

# Specific inactivation of two immunomodulatory *SIGLEC* genes during human evolution

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Sialic acid-recognizing Ig-like lectins (Siglecs) are signaling receptors that modulate immune responses, and are targeted for interactions by certain pathogens. We describe two primate Siglecs that were rendered nonfunctional by single genetic events during hominin evolution after our common ancestor with the chimpanzee. *SIGLEC13* was deleted by an *Alu*-mediated recombination event, and a single base pair deletion disrupted the ORF of *SIGLEC17*. Siglec-13 is expressed on chimpanzee monocytes, innate immune cells that react to bacteria. The human *SIGLEC17P* pseudogene mRNA is still expressed at high levels in human natural killer cells, which bridge innate and adaptive immune responses. As both resulting pseudogenes are homozygous in all human populations, we resurrected the originally encoded proteins and examined their functions. Chimpanzee Siglec-13 and the resurrected human Siglec-17 recruit a signaling adapter and bind sialic acids. Expression of either Siglec in innate immune cells alters inflammatory cytokine secretion in response to Toll-like receptor-4 stimulation. Both Siglecs can also be engaged by two potentially lethal sialylated bacterial pathogens of newborns and infants, agents with a potential impact on reproductive fitness. Neanderthal and Denisovan genomes show human-like sequences at both loci, corroborating estimates that the initial pseudogenization events occurred in the common ancestral population of these hominins. Both loci also show limited polymorphic diversity, suggesting selection forces predating the origin of modern humans. Taken together, these data suggest that genetic elimination of Siglec-13 and/or Siglec-17 represents signatures of infectious and/or other inflammatory selective processes contributing to population restrictions during hominin origins.

Sialic acids (Sias) are monosaccharides typically found at the outermost ends of complex glycan chains that decorate all vertebrate cell surfaces (1, 2). Sias are essential for embryonic development (3) and mediate important intrinsic organismal functions (1, 2). However, given their location and density, Sia-bearing glycans are also targets for recognition by many pathogen-binding proteins and toxins (1, 4, 5). Adding complexity to these opposing evolutionary selection forces, many important bacterial pathogens have evolved convergent mechanisms for molecular mimicry of host Sias (4, 6, 7). For all these reasons, both Sias and Sia-recognizing proteins are rapidly evolving in some taxa. Current data suggest that humans are an extreme example, with Sia-related genes representing a “hotspot” in human evolution (5). Of less than 70 human genes known to be involved in Sia biology, more than 10 have been documented to exhibit human-specific changes relative to the chimpanzee, our closest evolutionary cousins (5). Most of these human-specific genetic changes are in *Sia-recognizing Ig-like lectin (SIGLEC)* genes (5).

*SIGLEC* genes encode a family of transmembrane receptors that bind Sia-containing ligands via their amino-terminal extracellular Ig-like domains and modulate cellular responses via

cytosolic signaling motifs (7–10). The CD33-related subset of Siglecs (CD33rSiglecs) is rapidly evolving within vertebrates (7–10). The most likely reason is that CD33rSiglecs are prominently expressed on innate immune cells, and modulate responses to pathogens. In this regard, Siglec-3 and Siglecs-5 to -11 in humans seem to recognize sialylated ligands as “self-associated molecular patterns” (11), limiting unwanted reactivity against other cells in the same organism (7–10). However, certain immune-modulating bacterial pathogens carry out molecular mimicry of sialylated CD33rSiglec ligands (12), dampening host innate immune cell responses and facilitating infection (13). In one instance, a human bacterial pathogen evolved a more stable protein–protein interaction with an inhibitory CD33rSiglec to suppress innate immunity (14).

Evolution has also generated CD33rSiglecs with opposing activatory potential (7, 15, 16), transmitting positive signals to immune cells via recruitment of the immunoreceptor tyrosine-based activation motif (ITAM)-containing DAP12 adaptor protein (17). Some activatory Siglecs pair with inhibitory ones (15, 16), supporting the notion that they represent a host evolutionary response to pathogen mimicry and engagement of inhibitory CD33rSiglecs (7). However, low avidity engagement of activatory Siglecs can mediate paradoxical inhibitory responses (ITAMi) (18, 19).

