THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 48, Issue of November 28, pp. 48245–48250, 2003

Printed in U.S.A.

Human-specific Regulation of α2–6-linked Sialic Acids*[S]

Received for publication, September 4, 2003, and in revised form, September 18, 2003
Published, JBC Papers in Press, September 18, 2003, DOI 10.1074/jbc.M309813200

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Many microbial pathogens and toxins recognize animal cells via cell surface sialic acids (Sias) that are α2–3- or α2–8-linked to the underlying glycan chain. Human influenza A/B viruses are unusual in preferring α2–6-linked Sias, undergoing a switch from α2–3 linkage preference during adaptation from animals to humans. This correlates with the expression of α2–6-linked Sias on ciliated human airway epithelial target cells and of α2–3-linked Sias on secreted soluble airway mucins, which are unable to inhibit virus binding. Given several known differences in Sia biology between humans and apes, we asked whether this pattern of airway epithelial Sia linkages is also human-specific. Indeed, we show that since the last common ancestor with apes, humans underwent a concerted bidirectional switch in α2–6-linked Sia expression between airway epithelial cell surfaces and secreted mucins. This can explain why the chimpanzee appears relatively resistant to experimental infection with human Influenza viruses. Other tissues showed additional examples of human-specific increases or decreases in α2–6-linked Sia expression and only one example of a change specific to certain great apes. Furthermore, while human and great ape leukocytes both express α2–6-linked Sias, only human erythrocytes have markedly up-regulated expression. These cell type-specific changes in α2–6-Sia expression during human evolution represent another example of a human-specific change in Sia biology. Because the data set involves multiple great apes, we can also conclude that Sia linkage expression patterns can be conserved during millions of years of evolution within some vertebrate taxa while undergoing sudden major changes in other closely related ones.

Mammalian cells are covered by a dense glycocalyx consisting of glycolipids, glycoproteins, and proteoglycans. Many of these glycoconjugates carry glycan chains terminating with sialic acids (Sias),[1] a family of acidic 9-carbon sugars that are prominently expressed in animals of the deuterostome lineage (1, 2). By virtue of forming the "canopy" of the glycocalyx, Sias are recognized and used as attachment sites by a large number and wide variety of microbial pathogens and their toxins. Many Sia-binding proteins of different pathogens have been well characterized and generally show a high degree of specificity for different types of Sias and/or their linkage to the underlying glycan chain (1–5).

Perhaps partly because of the selection pressure exerted by such pathogens, the expression of Sias is highly regulated. Different cell and tissue types show marked differences in abundance, types, and linkages of Sias. Approximately 20 different sialyltransferases attach Sias to glycans via α2–3, α2–6, or α2–8 linkages (6–8). These linkages are often critical determinants for recognition by the Sia-binding proteins of pathogens. In general, most pathogen Sia-binding proteins seem to prefer α2–3-linked Sias and some prefer the α2–8 linkage (2, 3). Curiously, the human influenza A and B viruses are among rare examples of pathogens with known preference for α2–6-linked Sias (9). Notably, the few other microbes in which direct or indirect evidence suggests this type of linkage preference are also human pathogens or commensals such as human coronavirus OC43 (5), polyoma virus JC (10), adeno-associated virus serotype 5 (11), and *Streptococcus mutans* (12).

Influenza A and B viruses cause non-lethal gastrointestinal infections in wild waterfowl. Co-mingling of domestic fowl, pigs, and humans facilitates evolution of human-adapted viruses from avian reservoirs, generating influenza epidemics and pandemics (13). In contrast to human influenza viruses, avian and other mammalian influenza isolates prefer α2–3-linked Sias (14). Adaptation of animal influenza viruses to humans correlates with point mutations changing certain amino acids that switch the Sia linkage specificity from α2–3 to α2–6 (15) with the pig potentially acting as an intermediate reservoir (14). The reverse switch in linkage specificity occurs when human isolates are adapted for growth in the amniotic compartment of hen's eggs (13, 16). The detection of α2–6-linked Sias on the upper airway epithelial brush border of humans (17) is consistent with all of the above information as is the presence of α2–6-linked Sias on the upper airway epithelium of ferrets (the only effective mammalian model for human influenza infection) (18). An additional reason for human influenza susceptibility lies in the selective expression of α2–3-linked Sias in the goblet cells that secrete mucins into the human upper airway lumen (17, 19). These soluble mucins are

* This work was supported in part by U. S. Public Health Service Grant R01-GM323373, Yerkes National Institutes of Health Base Grant RR00165, American Lung Association Research Training Fellowship Award RT043N, and National Institutes of Health Training Grant 5T32DK07202 (to P. G.) and by the G. Harold and Leila Y. Mathers Charitable Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.jbc.org) contains Tables A and B.

