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Review article

Are humans prone to autoimmunity? Implications from evolutionary changes in hominin sialic acid biology

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ABSTRACT

Given varied intrinsic and extrinsic challenges to the immune system, it is unsurprising that each evolutionary lineage evolves distinctive features of immunoreactivity, and that tolerance mechanisms fail, allowing autoimmunity. Humans appear prone to many autoimmune diseases, with mechanisms both genetic and environmental. Another rapidly evolving biological system involves sialic acids, a family of monosaccharides that are terminal caps on cell surface and secreted molecules of vertebrates, and play multifarious roles in immunity. We have explored multiple genomic changes in sialic acid biology that occurred in human ancestors (hominins), some with implications for enhanced immunoreactivity, and hence for autoimmunity. Human ancestors lost the enzyme synthesizing the common mammalian sialic acid Neu5Gc, with an accumulation of the precursor sialic acid Neu5Ac. Resulting changes include an enhanced reactivity by some immune cells and increased ability of macrophages to kill bacteria, at the cost of increased endotoxin sensitivity. There are also multiple human-specific evolutionary changes in inhibitory and activating Siglecs, immune cell receptors that recognize sialic acids as "self-associated molecular patterns" (SAMPs) to modulate immunity, but can also be hijacked by pathogen molecular mimicry of SAMPs. Altered expression patterns and fixed or polymorphic *SIGLEC* pseudogenization in humans has modulated both innate and adaptive immunity, sometimes favoring over-reactivity. Meanwhile, dietary intake of Neu5Gc (derived primarily from red meats) allows metabolic incorporation of this non-human molecule into human cells——apparently the first example of "xeno-autoimmunity" involving "xeno-autoantigen" interactions with circulating "xeno-autoantibodies". Taken together, some of these factors may contribute to the apparent human propensity for autoimmunity.

Contents

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1. The immune system: a rapidly evolving feature of vertebrates

Different biological systems evolve at different rates, partly depending upon intrinsic and extrinsic selection pressures and the nature of trade-offs that determine reproductive fitness of the organism. With the possible exception of the reproductive system, the immune system seems to be the most prone to rapid evolution (including runaway "Red Queen" co-evolution) [1,2], being under a constant barrage of rapidly evolving pathogens and commensals/pathobionts, even while evading molecular mimicry by other microbes, and minimizing damage to the host, via misdirected and/or autoimmunity. Indeed, it has even been discussed whether the adaptive immune system of vertebrates was an "evolutionary misstep" [3], with great short time benefits––but a long-term price to pay, yet no possible turning back from the addiction to these elaborate and sophisticated immune response mechanisms. Meanwhile, the horseshoe crab lineage has survived for hundreds of millions of years, with nothing more than a simple innate immune system [4,5]. Of course, the innate immune system may also overreact, contributing inflammatory components to autoimmunity.

Thus, at any given time during the evolution of an organism or lineage (such as in the snapshot of life on earth that we currently live in) the reactivity of components of the immune system directly or indirectly reflects successful immune protection of a given lineage in the recent or distant past. In this light, while using mice as models for humans is extremely valuable, it also somewhat limited, given a common ancestor between these two species already existed before the dinosaurs went extinct. On the other hand immunological information regarding the closest relatives of humans (the non-human hominids or "great apes"–– chimpanzee, bonobo, gorilla and orangutan) [6] is constrained by the small number of individuals of these species that have been studied carefully in captivity, albeit under conditions that are somewhat similar, but obviously not identical to those of modern humans [7,8]. Even more limited information can be obtained by studying extinct human ancestors and their relatives (hominins) and by viewing the genomes of the more recently extinct Neanderthals and Denisovans [9].

2. Evolutionary biology of sialic acids and Siglecs: another rapidly evolving system

Diverse and complex glycan chains are a ubiquitous feature of most cell surface and secreted molecules, and of essentially every cell type that has emerged over >3 billion years of evolution [10]. But unlike the case with the genetic code, the expression and complexity of glycans has evolved and diverged much more rapidly in different taxa, and the potential for structural glycan variation is astronomical. Given these facts and the greater technical difficulties in analyzing glycans, it is not surprising that they have become the "dark matter of the biological universe" [11]. But unlike the situation with dark matter in the universe, we do know more than enough about glycans to incorporate them into the standard model of biological systems, and there is a need to train a new generation of biomedical investigators who view glycans as an information-rich and integral part of biological processes [12].

When it comes to self:non-self recognition by the innate immune system, glycans are often a conspicuous and major component of well-established pathogen-associated molecular patterns (PAMPs) [13], as well as the more recently appreciated self-associated molecular patterns (SAMPs) [14–19]. About 100 million years before the emergence of the adaptive immune system in jawed vertebrates during the Cambrian expansion [3], the Deuterostome lineage of animals (vertebrates and so-called "higher" invertebrates) irreversibly committed to include in their glycan repertoire a family of nine carbon backbone monosaccharides called sialic acids (Sias) [20,21], which are typically found at high densities the outermost ends of glycan chains of cell surface and secreted molecules in this lineage Angata and Varki, 2002, Chem Rev, 102, 439-69; Varki and Schauer, 2009, Essentials of Glycobiology, 199–218; Schnaar et al., 2014, Physiol Rev, 94, 461–518}. A recent calculation concludes that the concentration of sialic acids within the cell surface glycocalyx of a lymphocyte is > 100 mM! [22].