Two genomic loci encoding ITAM-containing primate Siglecs (*SIGLEC14* and *SIGLEC16*) are polymorphic, with their common alleles being nonfunctional (15, 16, 20). These polymorphisms exist in African populations, indicating that they likely originated before the migration of modern humans out of Africa about 60,000–70,000 y ago. Thus, functional and pseudogene alleles of *SIGLEC* genes can be maintained in populations over long periods. This finding likely reflects ongoing selection forces involving the need to maintain innate immune self-recognition and control damaging inflammatory responses, all against a backdrop of potential pathogen subversion of these mechanisms (7, 8, 20). An evolutionary balancing act is also supported by the high frequency of human-specific pathogens that carry out molecular mimicry of Sias through convergent evolutionary mechanisms (4, 6).

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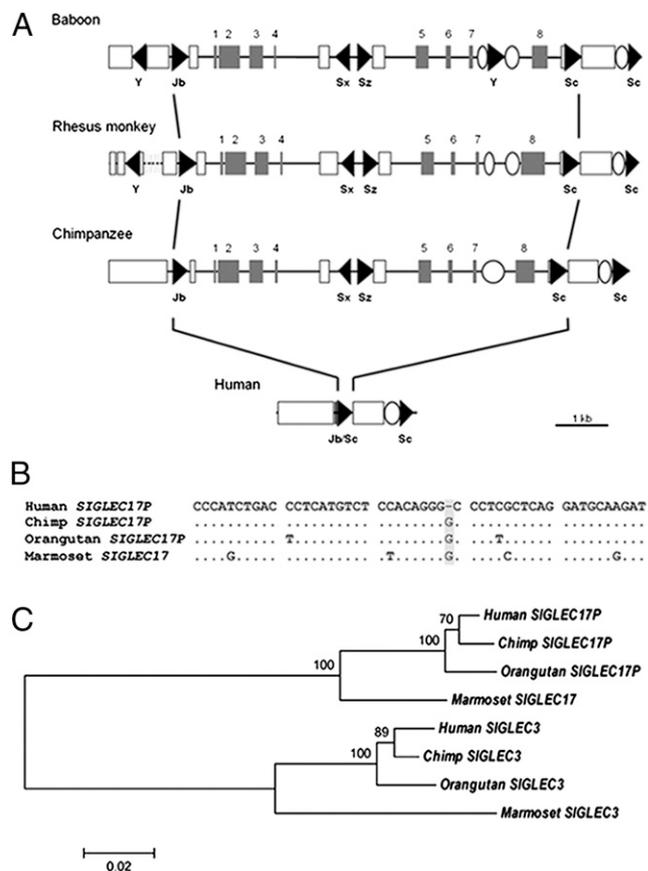
Current genetic, archeological, skeletal, and radioactive dating evidence indicates that all modern humans are derived from a population with an effective size of 10,000 or fewer that originated in Africa ~100,000–200,000 y ago (21–25) and later spread across the planet, replacing other hominin species and having limited interbreeding with our closest extinct cousins, the Neanderthals (26) and Denisovans (27). Even smaller estimates have been made of the effective population size of Neanderthals (28). The reasons for the small effective population sizes are unknown, and host–pathogen interactions have not been previously considered as contributors. Here, we report two genomic inactivation events in *SIGLEC* genes that became fixed before the emergence of modern humans in Africa. Our evidence indicates that these genes may have been inactivated in the hominin lineage, because they can be engaged by pathogens associated with life-threatening invasive infections in newborns and infants. It is also possible that the immunomodulatory capacity of these genes was costly in contexts beyond bacterial engagement of Siglecs, such as toxic inflammatory effects on ancestral hominin immune cells.