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[1] The abbreviations used are: Sias, sialic acids; Neu5Ac, *N*-acetylneuraminic acid; Neu5Gc, *N*-glycolylneuraminic acid; ELISA, enzyme-linked immunosorbent assay; CMAH, CMP-Neu5Ac hydroxylase; SNA, *S. nigra* agglutinin; MAH, *M. amurensis* hemagglutinin; TBS, Tris-buffered saline.

unable to adsorb out the α2–6-Sia binding human viruses before they reach and infect the airway epithelial cells.

The closest living evolutionary relatives of humans are the great apes (chimpanzee, bonobo, gorilla, and orangutan), which share 97–99% DNA sequence identity in alignable sections of the gene-rich/non-repetitive regions of the genome (20–23). This genetic similarity is reflected in the rarity of documented biochemical or structural differences among these species. For example, with respect to soluble plasma proteins of humans and great apes, there are remarkably few differences in overall glycosylation including the extent of Sia addition (24). However, among the few documented functional genomic differences between humans and great apes, two affect Sia biology: an exon deletion in the *CMAH* gene explaining the lack of expression of the Sia *N*-glycolylneuraminic acid (Neu5Gc) in humans (25) and a point mutation eliminating the Sia binding property of human Siglec-L1 (26). Another consequence of the human CMAH loss is an excess of the Sia *N*-acetylneuraminic acid (Neu5Ac), the precursor for Neu5Gc (27). This in turn causes a marked increase in ligands for sialoadhesin/Siglec-1 on human cells, possibly explaining a human-specific difference in the expression and distribution of this Neu5Ac-preferring lectin on macrophages (28). Our group has also recently found some other differences in sialic acid biology between humans and great apes.[2] Given <100 known genes involved in biosynthesis, transfer, metabolism, and recognition of Sias, it is surprising to find so many differences in Sia biology between humans and great apes. This prompted us to ask whether there are uniquely human features in the expression pattern of α2–6-linked Sias. In initiating this organism-wide exploration of human:great ape Sia linkages, we also wished to ask how much change in Sia linkage expression has occurred during ~13–14 million years of great ape and human evolution. Such data are currently not available for any such well defined clade of related vertebrates.

EXPERIMENTAL PROCEDURES

Lectin Histochemistry on Tissue Sections—Archival paraffin-embedded human tissue sections were from the University of California, San Diego Histology Resource. Archival paraffin blocks containing great ape tissues samples were from the Yerkes Primate Center. Biotinylated lectins were from Vector laboratories (Burlingame, CA). *Sambuccus nigra* agglutinin (SNA) recognizes α2–6-linked Sias, and *Maackia amurensis* hemagglutinin (MAH, also sometimes called MAL-II) recognizes α2–3- linked Sias (29). *Ulex europeaus* agglutinin, which recognizes α1–2-fucosylated linkages, was used as a positive control for the staining procedure because these linkages are found on the vasculature of humans and great apes. Thus, positive staining of the vasculature generally denotes that the tissue was optimally preserved, fixed, and processed and suitable for use in the analyses.

Paraffin-embedded formalin-fixed tissue sections mounted on glass slides were deparaffinized, blocked with bovine serum albumin to prevent spurious staining, and incubated with biotinylated lectins, appropriately diluted in 1% bovine serum albumin/TBS for 30 min, followed by washing with 0.1% Tween 20 in TBS. Lectin binding was detected using appropriately diluted alkaline phosphatase-labeled streptavidin followed by washes. Color was developed using Vector Blue substrate (Vector Laboratories), and nuclei were counterstained using Nuclear Fast Red. Slides were coverslipped in aquamount-mounting media and viewed using bright field illumination with a Zeiss microscope. Digitized images were captured with a 3-chip CCD Sony DKC-5000 digital photo camera using NIH Image software and Adobe PhotoShop. Pictures were examined by a minimum of two different investigators. Treatment with *Arthrobacter ureafaciens* sialidase (Calbiochem) was used to control for the Sia-specific binding of SNA and MAH. Incubation of sections with 2 mM sodium periodate to truncate Sia side chains was also used to confirm specificity of SNA binding (29).