While Sias are ancestrally derived from a more ancient family of nonulosonic acids that are found in many prokaryotes [21,23], they are sufficiently distinct enough in structure, and massively abundant across tissue types, as to have to been adopted as major SAMPs for recognition of self by the innate immune system [14]. This, in turn, seems to have resulted in selection pressure for prokaryotic pathogens and commensals to generate molecular mimics of such SAMPs, using every possible "trick in the book", including convergent evolution by exaptation of the genes of the ancient nonulosonic acid pathways [21]. Given that sialic acids are also the targets for numerous viral hemagglutinins, bacterial adhesins and toxins [24,25], it is not surprising that these molecules are extremely diverse in their structure and presentation, and vary greatly between species, cell types, and states of infection or inflammation [26–28].

3. Multifarious roles of sialic acids in the immune system

Given their ubiquity, diversity, and high density on all vertebrate cell surfaces, it should not be surprising that sialic acids have diverse and complex roles in the immune system, ranging from minor modulation to highly specific recognition processes. Space does not allow for the detailed discussion of this topic, and it has been extensively reviewed [18,29–38]. Figs. 1 and 2 from the 2012 review are reproduced here, and the details can be found in the cited articles. Most relevant to the question of autoimmunity are the following considerations: circulating plasma Factor H engages cell surface Sias, protecting cell surfaces from the alternative complement pathway; intrinsic Sia-binding Siglec molecules on immune cells detect sialylated ligands as SAMPs and can inhibit (or sometimes activate) immune cell reactivity; microbial sialidases targeted to cause loss of SAMPs also expose underlying glycoconjugate "cryptoantigens", Sias presented as microbial molecular mimics can induce autoantibodies; non-self Sias can be metabolically incorporated from dietary sources and become "xeno-autoantigens," targeted by intrinsic anti-Sia antibodies; O-acetylation of Sias can block Sia recognition by intrinsic lectins like Siglecs; and α2–6 sialylation of IgG-Fc region N-glycans can switch the effects of