## Results and Discussion

**Single *Alu*-Mediated Deletion Event Inactivated *SIGLEC13* in the Hominin Lineage.** Analysis of genomic BAC clones indicated that the primate *SIGLEC13* gene was missing from the human genome but present in chimpanzees and baboons (29). To understand events accounting for this apparently human-specific deletion, we analyzed the genomic region encompassing the *SIGLEC13* locus in the currently available sequence builds of the human, chimpanzee, baboon, and rhesus genomes. Repeat-Masker software identified several repetitive elements in this region in all of these species. These elements include *Alu* elements, which are primate-specific short interspersed elements (30). In the chimpanzee genome, five *Alu* elements are located in an ~10-kb genomic region containing the *SIGLEC13* locus (Fig. 1A). One *Alu* element belonging to the *AluJb* family is located upstream of the *SIGLEC13* locus, one *AluSx* and one *AluSz* elements are within the locus, and two *AluSc* elements are found downstream. These elements are also found in orthologous regions in the baboon and rhesus monkey genomes (Fig. 1A). In the human genome, we found just one composite *Alu* element (*AluJb/Sc*) occupying the region of ~7 kb that contains the *SIGLEC13* locus in chimpanzee, rhesus monkey, and baboon (Fig. 1A). The ~7-kb region deleted in the human genome is sandwiched between two ancestral *AluJb* and *AluSc* elements (Fig. 1A). Thus, a single *Alu*-mediated recombination event was the likely mechanism for human-specific deletion of *SIGLEC13*, leaving a single fused *Alu* element in the human genome.

**Human-Specific Mutational Events Functionally Altered and then, Pseudogenized Primate *SIGLEC17*.** We previously described the human *SIGLECP3* locus with an inactivating deletion in the predicted ORF (29). We now note that the rest of the predicted ORF remains intact. We found more than one cDNA clone predicting a full-length transcript derived from *SIGLECP3* (for example, BC041072 in *SI Appendix*, Fig. S1). In addition to the single nucleotide deletion, the remnant human *SIGLECP3* coding region harbors a human-unique missense mutation of the codon encoding an Arg residue that would have been involved in Sia recognition when the ORF was intact (*SI Appendix*, Fig. S24). Taken together with evidence for Sia-binding properties of the resurrected protein when the Arg codon is restored (see below) and evidence for an intact marmoset ortholog (*SI Appendix*, Fig. S24), we redesignate the original *SIGLECP3* locus as primate *SIGLEC17* and the corresponding human pseudogene as *SIGLEC17P*.

Phylogenetic sequence comparisons showed that the 1-bp deletion in *SIGLEC17P* is human-specific (Fig. 1B). A BLAST query using the human sequence identified an orthologous gene with a predicted intact ORF encoding Siglec-17 in the marmoset genome, with 93% DNA sequence similarity and 89% predicted protein



**Fig. 1.** Inactivation of two *SIGLEC* genes during hominin evolution. (A) Comparison of genomic structure surrounding the *SIGLEC13* locus among humans and other primates. Coding regions are represented by shaded boxes, and *Alu* elements are represented by triangles. Names of *Alu* subfamilies are shown. Open boxes and ellipses indicate LINE (long interspersed element) and LTR (long terminal repeat) elements, respectively. The dotted line indicates a sequence gap. (B) DNA sequence alignment using Clustal W in MEGA4 shows the human-specific loss of G in *SIGLEC17P* (highlighted in gray). (C) Reconstructed neighbor-joining (NJ) tree of *SIGLEC17P* and *SIGLEC3* among primates. Bootstrap values of 1,000 replicates are shown on internal branches. MEGA4 was used for NJ tree reconstruction and bootstrap analysis.

sequence similarity (*SI Appendix*, Fig. S24). Analysis of the RT-PCR–derived orthologous segment from chimpanzee peripheral blood mononuclear cell mRNA confirmed that, although the 1-bp deletion is human-specific, independent pseudogenization events have occurred in the chimpanzee as well as orangutan genomes. The homologous region containing the *SIGLEC17P* locus seems to be completely deleted in the rhesus and baboon genomes (29). Thus, although well-conserved in primate evolution from New World monkeys to ancestral hominins, the *SIGLEC17* gene has also undergone independent deletion or pseudogenization events in multiple primate taxa, with a distinct event in the hominin lineage.