Agglutination of Human and Chimpanzee Red Blood Cells—A fusion protein of Siglec-2 (CD22) and human Ig Fc region known to only bind to α2–6-linked Sias was used for human and chimpanzee red cell agglutination assays (28). Siglec-2-Fc was added in serial dilutions (0.5–5 μg in phosphate-buffered saline) to wells of round-bottomed 96-well plates. To each well was then added 25 μl of 0.5% fixed red blood cell suspension from each of two human subjects or from three chimpanzees. The plates were incubated for 1 h at room temperature and then observed for agglutination.

Flow Cytometry Analysis—Chimpanzee red cells and leukocytes were isolated from fresh blood samples collected in EDTA and shipped overnight on ice from the Yerkes Primate Center. Fresh bonobo blood from the Zoological Society of San Diego was transported on ice and stored overnight. To assure comparability, human blood samples were collected locally at around the same time and stored overnight on ice, awaiting the arrival of the ape samples. Leukocytes were prepared from whole blood (30) by phosphate-buffered saline wash and two consecutive steps of erythrocytes lysis in 5 volumes of ACK buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.2). Cells ($0.3–1 \times 10^6$) were stained for 1 h at 4 °C with phycoerythrin-conjugated SNA or MAH (5 μg/ml) and for leukocytes with cell type-specific markers (Cy5-conjugated mAbs: CD14 for monocytes; CD13 for monocytes/granulocytes; CD3 or CD19 for lymphocytes). In some experiments, the lectin was replaced with Siglec-2-Fc (10 μg/ml) that had been preincubated (at least 15 min at 4 °C) with 100× diluted phycoerythrin-conjugated goat $F(ab')_2$ anti-human IgG (dilution optimized for the batch of secondary antibody). Following washing, cells were resuspended in phosphate-buffered saline and immediately analyzed on a FACSCalibur (BD Biosciences Immunocytometry Systems). Controls to show dependence on Sia for lectin, or Siglec binding included prior treatment with mild periodate (31) or sialidase (*A. ureafaciens* sialidase) treatment following established protocols.

ELISA for quantitation of α2–3- and α2–6-linked Sialic Acids on Plasma Proteins—Human plasma samples from EDTA anti-coagulated blood of eight human volunteers (with Institutional Review Board approval) were stored frozen at −80 °C. Great ape blood samples (9 chimpanzees, 5 bonobos, 7 gorillas, and 6 orangutans) were collected in EDTA at the Yerkes Primate Center and shipped on ice to the laboratory where plasma was collected after centrifugation and then stored at −80 °C. Samples were diluted to concentrations of approximately 3 μg/ml (corresponding to a 1:1000 dilution for most samples, giving saturation of binding sites on the wells). Diluted samples were coated on Costar ELISA plates in triplicate overnight at 4 °C. The plate was washed four times with TBS and then blocked for 1 h with freshly made TBS-Tween 20. Plates were then incubated for 2 h with biotinylated SNA or MAH (each diluted 1:10,000), washed four times with TBS-Tween 20, and then incubated at room temperature with alkaline phosphatase-conjugated Streptavidin at 1:1000 dilution for 1 h. Plates were developed with alkaline phosphatase substrate for 15 min, and absorption readings were taken at 405 nm.

RESULTS

Unlike humans, Great Apes Do Not Express High Levels of α2–6-linked Sialic Acids on Airway Epithelial Cells—As indicated in Table A (see supplemental data), a large number of samples (295) comprising many different tissue types from humans and all of the great apes were analyzed using lectin histochemistry. The most striking difference between humans and great apes was evident in the epithelium lining of the trachea and lung airways. All 11 samples of human trachea and lung samples showed intense SNA lectin staining of the cilia and their basal bodies at the luminal edge of the bronchiolar epithelial cells, indicating the presence of α2–6-linked sialic acids (see Fig. 1 for an example). In contrast, SNA staining was not observed in the trachea or lung airways from chimpanzees, gorillas, or orangutans (see Fig. 1 for examples). Interestingly, similar findings were made in the mouse (Fig. 1), which is not a good host for human influenza viruses unless subjected to multiple rounds of adaptation. False-negatives were ruled out by virtue of positive staining in endothelia and stroma of the same great ape tissue sections. 3 of 10 chimpanzee trachea samples showed occasional epithelial edge staining. However, these particular samples had some underlying inflammation during which up-regulation of α2–6-linked sialylation is known to occur in some other tissues (32, 33). Thus, human (and not

[2] T. Angata and A. Varki, unpublished data.