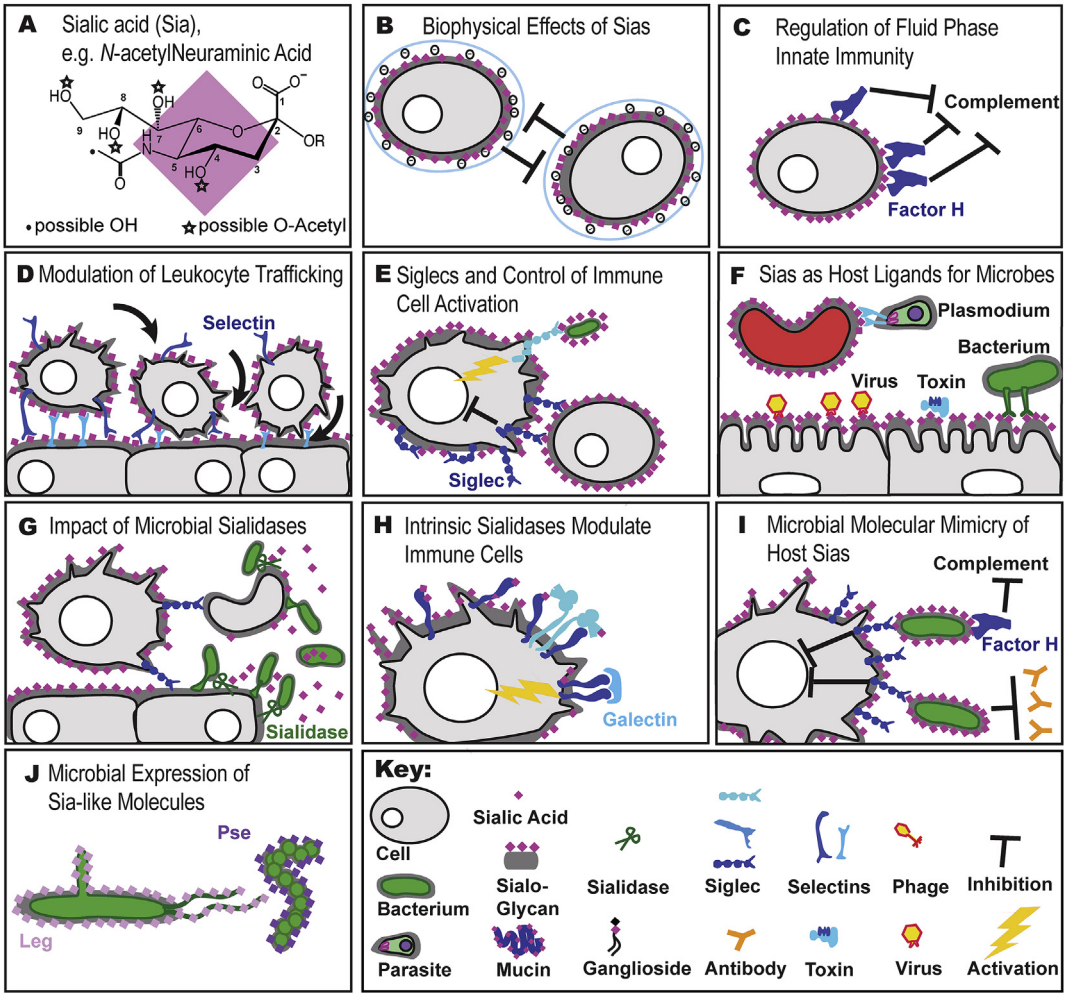

Fig. 1. Some Examples of Roles of Sialic Acids in immunity. Sialic acids are shown as pink diamonds. See the text for discussion. (A) Neu5Ac, the most common sialic acid in mammals. These acidic sugars share a nine-carbon backbone and can be modified in many ways. (B) The high density of terminal sialic acids on the glycocalyx of vertebrate cells imparts negative charge and hydrophilicity to cell surfaces, altering biophysical properties. (C) Factor H binds cell surface Sias, protecting cell surfaces from the alternative complement pathway. (D) Intrinsic Sia-binding molecules such as selectins on endothelia, leukocytes, and platelets initiate leukocyte rolling on endothelial surfaces, a key initial step for leukocyte extravasation. (E) Intrinsic Sia-binding Siglec molecules on immune cells detect sialylated ligands and can inhibit immune cell activation. There are also activatory Siglecs. (F) Host Sias are frequently exploited as attachment sites ("receptors") by pathogens including protozoa, viruses, bacteria, and toxins. (G) Microbial sialidases can help pathogens to expose underlying glycan-binding sites, to avoid sialylated decoys (see below), and/or provide Sias as food sources. The loss of SAMPs from cells may then be used by host immune cells to react to pathogens, and/or to clear away desialylated cells or glycoproteins. (H) Endogenous sialidases such as Neu1 can modulate immune cell function by modulating receptor clustering, possibly by exposing underlying galactose residues and facilitating galectin-mediated cross-linking of surface molecules. (I) Microbial mimicry of host Sias allows manipulation of host immune response by engaging inhibitory Siglecs, inhibiting complement via factor H binding, and reducing the opportunity of the host to form antibodies. (J) Microbial synthesis of Sia-like molecules, such as legionaminic acid and pseudaminic acid stabilizes fimbriae. (Reproduced with permission from Varki, A., and Gagneux, P. *Ann. N.Y. Acad. Sci.*, 1253:16–36, 2012.)

some IgG antibodies from activating to inhibitory.

4. CD33-related Siglecs as modulators of adaptive and innate immunity

Among the many roles of sialic acids in immunity, their recognition by a family of sialic acid recognizing immunoglobulin superfamily members (Siglecs) has become a topic of interest, including the subset of Siglecs evolutionarily related to CD33(Siglec-3)—a receptor that is prominently but differentially displayed on several cells of the immune system (Fig. 3). Again, space does not allow a detailed discussion regarding these CD33rSiglec receptors, and details can be found in various reviews [30–34,36–40]. The key features relevant to this discussion are their recognition of endogenous sialic acids (or their microbial evolutionary mimics) via an extracellular amino terminal V-set domain, and signaling via cytosolic tail inhibitory ITIM motifs, or less commonly by recruiting a DAP12 adapter which carries cytosolic activating ITAM motifs.

5. Evolutionary chain of Red Queen effects involving sialic acids and Siglecs

It is evident from the above discussion that Sias and Siglecs are at the nexus of multiple ongoing evolutionary arms races, which are summarized in Fig. 4, and discussed elsewhere [15]. Taken together, such data explain the rapid evolution of the CD33rSiglec-encoding gene cluster, driven by a selection pressure for maintain self-recognition by innate immune cells in the face of pathogen subversion. Even amongst the "higher" primates, Siglecs show quantitative and qualitative intra- and interspecies variations in expression patterns on leukocytes, both in circulation and in tissues [15].