Additional BLAST analyses showed that *SIGLEC17* is most closely related to *SIGLEC3* (encoding Siglec-3/CD33), with the two loci evolving as paralogs in primates (Fig. 1C). The predicted V-set and C2-set domains of the resurrected human *SIGLEC17* and human *SIGLEC3* genes share 66% DNA sequence similarity (*SI Appendix*, Fig. S2B). The human pseudogene *SIGLECP6* also has homology to human *SIGLEC17P*. A phylogenetic tree of primate *SIGLEC3*, *SIGLECP6*, and *SIGLEC17P* gave no evidence for a gene conversion during primate evolution among these loci.

**Inactivation Events of *SIGLEC13* and *SIGLEC17* Are Human-Universal.** The *Alu*-mediated *SIGLEC13* deletion and the *SIGLEC17* frame-



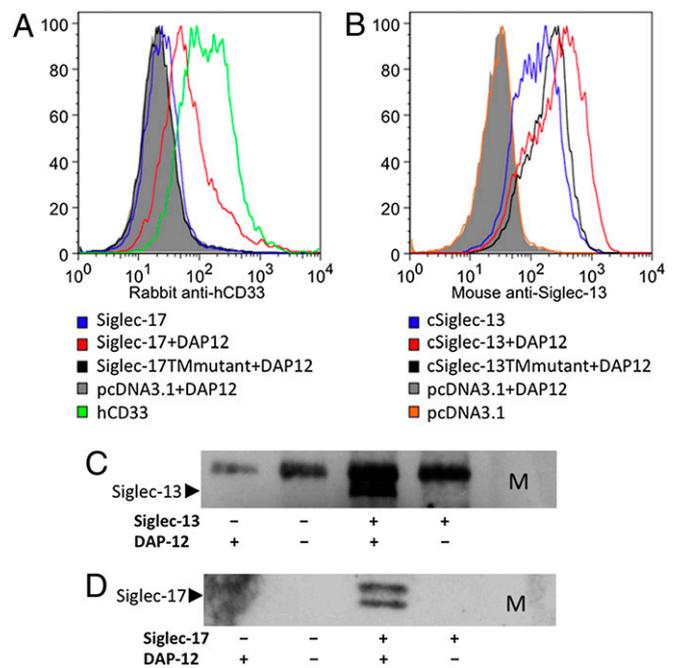
cells sometimes displayed constitutive TNF production, which was further boosted by LPS (*SI Appendix, Fig. S5A*). Thus, Siglec-13 and -17 likely mediated signaling through DAP12 in ancestral monocytes and NK cells, modulating cytokine secretion.

**Specific Interactions of Siglec-13 and -17 with Sialylated Bacterial Pathogens.** Some other CD33-related Siglecs are known to recognize certain pathogenic bacteria (13, 14) [e.g., Group B *Streptococcus* (GBS), a Gram-positive bacterial pathogen that expresses Sias and causes invasive infections in human newborn infants (33)]. GBS engagement of Siglecs can involve the Sias (13) and/or a specific cell surface-anchored protein, the  $\beta$ -protein (14). Because Siglec-13 and -17 could respond to a bacterial product (LPS) (*SI Appendix, Fig. S5*), we hypothesized that they would also interact with certain important sialylated human pathogens. Indeed, the extracellular domain of Siglec-13 bound to sialylated GBS A909 but not the nonpathogenic Gram-positive bacteria *Lactococcus lactis* (Fig. 4A). Extracellular domains of both Siglec-13 and -17 also bound to the sialylated Gram-negative pathogen *Escherichia coli* K1 (another leading cause of sepsis and meningitis in human newborns) but not nonpathogenic *E. coli* K-12 (Fig. 4A). Interestingly, although some Sia-dependent binding is observed, these interactions prominently involved trypsin-sensitive protein-protein interactions (Fig. 4 and *SI Appendix, Fig. S6*). With GBS, analysis of an isogenic bacterial mutant identified the likely binding partner for Siglec-13 as the GBS  $\beta$ -protein (Fig. 4C), an interaction previously shown to suppress human leukocyte responses through Siglec-5 (14).