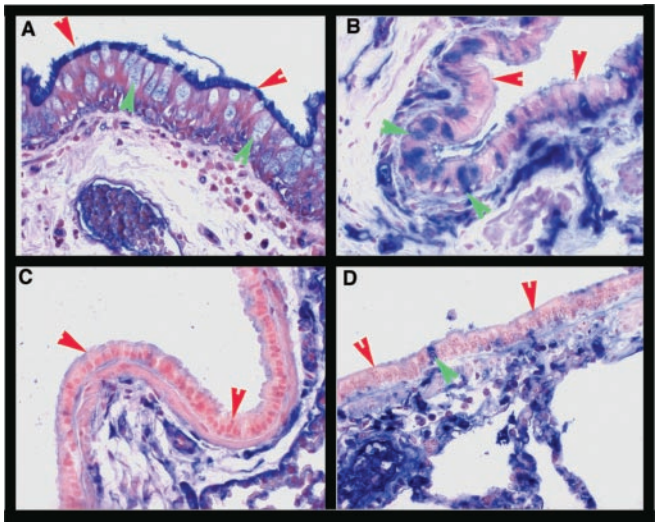

FIG. 1. **Differential expression of α2–6-linked sialic acids between humans and great apes.** Examples of ×100 oil-immersion microphotographs of SNA staining of tracheal epithelium in human (*A*), chimpanzee (*B*), mouse (*C*), and gorilla (*D*). SNA is visualized by the Vector Blue substrate, and cell nuclei is counterstained with Nuclear Fast Red. *Red arrows* indicate the edge of the ciliated epithelium facing the lumen. *Green arrows* indicate examples of mucin-filled goblet cells. Bonobo and orangutan samples appeared similar to those of chimpanzees and gorillas (data not shown).

great ape) bronchial epithelial cells preferentially express α2–6-linked Sias at their ciliated borders.

Goblet Cells and Secreted Mucins Show an Inverse Staining Pattern—The lumen of airways contains heavily sialylated soluble mucins, which are secreted by specialized epithelial goblet cells that are interspersed among the columnar epithelial cells. The number of such goblet cells can vary considerably among different samples. In keeping with a previous report (18), we noted that in human samples in which goblet cells were seen, almost all did not stain strongly with SNA (see Fig. 1 for an example). In contrast, those ape samples in which goblet cells were seen showed intense staining with SNA in six of seven samples, indicating abundant α2–6-linked Sias. Similar SNA staining of secreted mucin in the lumen of ape samples was detected in some instances (data not shown). Thus, sometime after the last common ancestor with chimpanzees and bonobos, human airway epithelium underwent a concerted switch in the expression of α2–6-linked Sias with the ciliary border of the columnar epithelium strongly up-regulating this structure and the goblet cells and secreted mucins down-regulating it.

Other Human-specific Differences in Tissue Expression of α2–6-linked Sias—As shown in the examples in Fig. 2 and detailed in Table I, some tissues other than trachea/lung showed consistent differences between humans and great apes. In human skin, the SNA lectin bound to secretions in eccrine sweat glands. This was not seen in samples from the great apes with the exception of one orangutan sample. In all of the samples from great apes (but not humans), the SNA lectin bound strongly to the stromal elements in the spleen (splenic cords) in addition to binding to lymphoid follicles and vessels (Fig. 2). In liver samples from most great apes (but not humans), the SNA lectin bound strongly to the luminal edges of the bile ducts in the portal triads (Fig. 2). In ileal samples from some great apes (but not humans), the SNA lectin highlighted the external longitudinal muscular layer of the muscularis externa. Pancreatic islet cell staining was seen only in the gorilla and orangutan samples. The human prostate samples showed intense MAH staining of myoepithelial cells (below the epithelial cell layer), which was not seen in corresponding great