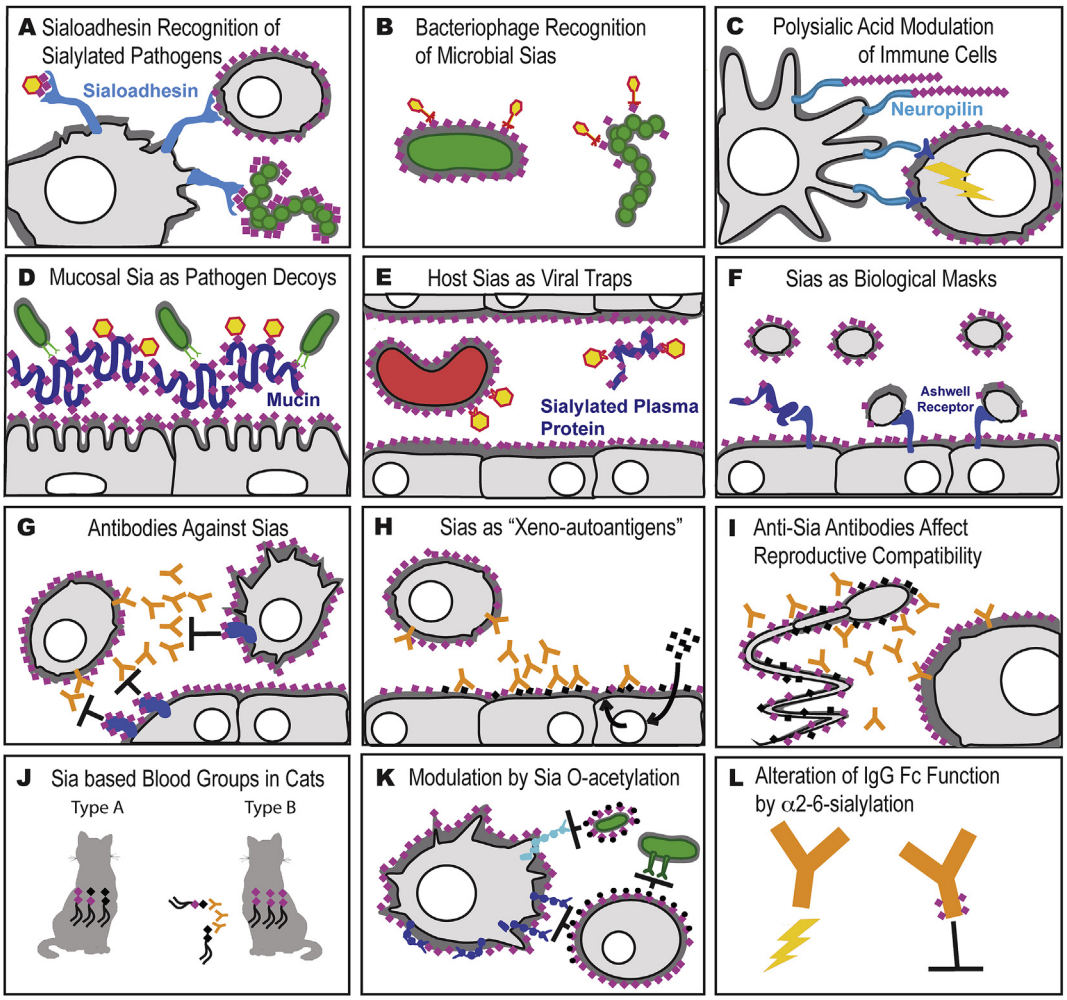

Fig. 2. More Examples of Roles of Sialic Acids in Immunity. Sialic acids are shown as pink diamonds. See key in Fig. 1, and the text for discussion. (A) Siglec-1 (sialoadhesin) expressed on macrophages recognizes Sias in patterns commonly found on microbial pathogens and facilitates phagocytosis. Siglec-1 may also mediate immune cell interactions with one another. Some viruses exploit Siglec-1 binding to gain access to host cells. (B) Certain bacteriophages use Sias on their microbial hosts as "receptors" for invasion. (C) Polysialic acid on immune molecules such as neuropilin on dendritic cells modulates interactions with T cells. (D) Sia-rich secretions on host epithelia can act as decoys for Sia-binding microbes. (E) Sia-covered erythrocytes and Sia-rich plasma proteins can act as "viral traps." (F) Sias act as biological masks by blocking interactions between intrinsic receptors and underlying glycan structures. (G) Sias on potentially antigenic glycoconjugates prevent the formation of antibodies to "cryptoantigens." Less commonly, Sias can be autoantigens. (H) Nonself Sias can be metabolically incorporated from dietary sources and become "xeno-autoantigens," targeted by intrinsic anti-Sia antibodies. (I) Female genital tract reactions to nonself Sia on sperm can lead to reproductive incompatibility. (J) Some mammals, such as cats, have blood groups defined by Sia-containing glycolipids. (K) O -acetylation of Sias can block Sia recognition by intrinsic lectin s like Siglecs, and modulate microbial lectin interactions, in a positive or negative fashion. (L) Alpha-2–6 sialylation of IgG-Fc region N -glycans can change the effects of IgG antibodies from activating to inhibitory. (Reproduced with permission from Varki, A., and Gagneux, P. *Ann. N.Y. Acad. Sci.*, 1253:16–36, 2012).

6. Multiple changes in sialic acid biology during human evolution

Given rapid evolution, it is not uncommon to find significant genomic differences in sialic acid biology between lineages like rodents and primates. Most such differences tend not to be found in the genes involved in biosynthesis, activation or transfer of sialic acid, rather they are prominently seen in the receptors that recognize Sias of intrinsic and extrinsic origin. The human lineage appears to be unusual in having a substantial load of genetic and genomic changes affecting sialic acid biology. Again, this issue has been addressed in detail elsewhere [34,41], and only the key points are further discussed below.

