

A Structural Difference Between the Cell Surfaces of Humans and the Great Apes

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ABSTRACT The sialic acids are major components of the cell surfaces of animals of the deuterostome lineage. Earlier studies suggested that humans may not express N-glycolyl-neuraminic acid (Neu5Gc), a hydroxylated form of the common sialic acid N-acetyl-neuraminic acid (Neu5Ac). We find that while Neu5Gc is essentially undetectable on human plasma proteins and erythrocytes, it is a major component in all the four extant great apes (chimpanzee, bonobo, gorilla and orangutan) as well as in many other mammals. This marked difference is also seen amongst cultured lymphoblastoid cells from humans and great apes, as well as in a variety of other tissues compared between humans and chimpanzees, including the cerebral cortex and the cerebrospinal fluid. Biosynthetically, Neu5Gc arises from the action of a hydroxylase that converts the nucleotide donor CMP-Neu5Ac to CMP-Neu5Gc. This enzymatic activity is present in chimpanzee cells, but not in human cells. However, traces of Neu5Gc occur in some human tissues, and others have reported expression of Neu5Gc in human cancers and fetal tissues. Thus, the enzymatic capacity to express Neu5Gc appears to have been suppressed sometime after the great ape-hominid divergence. As terminal structures on cell surfaces, sialic acids are involved in intercellular cross-talk involving specific vertebrate lectins, as well as in microbe-host recognition involving a wide variety of pathogens. The level of sialic acid hydroxylation (level of Neu5Ac versus Neu5Gc) is known to positively or negatively affect several of these endogenous and exogenous interactions. Thus, there are potential functional consequences of this widespread structural change in humans affecting the surfaces of cells throughout the body. *Am J Phys Anthropol* 107:187-198, 1998. © 1998 Wiley-Liss, Inc.

All living humans belong to one species with limited genetic diversity (Barbujani et al., 1997). Darwin and Huxley correctly surmised that, from the evolutionary point of view, humans are closely related to the great apes (Darwin, 1871; Huxley, 1863), and this was confirmed in modern times by biochemical, immunological and genetic data (Sarich and Wilson, 1967; Doolittle et al., 1971; Goodman et al., 1983; Caccone and Powell, 1989; Djian and Green, 1989; Sibley et al., 1990; Royle et al., 1994; Arnason et al., 1996;

Ruvolo, 1997; Takahata and Satta, 1997). Although there remains some controversy regarding the precise dating and taxonomic nomenclature, there is near general agree-

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ment regarding the evolutionary relationships amongst the great apes and humans. Indeed, humans can be considered specialized members of the great ape family, sharing an almost 99% genetic identity with *Pan troglodytes* (the chimpanzee) and *Pan paniscus* (the bonobo), which, in turn, seem to have a lesser genetic identity with *Gorilla gorilla* (the gorilla) and *Pongo pygmaeus* (the orangutan) (Arnason et al., 1996; Takahata and Satta, 1997; Ruvolo, 1997). The morphological and functional differences between humans and these great apes should therefore be based on very few changes in gene expression or function. However, to date no major differences in gene expression or molecular structure have been reported between humans and these closely related species.

One class of genes that has the potential to simultaneously affect many functions in many cell types are those mediating post-translational modifications such as glycosylation. Glycosylation is a general term to describe the attachment and modification of sugar chains (oligosaccharides or glycans) on proteins and lipids. This occurs while these macromolecules are being synthesized and transported through the endoplasmic reticulum (ER) and the Golgi apparatus (Varki and Marth, 1995). A cascade of sequential biochemical reactions results in the formation of glycoproteins and glycolipids, which can then present a dense and complex array of sugar chains on the surface of all cells in the body. There is now abundant evidence that the precise structure of these glycans varies in a tissue-specific and developmentally regulated manner (Varki and Marth, 1995), and that they can mediate or modulate a wide variety of biological functions (Varki, 1993). Unlike the case with DNA, RNA and proteins, glycan biosynthesis is not template driven, but follows an "assembly-line" type of mechanism (Varki, 1998). Thus, a change in a single enzyme involved in the ER-Golgi biosynthetic pathway can result in a marked change in the glycans of a large number of glycoproteins and glycolipids. Here we have studied the structure and expression of the sialic acids, a family of monosaccharide units that are attached to the outer ends of glycan chains on cell surfaces throughout the body.