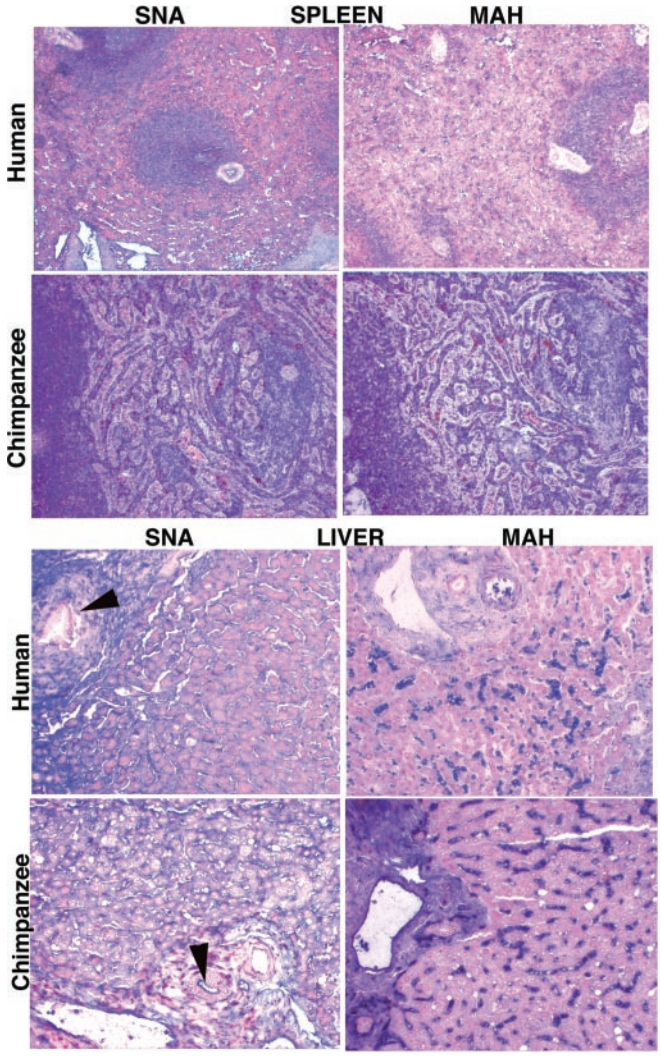

FIG. 2. **Examples of differences in SNA or MAH staining of human and chimpanzee tissues.** Paraffin-embedded sections were stained with SNA or MAH as described under "Experimental Procedures." Examples shown are spleen and liver. In the samples from the great apes (but not humans), the SNA lectin bound to the splenic cords (stromal elements) in the spleen in addition to binding to lymphoid follicles and vessels. In liver samples of great apes (but not humans), SNA bound to the luminal edges of bile ducts in the portal triads (see *black arrows* for an example). A complete listing of human:ape differences is shown in Table I.

ape samples. In other tissues such as the ovary, testes, tonsils, and appendix, the SNA lectin bound to supporting stroma more intensely in the great ape tissues than in corresponding human samples (data not shown). Overall, the increased expression of α2–6-linked Sias on human upper airway epithelial cells is accompanied by similar up-regulation on skin eccrine gland secretions. In contrast, the same structure appears markedly down-regulated in several human tissues such as splenic cords, liver bile ducts, ileal smooth muscle, ovary, testes, appendix, tonsils, and skin. Thus, we present evidence for multiple changes in α2–6-Sia linkage expression (up- and down-regulation) occurring specifically in humans. In contrast, the expression pattern of α2–6-linked Sias has remained relatively unchanged through 13+ million years of evolution of the great apes with the exception of pancreatic islet cells where α2–6-sialylation was apparently turned off in the chimpanzee-human-bonobo clade after their common ancestor with the gorilla and orangutan.

Analysis of Blood Cells Also Shows Human-specific Expression Pattern of α2–6-linked Sialic Acids—Flow cytometry of

TABLE I
Summary of significant differences observed with SNA and MAH lectin binding

Only cell types in which significant differences between humans and great apes were found are listed (for more details on all of the tissues studied, see Supplementary information). Indicated are the numbers of samples in which the observation was made/number of samples in which the cell type could be evaluated accurately. With the exception of pancreatic islets, all of the differences are human-specific and are general to the great apes as a group.