7. Human loss of a major mammalian sialic acid, Neu5Gc

One of the remarkable evolutionary biochemical "inventions" during the Cambrian expansion was a novel enzymatic mechanism for the hydroxylation of the common sialic acid Neu5Ac, generating a rare example in nature of an N-glycolyl group (addition of one oxygen atom to the N-acetyl group of Neu5Ac, a most difficult feat from a chemist's perspective). This one-time invention involves a complex set of co-factors that support the primary Cytidine monophosphate N-acetylneuraminic acid hydroxylase (*CMAH*) gene product, whose activity has apparently never been reinvented by any microbe pathogen or commensal. Given the timing of evolutionary appearance and confinement to the Deuterostome lineage Neu5Gc can be considered one of most reliable signals of "self" for a vertebrate. However, because Neu5Gc synthesis is dependent on a single gene, it is also prone to inactivation in some evolutionary lineages, the first reported example being that of and Alu-Alu fusion-mediated exon deletion that inactivated the *CMAH* gene, likely sometime prior to the emergence of the genus *Homo* about 2 million years ago [42,43]. Note that besides the loss of this

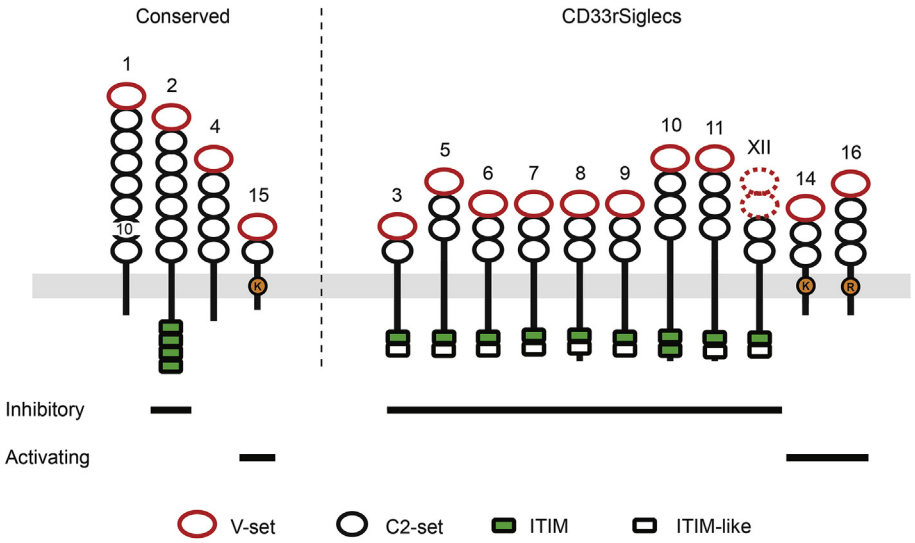

Fig. 3. The Family of Human Siglec Receptors. Conserved (left) and CD33-related (right) Siglecs are cell surface receptors with a variable number of extracellular immunoglobulin-like domains. Structural elements for each protein were derived from the Uniprot database. The outermost domain (V-set, in red) binds to sialylated molecules through a critical arginine residue. Siglecs may contain intracellular signaling motifs that are ITIM or ITIM-like. The transmembrane segment of Siglec-14, -15, and -16 contain a basic amino acid (lysine or arginine) that can interact with negatively charged amino acids of protein adapters like DAP-12 which have ITAM motifs. The V-set domains of Siglec-XII cannot bind sialic acid due to a mutation in a critical arginine residue, and are indicated with dotted lines. The gene for Siglec-13 is deleted in humans, and Siglec-17 is inactivated by a frame-shift mutation in humans. (Figure reproduced with permission from Schwarz, F., Fong, J.J., and Varki, A. *Adv. Exp. Med. Biol.*, 842:1–16, 2015).

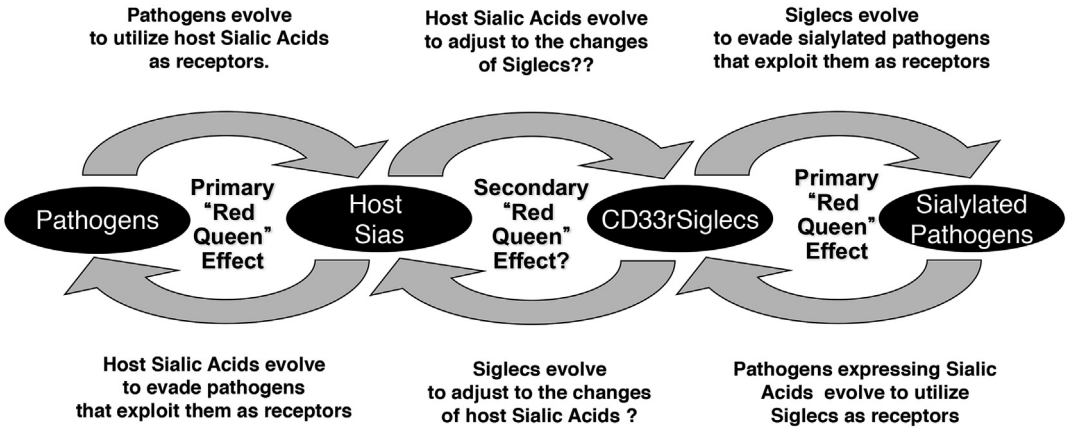

Fig. 4. Probable evolutionary chain of Red Queen effects involving Sialic Acids and CD33rSiglecs. (Reproduced with permission from Padler-Karavani et al. *FASEB J.* 28:1280–93, 2014.) See text for a brief discussion.

unusual sialic acid, a secondary result of the mutation is an excess of the Neu5Ac precursor. While the initial selection might have been by a pathogen that selectively recognized Neu5Gc (of which several are known) [44–49], there is a possibility that this mutation was eventually driven to fixation by altered fertility, involving CMAH null female anti-Neu5Gc antibodies against CMAH positive sperm, perhaps even contributing to the origins of the *Homo* lineage [50,51].