**Reduced Intracellular TNF in Siglec-13–Transfected RAW 264.7 Cells in Response to the Bacterial Infection.** Semistably transfected RAW264.7 cells with Siglec-13 cDNA in pcDNA3.1 were acquired as mentioned above. Cells were infected with *E. coli* K1 or GBS A909 for 1 h at low multiplicity of infection (MOI; 0.6 and 0.1, respectively), and the level of intracellular TNF was then measured by an APC (Allophycocyanin) rat anti-mouse TNF antibody for all of the infected cells. Interestingly, compared with the mock control, the Siglec-13–transfected cells showed reduced intracellular TNF (Fig. 5A and B). In this regard, it is known from work on other DAP12-interacting proteins that engagement of corresponding receptors can mediate either activating responses (through classical ITAM-Syk kinase signaling) or paradoxical inhibitory responses [ITAMi through Src kinase and Src homology phosphatase (SHP-1)] (18, 19). Thus, pathogenic bacteria might have been taking advantage of the cell surface-expressed Siglec-13 in ancestral hominins to dampen host innate immune cell responses and facilitate infection. Similar studies with Siglec-17 were not possible, because semistable expression in these cells resulted in markedly retarded growth.

Attempting to further recreate ancestral interactions of cells expressing Siglec-13 and -17 with human pathogenic bacteria, we tried to generate stable macrophage cell lines expressing them. However, compared with control-transfected cells, those expressing Siglec-13 or -17 grew very slowly in culture, and we could not generate long-term stable lines in either mouse RAW264.7 or human THP-1 macrophage cell lines. Similar difficulties were encountered with making stable transfectants of Siglec-17 in the human NK cell line NK-92. Apparently, sustained expression of these molecules is toxic to these cell types. Given the expression of Siglec-13 on chimpanzee monocytes, we assume that ancestral hominin macrophages were able to tolerate expression of this Siglec. Regardless, it is reasonable to suggest that Siglec-13–positive monocytes and/or Siglec-17–positive NK cells on ancestral innate immune cells may have influenced interactions with pathogenic bacteria and/or have had toxic effects.

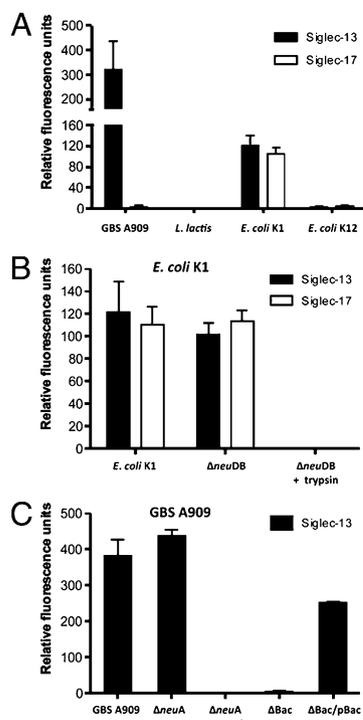
**Timing of SIGLEC Pseudogenization Events During Hominin Evolution.** The two hominin pseudogenization events occurred after a common ancestor with the chimpanzee, but predated the common origin of modern humans. Fresh analysis of genomic sequence data from a newly obtained well-preserved Neanderthal sample from Monti Lessini (MLS3) (*SI Appendix, see ancient DNA analysis*) showed that the pseudogenized allele of *SIGLEC17*