Cell type	SNA staining		MAH staining	
	Human	Great ape	Human	Great ape
Cilia and basal plate of epithelial cells in trachea and lung bronchioles	11/11	3/10[a]	0/11	4/10
Goblet cells of tracheal/bronchial epithelium	1/9	6/7	3/8	8/10
Eccrine sweat gland secretions in skin	8/11	1/6[b]	4/11	1/6[a]
Splenic cords (stromal elements)	3/7[a]	10/10	1/7	6/10
Liver bile ducts	2/7[a]	11/14	0/7	0/14
Ileal smooth muscle	0/5	5/9	0/5	0/9
Pancreatic islet cells	0/7	4/14[c]	0/7	0/14
Prostatic myoepithelial cells	0/5	0/4	5/5	0/4

[a] Only faint and/or focal expression.
[b] Positive in orangutan only.
[c] Positive in gorilla and orangutan only.

erythrocytes using fluorescently labeled lectins showed that human but not chimpanzee or bonobo erythrocytes are strongly positive for α2–6-linked Sia (see Fig. 3*B* for representative example; note that the *x* axis is a log scale). Also, although >95% erythrocytes are strongly positive in humans, only of chimpanzee and 18–28% bonobo erythrocytes are weakly 40–68% positive. This indicates that the change in the upper airway epithelia is at least partially mirrored in cells of the erythroid lineage. In contrast, peripheral blood leukocytes showed comparably high levels of α2–6-linked Sia in humans, chimpanzees, and bonobos (see Fig. 3*A*).

Plasma Proteins Show a Chimpanzee-specific Increase in α2–3-linked Sialic Acids—Most plasma proteins carry *N*-glycans that terminate in α2–3- and/or α2–6-linked Sias. The total amount of plasma proteins and the amount of Sias per milligram protein are similar among humans and all of the great ape plasma samples (data not shown). ELISAs on human and great ape plasma proteins revealed that all five species show similar levels of SNA binding (Fig. 4). However, whereas human, bonobo, gorilla, and orangutan plasma samples show no statistically significant differences in MAH binding, chimpanzees have uniquely up-regulated binding of this lectin (Fig. 4). Because plasma glycoproteins come primarily from plasma cells (immunoglobulins) and liver hepatocytes (most other glycoproteins), this result suggests that chimpanzee hepatocytes and/or plasma cells have increased activity of one or more α2–3-sialyltransferase(s) and that this change occurred after the common ancestor with the bonobo.

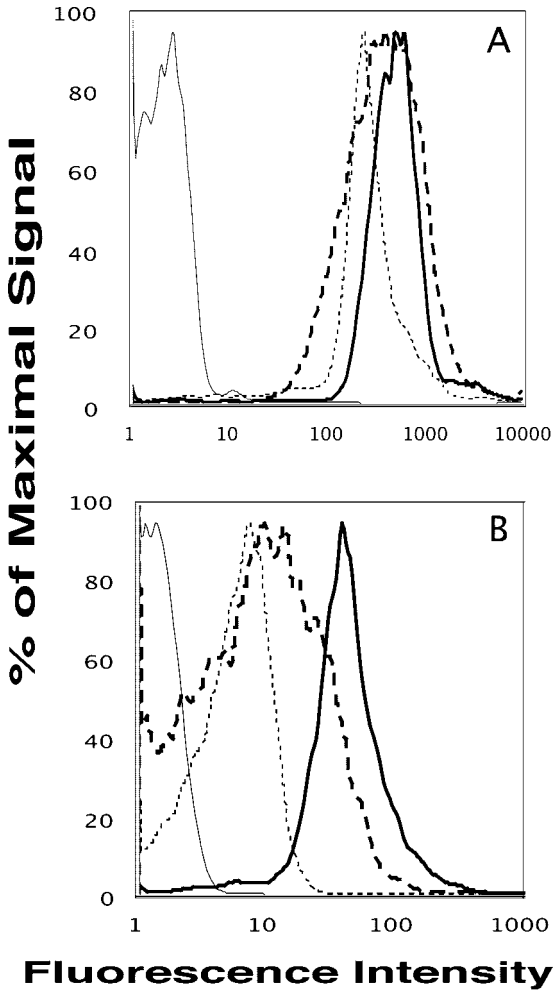

FIG. 3. **Selective expression of α2–6-linked sialic acids on human red blood cells.** Flow cytometry for SNA staining of peripheral blood cells (note that the *x* axis is a log scale) is shown. Human (*solid line*); chimpanzee (*long dashes*); bonobo (*dashed line*); unstained control (*thin solid line*). *A*, total leukocytes. *B*, erythrocytes. Results shown are typical of those seen in samples from 9 humans, 4 chimpanzees, and 4 bonobos.