8. Impact of Neu5Gc loss on general reactivity of immune cells

Regardless of the original mechanisms by which the *CMAH* inactivation mutation became fixed in human ancestors, one should expect a significant impact of the loss of millions of hydroxyl groups on the surface of many cell types. Indeed, a mouse model with a *Cmah* mutation similar to that of humans has many phenotypes [52,53], some of which affect the immune system, and potentially contribute to autoimmunity. A general effect on T cells [54] and macrophages [55] is that *Cmah*-null cells are prone to hyper-reactivity, a finding corroborated by feeding of such cells with exogenous Neu5Gc to obtain metabolic incorporation and surface expression, and the resulting suppression of the hyperactivity [54,55]. The mechanisms involved in this effect are largely unknown, but may include multiple effects of this biochemical and biophysical change on signaling receptors and downstream transcription factors [53]. Notably, *Cmah* null mice are, like humans, very sensitive to the toxic effects of gram negative bacterial lipolysaccharide [55]. On the other hand, macrophage phagocytosis of bacteria is substantially improved by the *CMAH* null state, in humans and mice [55]. One can speculate that this combination might have improved survival following transient bacterial exposure during scavenging, hunting and butchering of carcasses by the early genus *Homo*––but at the price of a more severe toxic response to a less frequent major infection. Now that many ancient infectious risks have been diminished, the intrinsic hyper-reactivity

of human lymphocytes and macrophages and attendant autoimmunity may reveal a major evolutionary liability.

9. Impact of Neu5Gc loss on Siglec ligands and function

Given that the CMAH gene appears to be intact and functional in all non-human Old World primates, it is not surprising that several of the CD33-related Siglecs of such primates preferentially bind to Neu5Gc containing glycans, a signal of "self" that is far more difficult for microbes to mimic. Thus, at the time that human ancestors lost this preferred "self" signal, the residual SAMPs would have consisted of sialoglycans much richer in Neu5Ac. It is reasonable to suggest that some loss of self-recognition by innate immune cells might have occurred with attendant hyper-reactivity, but it is unclear how much of an evolutionary adjustment has occurred since then. It is reasonable to suggest that some of the relative hyper-reactivity of human immune cells is related to this process, but further studies are needed to determine the extent of its significance. In this regard, studies of CD33rSiglecs in the New World primate lineage (wherein an independent loss of *CMAH* seems to have occurred) [56] would be of interest in a comparative sense; however, the depth of time of that loss (>30 million years) is much greater than that of the hominin loss, so there may have been more time for evolutionary adjustment.

10. Consequences of Neu5Gc loss for pathogen molecular mimicry and secondary autoimmunity

As discussed earlier, pathogens have reinvented the synthesis of Neu5Ac many times, but never that of Neu5Gc. Such sialylated molecular mimics not only suppress complement activation and block antibody recognition of underlying epitopes, but can also engage innate immune Siglecs that preferentially recognize Neu5Ac, thus dampening immune responses [57]. Once the hominin lineage lost Neu5Gc, with a consequent excess of Neu5Ac, the change in the "self" sialome would have given these types of pathogens and added opportunity for successful molecular mimicry. Indeed, several human specific pathogens such as group B *streptococcus* [58,59], *E. coli* K1 [60] and non-encapsulated *Haemophilus influenzae* [61,62] appear to use this strategy successfully.

Logically, the presentation of the "self" sialic acid Neu5Ac on foreign structures such lipooligosaccharides might also trigger autoimmune responses. While this is fortunately rare, it clearly occurs following infections with *Campylobacter* species that express human-like sialoganglioside structures on their lipooligosaccharides [63,64], and less commonly following infections with *Haemophilus influenzae* [65]. The resulting circulating autoantibodies typically attack sialoglycans in the nervous system, resulting in serious outcomes such as Guillain-Barré and Miller-Fisher syndromes. It is possible that these devastating diseases are the proverbial tip of the iceberg, and that there are less pathogenic but clinically significant antibodies directed against other Neu5Ac glycans in humans, which are generated by interactions with such microbial mimics.