**Fig. 3.** Importance of DAP12 for optimal surface expression of Siglec-13 and -17. (A) 293T cells were transiently transfected with a Siglec-17 cDNA in pcDNA3.1 with or without cotransfection with FLAG-tagged DAP12. Human CD33-transfected cells were used positive controls for detection by a rabbit anti-human CD33 antibody, which partially cross-reacts with human Siglec-17. Cells cotransfected with pcDNA3.1 and DAP12 were used as a negative control. Fluorescence was measured after staining with rabbit anti-human CD33 and then Alexa Fluor 647 donkey anti-rabbit IgG. The Siglec-17Tmmutant in pcDNA3.1 was made from Siglec-17–pcDNA3.1 by introducing a K253A mutation. Cotransfected pRES2-EGFP (Clontech) was used to gate positively transfected cells. (B) The 293T cells were transiently transfected with a Siglec-13 cDNA in pcDNA3.1 with or without cotransfection with FLAG-tagged DAP12. Controls were as in A. Fluorescence was measured after staining with mouse anti-Siglec-13 and then Alexa Fluor 647 goat anti-mouse IgG. The cSiglec-13Tmmutant in pcDNA3.1 was made from cSiglec-13–pcDNA3.1 by introducing a K352A mutation. Cotransfected pRES2-EGFP (Clontech) was used to gate positively transfected cells. (C and D) 293T cells transiently transfected with cDNAs for chimpanzee Siglec-13 (C) or human Siglec-17 (D) with/without DAP12 were lysed. M2 agarose beads were used to pull down FLAG tagged DAP12. Mouse anti-Siglec-13 or rabbit anti-human CD33 (which cross reacts with Siglec-17) were used in Western blots followed by HRP conjugated secondary antibodies, as shown in C and D, respectively. The upper band on the coimmunoprecipitation is nonspecific because of the use of M2 beads carrying a mouse antibody (C). M indicates All Blue protein standard (BIO-RAD).

was already present before the population divergence of Neanderthals and modern humans (between 270,000 and 440,000 y ago) (26). The same genotype was noted in published Neanderthal genomic sequences. Whether *SIGLEC13* was also deleted from the Neanderthal genome could not be determined with certainty. However, we found no evidence for the chimpanzee version of the gene in the Neanderthal sample analyzed here (*SI Appendix*) or the published Neanderthal genome sequence. The recently published Denisovan genome sequence (27) also showed evidence of the modern human versions of *SIGLEC17P* and the possible deletion of *SIGLEC13*.

Taken together, the data indicate that both the *SIGLEC13* deletion allele and the modern human *SIGLEC17P* allele were already present in the common ancestral populations of Neanderthals, Denisovans, and humans. However, other similar *SIGLEC* genes (*SIGLEC14* and *SIGLEC16*) show polymorphic pseudogenization in modern humans, with moderate frequency persistence of the intact functional allele in all human populations (15, 16). Initial pseudogenization of *SIGLEC16* was estimated to occur at least 3 Mya (20). Given the small

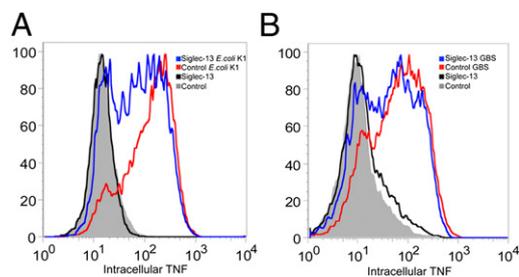


**Fig. 4.** Chimpanzee Siglec-13 or resurrected Siglec-17 interacts selectively with bacterial pathogens. (A) Chimpanzee Siglec-13-Fc or resurrected human Siglec-17-Fc (with Arg) chimeras were immobilized to ELISA wells by protein A, and binding of FITC-labeled sialylated strains GBS A909 (serotype Ia) or *E. coli* K1 RS218 (Str<sup>R</sup>) was studied. Negative controls were *L. lactis* and non-encapsulated laboratory *E. coli* K-12 strain DH5 $\alpha$ . (B) Binding of *E. coli* K1 strain RS218 (Str<sup>R</sup>), isogenic Sia-deficient *E. coli* K1  $\Delta neuDB$ , or *E. coli* K1  $\Delta neuDB$  pretreated with trypsin was studied as in A. (C) Binding of GBS A909 serotype Ia, isogenic Sia-deficient GBS  $\Delta neuA$ , or GBS  $\Delta neuA$  pretreated with trypsin was studied as in A. Strains  $\Delta Bac$  (lacking  $\beta$ -protein) or the plasmid complemented mutant  $\Delta Bac$  + pBac were also studied. All values are means from three independent experiments  $\pm$  SD.