DISCUSSION

Sias have been shown to be essential for the development of the mouse embryo (34). However, they are also known to be targets for a wide variety of microbial pathogens and toxins. Thus, despite the highly conserved nature of mammalian sialic acid biosynthesis pathways and sialyltransferase gene sequences, the tissue and cell type-specific patterns generated by differential and combinatorial expression of these enzymes may be an important mechanism for rapid pathogen-driven host evolution (35, 36). Here we have investigated the Sia linkage type on most major tissues and cell types of humans in comparison with our closest evolutionary relatives, the great apes. The most obvious and striking difference was found in the airway epithelial cells where only humans prominently up-regulated α2–6-linked Sias on the ciliary border of columnar epithelial cells, even while down-regulating expression of the same structure in goblet cells and secreted mucins. This pattern of Sia linkage expression has been implicated in the adaptation of influenza viruses to infection of humans (15, 37).

Although circulating antibodies indicate that chimpanzees are exposed to human Influenza A in captivity (38), there are no reports of symptomatic influenza illnesses in these closest evolutionary relatives. Indeed, in the few published reports of experimental infection, very high virus titers delivered directly into the trachea of chimpanzees did not result in symptomatology, despite some evidence of virus replication (39–41). This

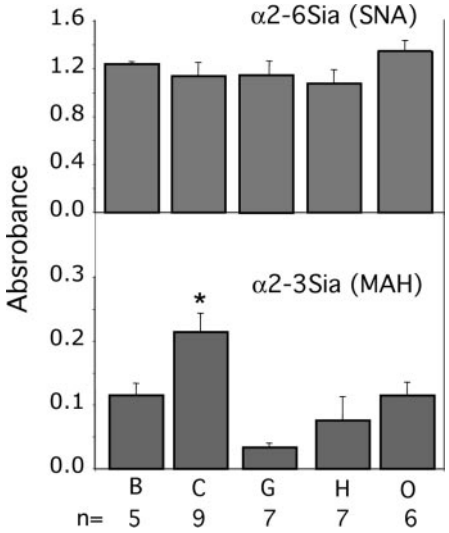

FIG. 4. **Comparison of α2–3- and α2–6-linked sialic acids in blood plasma proteins.** Mean absorbance for SNA and MAH staining in ELISA on plated plasma proteins from humans and great apes. *Bars* indicate mean ± S.E., and *asterisk* indicates significant difference for MAH binding between chimpanzee and human plasma (unpaired Student's *t* test, $p < 0.01$). *B*, bonobo; *C*, chimpanzee; *G*, gorilla; *H*, human; and *O*, orangutan.

lack of symptoms is surprising, given that chimpanzees are genetically so similar to humans. We suggest that this apparent attenuation of human influenza infection in chimpanzees is most probably because of the absence or low amounts of the receptor (α2–6-linked Sia) on their target respiratory epithelia as well as the abundance of the same structure on secreted mucins. Such mucins can act as soluble decoy for the virus, adsorbing it before it can reach the target cell surfaces.

We also address the general question of how much change in overall sialylation patterns occurs over millions of years of vertebrate evolution, *i.e.* the 13+ million years since the last common ancestor of great apes and humans (42). Our results indicate that while the expression pattern of α2–6-linked Sias has remained largely conserved during the independent evolution of the four great ape species, several changes have occurred during human evolution. Taken together with the other human-specific changes in Sia biology (see Introduction and discussion below), these findings suggest that the human lineage underwent one or more selective sweeps driven by Sia-binding pathogens.

If changes in externally exposed Sia residues are being driven by exposure to Sia-binding pathogens, one might expect internal structures to exhibit fewer changes among species because of less direct interaction with pathogens. However, the subepithelial stroma should be in an intermediate position because it is the next target following epithelial invasion by a Sia-binding pathogen. The question arises whether up-regulation of a structure in certain epithelia accompanied by down-regulation of the same structure in nearby stroma could allow the organism to generate spatial heterogeneity of receptor molecules to impede rapid systemic spread of Sia-binding pathogens. This possibility is partially supported by our findings that stroma appears positive for SNA in all of the hominoid species.