11. Relative over-reactivity of human T and B cells related to suppressed expression of Siglecs

Quite apart from any evolutionary changes in the binding specificity of CD33rSiglecs, their actual level of expression on immune cells is likely to have an impact on cellular reactivity. In this regard a striking finding is that the complement of CD33rSiglecs that are displayed on chimpanzee T and B cells are much less prominent on human T cells, even though the genes are shared [66]. Studies showed that this suppression of lymphocyte Siglec expression may at least partially account for the relative hyper-reactivity of human T and B cells [67], including the marked apoptotic over-reaction of human CD4 T cells to HIV infection [68]. The latter finding perhaps accounts for the much more frequent and rapid progression of HIV infection to full-blown AIDS in humans versus chimpanzees [69]. Of course, it is very popular to say that "chimpanzees also get AIDS". While AIDS-like syndromes do occur in SIV infected chimpanzees [70], the vast majority of chimpanzees that were experimentally infected with HIV in the previous century have stayed alive through natural lifespans, despite circulating virus load. Of course other mechanisms likely also contribute to the difference [71,72]. In this regard it is interesting that the severe complications of hepatitis B and C that are also related to immunoreactivity (rather than the virus itself) appeared to be much more common in humans than in the experimentally infected chimpanzees of the last centuries experiments [73–75]. Note that in times past the notion persisted that chimpanzees must be "good models for human diseases", so there was likely a reporting bias, favoring similarities rather than differences. Now that all invasive chimpanzee research has been appropriately stopped [76], we will never know the real answer, but the overall picture seems reasonably clear: human T cells have a propensity for hyper-reactivity to various stimuli.

12. Fixed and polymorphic genomic changes in human *SIGLEC* genes

While the homology and expression patterns of CD33rSiglecs found on immune cells varies between different evolutionary lineages, human ancestors appear to have accumulated a large number of fixed and polymorphic changes in these genes that likely predated the origin of our species in Africa. Space does not allow for a detailed discussion of all these genomic events and their potential functional consequences, which are discussed in recent reviews and papers [34,77–81], but it is likely that they are altering the immune reactivity of individuals and that combinations of these polymorphisms are contributing to relative propensities to autoimmune disease. Careful population studies are needed to address this issue further.

13. "Xeno-autoimmunity", due to metabolic incorporation of a non-human molecule into humans

A rather unusual cause of auto-immune reactivity in humans arises from the fact that following the elimination of the CMAH gene and loss of expression of the Neu5Gc sialic acid, human ancestors began to consume larger amounts of animal foods that contain this molecule (in current times, primarily red meats of mammalian origin, such as beef, pork, and lamb) [82] (see Fig. 5). As mentioned earlier human cells are capable of metabolically incorporating this non-human molecule and presenting it on cell surfaces as if it originated in the same cell. Detection of small amounts of Neu5Gc in human tissues and studies of mice fed with the molecule affirm that this process is ongoing in humans who consume foods rich in Neu5Gc [82]. Effectively this appears to be the first example of a "xeno-autoantigen" i.e., arising from another animal source, but being metabolically incorporated as if it is part of the human cell surface. Although the amounts incorporated are very small, they are immunologically significant because humans have circulating anti-Neu5Gc antibodies. Notably the mechanism by which humans generate such antibodies does not appear to be related to oral consumption. Rather it may be due to another mechanism, in which the foreign sialic acid is incorporated from food into the lipooligosaccharides of commensal gram-negative non-encapsulated *Haemophilus influenzae*, molecules which then