population of Neanderthals and Denisovans analyzed and the limited quality of these data, we cannot be certain regarding the timing of fixation of the *SIGLEC13* and *SIGLEC17* pseudogenes in hominin populations. Nevertheless, we hypothesize that expression of these genes became detrimental to survival under the selective pressure of pathogenic bacteria that were able to bind to the Siglecs and subvert their homeostatic immune functions. Another (not mutually exclusive) selection pressure for Siglec elimination could have been toxic overactivation of the immune system. To seek evidence for these hypotheses, we looked for residual signatures of selection surrounding these loci.

#### Genomic Evidence for Selection at the *SIGLEC13* and *SIGLEC17* Loci.

When positive directional selection involves specific genes or pseudogenes, the genomic regions encompassing such loci can show limited variation in the time period immediately after (34, 35) depending on the type of selection and whether the allele has reached fixation. This kind of signature of selection will be eroded over time by additional random mutations and/or recombination. The deepest time at which such signatures can still be confidently detected is thought to be 5,000–10,000 generations or about 100,000–200,000 y (34, 35). This depth of time happens to be similar to the depth of time proposed for the origin of modern humans (21, 22, 25). Thus, it is impossible to conclusively prove a classical “selective sweep” before the origin of modern humans  $\sim$ 200,000 y ago. However, we decided to look for any residual evidence of selection surrounding these two loci compared with data for adjacent genes.



**Fig. 5.** Reduced intracellular TNF of Siglec-13–transfected RAW264.7 cells in response to bacterial pathogen infection; 500,000 cells semistably transfected with Siglec-13 or vector only and selected with G418 for 3 wk were seeded in a 12-well plate. The next day, 2 h before infection, cells were washed three times with HBSS, and regular culture medium without G418 added. Cells were infected with bacterial pathogens at an appropriate MOI for 1 h at 37 °C. The BD Fixation and Permeabilization Solution Kit (555028) with APC rat anti-mouse TNF- $\alpha$  was used to detect the intracellular TNF level using the recommended protocol. Cells without bacterial infection were used as controls. Cells transfected with pcDNA3.1 vector were used as a control. (A) Cells infected by *E. coli* K1 RS218 (Str<sup>R</sup>) at MOI = 0.6. (B) Cells infected by GBS A909 (serotype Ia) at MOI = 0.1.

We, indeed, found low  $\pi$ - (nucleotide diversity) and  $\theta$ - (nucleotide polymorphism) values for *SIGLEC17P* and the *SIGLEC13* flanking regions relative to adjacent loci such as *SIGLEC8* and *SIGLEC10*. Using GENECONV, we did not find any evidence that either locus was involved in gene conversions that may explain our observations. Furthermore, the estimated  $H$  values of Fay and Wu of human *SIGLEC13* flanking region and *SIGLEC17* are significantly negative, and the DH and DHEW tests (36, 37) show significant results for *SIGLEC17P* (SI Appendix, Table S4). However, not all of the statistics significantly rejected neutral evolution of two loci in our coalescent analysis (SI Appendix, Table S4). Notably, relatively low polymorphisms were also observed in *SIGLEC7*, which is  $\sim$ 10 kb away from *SIGLEC17P*. Like *SIGLEC17P* (SI Appendix, Table S4), *SIGLEC7* also showed significant deviation from neutrality in some statistical tests. However, *SIGLEC9*, another adjacent locus at a comparable distance, showed neither low polymorphisms (SI Appendix, Table S2) nor statistical significance in any of the tests. Thus, *SIGLEC7* could not be the locus of selection. In addition, an HKA (Hudson–Kreitman–Aguadé) test (38) between *SIGLEC17P* and *SIGLEC7* loci indicated the significantly lower polymorphism in *SIGLEC17P* compared with *SIGLEC7* with the chimpanzee sequence as an outgroup ( $P < 0.05$ ), supporting the notion that *SIGLEC17P* might be the center of the proposed ancient selective event.