Once a Sia-binding pathogen has invaded past an epithelial surface and the surrounding stroma, it can enter the bloodstream. Here, it would interact with four general classes of Sia-bearing endogenous molecules: erythrocyte; leukocyte; and endothelial cell surface glycans; and soluble plasma proteins. With regard to erythrocytes, humans again appear to be unusual in strongly expressing α2–6-linked Sias (Fig. 3*B*). This fits with our prior suggestion that these non-nucleated cells can act as a "sink" to adsorb away viral agents such as human influenza A that cannot proliferate in them (35). With regard to plasma proteins, it appears that chimpanzees are the unusual species, having an increased level of α2–3-linked-Sias (Fig. 4). Again, it is presumed that these species-specific differences were selected by the regime of Sia-binding pathogens encountered in their past. Interestingly, endothelial cells in the vasculature of all of the hominoids appear to carry similar amounts of α2–6-linked-Sias (data not shown).

Ultimately, these changes reflect the consequence of altered action of sialyltransferases, the Golgi enzymes that attach Sia in different linkages to glycoconjugates destined for the cell surface or the extracellular milieu (6–8). The increase in the common α2–6 linkage of Sia to galactose on *N*-glycan side chains probably explains changes in SNA-staining in human cells. This Siaα2–6Gal structure is produced primarily by the enzyme ST6Gal-I, which is encoded by the highly conserved mammalian gene *SIAT1* (43, 44). Although a second ST6Gal isozyme has been recently reported (45), it is only expressed in the brain. The expression of the *SIAT1* gene is controlled by multiple promoter-driven alternatively spliced non-coding exons spanning a large genomic region upstream of the *SIAT1* gene (46). Apart from such spatial regulation of ST6Gal-I in different tissues (47), there is also well documented temporal regulation. In mammals, transient up-regulation occurs during acute phase reaction when the organism experiences trauma or infection (32, 33). This up-regulation ceases when the trauma or infection is resolved.

Altered regulation of the *SIAT1* gene seems a logical explanation for the human:ape differences presented here. Future studies need to determine the nature and content of the ST6Gal-I mRNAs in tissues from humans and great apes. This task is rendered difficult by the very limited availability of fresh non-fixed great ape tissues and by the very low levels of ST6Gal-I mRNAs in cells (we were unable to isolate full-length cDNAs from the few frozen ape lung samples available to us). Another difficulty is the very complex promoter region of *SIAT1*, which stretches over 160 kb of chromosome 3, including several 5′ non-coding exons alternatively transcribed to form highly regulated tissue-specific mRNAs (48). Furthermore, the same tissue may contain mRNAs from different cell types that are showing different levels of up-regulation or down-regulation (*e.g.* the ciliary epithelial *versus* goblet cells in the airway epithelium). On the other hand, it is difficult to carry out *in situ* hybridization studies on the limited samples available without knowing which of the many types of mRNAs might be present.

Whereas mice that are experimentally rendered null for several of the α2–3-sialyltransferases show compensatory sialylation because of the activity of related isozymes (49), ST6Gal-I null mice (50) completely lack Sia α2–6 linked to galactose (44). Despite this finding, they are viable and fertile and show only impaired B-cell function (that may be because of the loss of ligands for CD22/Siglec-2) (50). Thus, it remains a mystery why the sialylated structure produced by ST6Gal-I is so privileged as compared with the other related Sia-containing structures. In this regard, the selective forces responsible for the human-specific Sia linkage changes are also unknown. Regardless, these changes could be responsible for other disease susceptibility or phenotypic differences between great apes and humans.

At present, it is also not possible to determine whether this difference in Sia biology between humans and great apes is functionally related to all of the other changes reported to date: human *CMAH* gene inactivation and loss of Neu5Gc (51); the resulting excess of Neu5Ac; a point mutation eliminating Sia binding by human Siglec-L1 (26); human-specific increase in

ligands for sialoadhesin/Siglec-1 (28); and the change in expression and distribution of this Neu5Ac-preferring lectin on macrophages (28). Given that the human genome contains <60 known genes directly involved in Sia biology (52), it is reasonable to speculate that these are all related changes and that they represent the signature of one or more serious infectious challenges faced by our hominid ancestors sometime before the common origin of modern humans, ~100–200 thousand years ago. It also remains to be seen whether these differences are relevant to any other current day human-specific infectious disease patterns and whether any of these changes have any direct bearing on the evolution of other human-specific features that distinguish us from the great apes.

Finally, a recent study found that the rate of gene expression changes for numerous genes has dramatically changed in the brain but not in other tissues of the human lineage as compared with the rhesus monkey and the chimpanzee (53). In contrast, the results of this study suggest that a single gene (*ST6Gal-I*) has undergone up- or down-regulation in several human tissues.

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