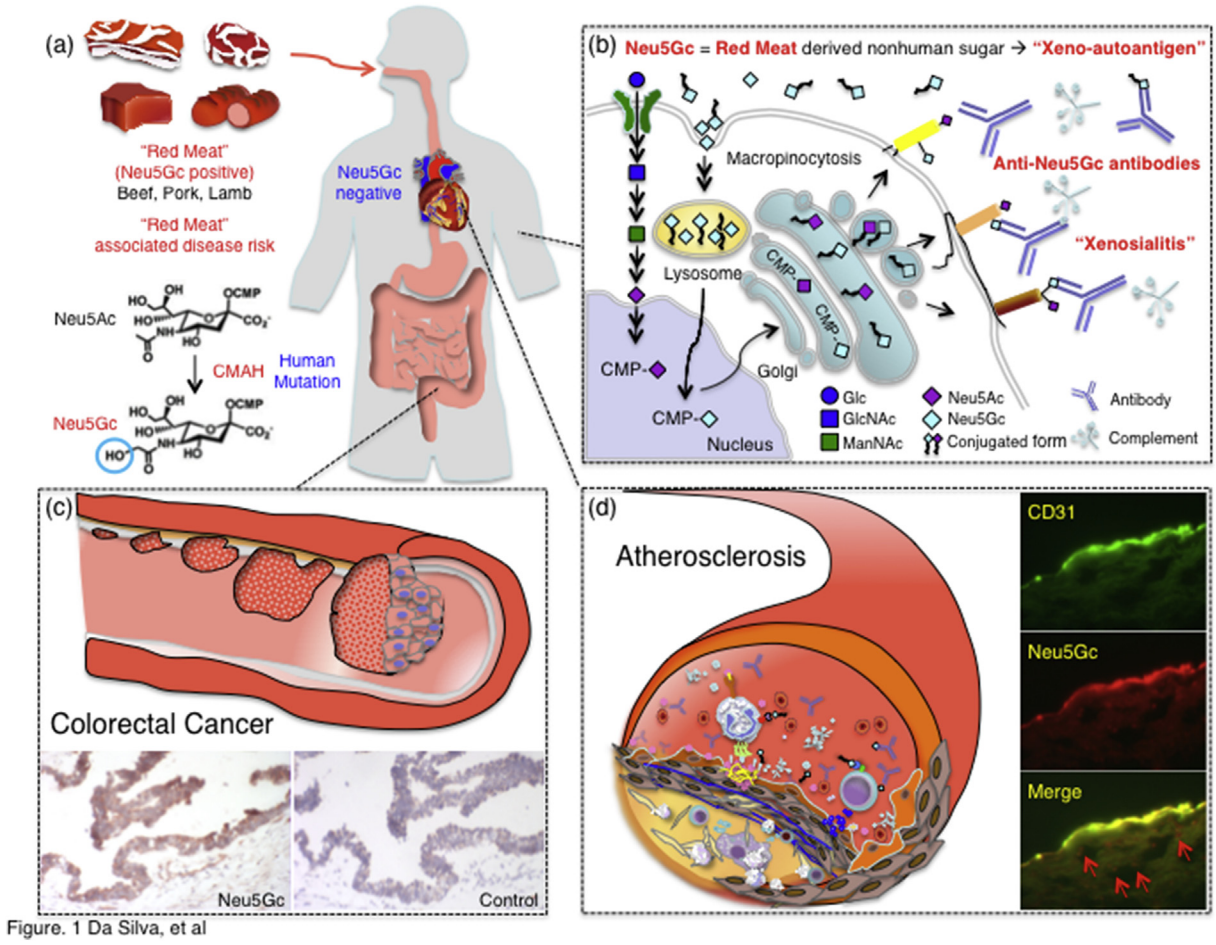

Fig. 5. Potential disease risks associated with metabolic incorporation of the red meat-derived non-human sialic acid. (a) Red meat (beef, pork, lamb) are food sources rich in Neu5Gc. Humans cannot synthesize Neu5Gc from the precursor sialic acid Neu5Ac, due to inactivation of CMAH. (b) Neu5Gc can be incorporated into human cells through the same pathway used for Neu5Ac recycling. Endocytosed Neu5Gc is used as substrate for the synthesis of sialylated glycans in the Golgi. Cell surface glycans containing Neu5Gc may be targeted by circulating anti-Neu5Gc antibodies and complement, leading to a human specific inflammation, termed "xenosialitis". (c) Neu5Gc incorporation in human epithelia or endothelia and subsequent xenosialitis may be a risk factor for the promotion of carcinomas or atherosclerosis (d), or other inflammatory diseases associated with red meat consumption. Neu5Gc is detected in human colorectal cancer cells (c), in endothelial cells (CD31 positive) and subendothelial components (red arrow) in human atherosclerotic lesions (d). (Reproduced with permission from Alisson-Silva, F., Kawanishi, K., and Varki, A. *Mol Aspects Med*. 51:16–30, 2016).

appear to trigger the immune response [83]. It is of course possible that other gut bacteria that take up Neu5Gc also participate in this unusual form of "xeno-autoimmunization". Given the presence of the circulating antibodies, it is reasonable to suggest that the interaction with the tissue-incorporated diet-derived Neu5Gc could result in inflammation i.e., "xenosialitis". If this process triggered autoimmune disease, then it would be a case of "xeno-autoimmunity".

There is little doubt that the ingestion of red meats (particularly easily digestible processed red meats is associated with a human specific propensity to increase risk of inflammation-associated diseases such as carcinomas and atherosclerosis [84–88]. Studies of the contribution of Neu5Gc-induced xenosialitis in such processes are underway in human-like *Cmah* null mice, and the data to date are consistent with the hypothesis [82]. However, given the wide range of antibody levels and the large variety of glycan structures bearing Neu5Gc, and the difficulties in getting accurate dietary histories from human populations, it is not a trivial matter to sort out the relationship of certain specific anti-Neu5Gc antibodies with tissue incorporation from diet, in relation to disease and inflammation.

One autoimmune disease that would be worth pursuing is rheumatoid arthritis (a disease apparently unique to humans) in which anecdotal studies (and a few limited trials) suggest that elimination of meat from the diet may reduce disease severity [89–91]. However, is difficult to be certain that any effect is not due to other simultaneous changes in diet or behavior of such motivated individuals. The N of 1 study to be done is to ask patients with autoimmune disease that is relatively stable on current therapy to make a single change only in their diet, to substitute poultry (which is free of Neu5Gc) for red meats (lamb pork and beef) and to then follow the levels of inflammation and disease severity. If positive results are seen, one could have that patient alternate off and on with this change in diet, and follow the symptoms in relationship to each diet switch.

Lastly, there is one theoretical mechanism by which the existence of the xenoantigens *in vivo* might facilitate the onset of true autoimmunity directed against self-molecules. If endogenous glycoproteins bearing small amounts of Neu5Gc formed immune complexes with circulating anti-Neu5Gc antibodies, these complexes could potentially have an adjuvant effect, to increase the probability of autoimmune reactions to endogenous peptides [92]. Limited studies of therapeutic agents that carry Neu5Gc suggests that this is a possibility. Further explorations are needed.

14. Conclusions and perspectives

While no single one of the above-mentioned evolutionary changes in human sialic acid biology is a strong and dominant cause of the human propensity to autoimmunity, several of them operating in combination could well be significant contributing factors. Further in-depth studies of each possibility need to be pursued, with the eventual goal of reducing the clinical burden of autoimmunity. On a more general note, the complex roles of glycans in many relevant aspects of the immune system and the environment may deserve more attention in studies of autoimmunity.

Acknowledgments

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