Interestingly, the resurrected Siglec-17 also shows a low non-synonymous/synonymous rate ratio (dN/dS) that is similar to most other currently functional Siglecs, suggesting that it was subject to purifying selection before its pseudogenization (SI Appendix, Fig. S7). Notably, with the exception of the human-universal single base pair deletion, the *SIGLEC17* ORF remains conserved in all 28 HapMap humans studied, suggesting inadequate time for accumulation of other random mutations. We, of course, cannot rule out the possibility that this pseudogene is still undergoing purifying selection because of some other unknown function (39) (e.g., as a small RNAi-altering gene expression (40)).

Although no single test can be conclusive at this depth of evolutionary time, our collective data indicate a residual signature of ancient selection forces acting on both of these loci, which must have predated the common origin of modern humans. However, such signatures should have faded in 100,000–200,000 y (34, 35). Thus, it is reasonable to speculate that positive selection on these pseudogenization events may have been involved in population bottlenecks close to the origin of modern humans.

**Dating the Selection on the Inactivation of *SIGLEC13* and *SIGLEC17*.** The hominin-specific events inactivating *SIGLEC13* and *SIGLEC17*

likely occurred first in the common ancestral populations of modern humans, Neanderthal, and Denisovans. In keeping with this finding, coalescence analysis estimated the time of the most recent common ancestor of identified haplotypes at 800,000–900,000 for the *SIGLEC13* deletion locus and *SIGLEC17P* (*SI Appendix*). At first glance, this timing may seem at odds with aforementioned signals of selection at both loci, which should have been completely erased over such long periods of time. However, we can suggest a scenario consistent with all of the data—that some active alleles of these genes persisted in the common ancestral population of modern humans, Neanderthal, and Denisovans until selection eliminated them at some point close to the common origin of modern humans. To seek additional evidence for this possibility, we calculated dates for selection acting on these events (*SI Appendix*). Approximate times were calculated as ~105,000 y for *SIGLEC17P* and ~46,000 y for *SIGLEC13*. These numbers are approximations, and the method does not allow error estimations. Because both pseudogenization events are universal to all modern human populations, they must actually date back to at least the common origin of modern humans ~100,000–200,000 y ago. Regardless of exact timing, these data provide support for selection acting on these pseudogenes close to the common origin of modern humans.

**Bacterial Pathogens as Selective Agents in Hominin Evolution?** Independent of exactly when fixation of these pseudogenes occurred in ancestral hominins, the question arises as to what selective forces were involved. Alteration of innate immune defense against invasive human neonatal pathogens such as GBS and *E. coli* K1 would exert a powerful selection pressure on reproductive success, and other prevalent microbial pathogens

could also have subverted Siglec signaling to promote infection. Thus, in addition to other extant theories about the origin of modern humans, interactions of infectious agents with the innate immune system of early humans should be examined as a source of potential selection. After all, infectious agents are already widely recognized as selective agents to explain human polymorphisms (e.g., the role of malaria in selecting for the sickle cell hemoglobin trait). Alternative (but not mutually exclusive) hypotheses include Siglec-mediated overactivation of immune cells and/or changes in commensal–host interactions, which could have compromised reproductive success or the ability to care for the young. Additional studies are needed to further investigate the theoretical role of bacterial selection pressure during human origins. Strong selection by pathogens could result in severe population restrictions and bottlenecks, such as seen in hominin origins. If so, we may need to consider the possibility of an “infectious origin” of modern humans.

## Materials and Methods

DNA samples and population groups are described in *Results*. PCR, RT-PCR, sequencing details, and details of flow cytometry for detection of Siglecs or intracellular TNF expression are in *SI Appendix*. Cells transfected with expression constructs for Siglec-13 or -17 with or without DAP12–FLAG were analyzed by coimmunoprecipitation and Western blotting (*SI Appendix*). Details of array fabrication and binding assays, transfection of Siglecs in mouse Raw264.7 cells, molecular and cellular assays, and human population genetic analysis are in *SI Appendix*. Interaction of Siglec-Fcs with bacteria was studied as described (33) with minor modifications (*SI Appendix*).

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