161

Ulyanova T, Blasioli J, Woodford-Thomas T, Thomas M (1999) The sialoadhesin CD33 is a myeloid-specific inhibitory receptor. Eur J Immunol 29:3440–3449

Varki A (2011) Since there are PAMPs and DAMPs, there must be SAMPs? Glycan "self-associated molecular patterns" dampen innate immunity, but pathogens can mimic them. Glycobiology 21:1121–1124

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

Wang X, Chow R, Deng L, Anderson D, Weidner N, Godwin A, Bewtra C, Zlotnik A, Bui J, Varki A, Varki N (2011) Expression of Siglec-11 by human and chimpanzee ovarian stromal cells, with uniquely human ligands: implications for human ovarian physiology and pathology. Glycobiology 21:1038–1048

Wang X, Mitra N, Cruz P, Deng L, Varki N, Angata T, Green E, Mullikin J, Hayakawa T, Varki A (2012a) Evolution of siglec-11 and siglec-16 genes in hominins. Mol Biol Evol 29:2073–2086

Wang X, Mitra N, Secundino I, Banda K, Cruz P, Padler-Karavani V, Verhagen A, Reid C, Lari M, Rizzi E, Balsamo C, Corti G, de Bellis G, Longo L, Beggs W, Caramelli D, Tishkoff S, Hayakawa T, Green E, Mullikin J, Nizet V, Bui J, Varki A (2012b) Specific inactivation of two immunomodulatory SIGLEC genes during human evolution. Proc Natl Acad Sci U S A 109:9935–9940

Wu Y, Lan C, Ren D, Chen GY (2016) Induction of Siglec-1 by endotoxin tolerance suppresses the innate immune response by promoting TGF-beta1 production. J Biol Chem 291:12370–12382

Yamanaka M, Kato Y, Angata T, Narimatsu H (2009) Deletion polymorphism of SIGLEC14 and its functional implications. Glycobiology 19:841–846

Zhang J, Raper A, Sugita N, Hingorani R, Salio M, Palmowski M, Cerundolo V, Crocker P (2006) Characterization of Siglec-H as a novel endocytic receptor expressed on murine plasmacytoid dendritic cell precursors. Blood 107:3600–3608

Zheng Q, Hou J, Zhou Y, Yang Y, Xie B, Cao X (2015) Siglec1 suppresses antiviral innate immune response by inducing TBK1 degradation via the ubiquitin ligase TRIM27. Cell Res 25:1121–1136

Zoller M (2011) CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? Nat Rev Cancer 11:254–267