The common sialic acid N-acetyl-neuraminic acid (Neu5Ac) is a structural component of most glycosylated molecules in all animals of the deuterostome lineage (Schauer, 1982; Varki, 1992; Kelm and Schauer, 1997; Troy, 1992; Ye et al., 1994). Being at the terminal (outer) position of many cell surface macromolecules, Neu5Ac can mediate cell-cell interactions via recognition by specific sialic acid-binding lectins (Varki, 1997; Kelm and Schauer, 1997; Crocker and Feizi, 1996) and by selectively modulating other interactions in many systems (Rutishauser and Landmesser, 1996; Rutishauser, 1996). For example, the selectins, CD22, CD33 and sialoadhesin are receptors that recognize sialic acid-containing ligands in the vascular, hematopoietic and immune systems, myelin-associated glycoprotein (MAG) recognizes sialylated ligands in the brain, and polysialic acids on the neural cell adhesion molecule can modulate neural plasticity. A variety of structural modifications and variations of Neu5Ac have been reported (Schauer, 1982; Varki, 1992; Kelm and Schauer, 1997). This results in a remarkable diversity in the types of sialic acids found on cell surfaces, which is further accentuated by many different types of linkages to the underlying sugar chain. This diversity in sialic acid structure and presentation is known to modulate sialic acid functions in endogenous intercellular cross-talk as well as in exogenous microbe-host recognition (Schauer, 1982; Varki, 1992; Kelm and Schauer, 1997; Troy, 1992; Ye et al., 1994).

One of the most common modifications of Neu5Ac is the hydroxylated form N-glycolylneuraminic acid (Neu5Gc), which arises from the addition of a single oxygen atom to the parent molecule (see Fig. 1). This oxygen atom is added by the enzymatic action of a specific hydroxylase which converts the nucleotide sugar donor CMP-Neu5Ac to CMP-Neu5Gc (Shaw and Schauer, 1988; Bouhours and Bouhours, 1989; Muchmore et al., 1989; Kozutsumi et al., 1990; Kozutsumi et al., 1991; Shaw et al., 1992; Kawano et al., 1993; Schneckenburger et al., 1994; Shaw et al., 1994; Takematsu et al., 1994; Kawano et al., 1995). The expression of Neu5Gc is widespread in mammalian cells (it can be the major sialic acid in some cell types), and shows tissue-specific and developmentally

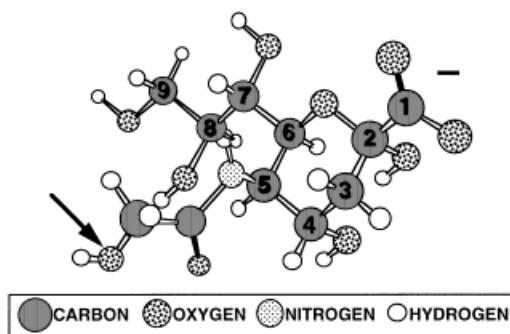


Fig. 1. α -N-glycolyl-neuraminic acid (Neu5Gc). Ball and stick model of the pyranose form of Neu5Gc, with the carbon atoms numbered. There is a negatively charged carboxyl group at the 1-position, and linkages to the underlying glycoconjugate (not shown) occur via the 2-position. The arrow points to the single additional oxygen atom that distinguishes Neu5Gc from its metabolic precursor, N-acetyl-neuraminic acid (Neu5Ac).

regulated expression in a variety of systems (Schauer, 1982; Bouhours and Bouhours, 1983; Muchmore et al., 1987; Muchmore, 1992; Varki, 1992). The addition of this single oxygen atom to Neu5Ac is also known to affect interactions involving several of the known endogenous and exogenous receptors for sialic acids such as CD22, myelin associated glycoprotein, sialoadhesin and the Influenza A virus hemagglutinin (Kelm et al., 1994; Powell and Varki, 1994; Collins et al., 1997a, 1997b).

Using techniques like thin layer chromatography (TLC), early workers noted that, in contrast to many other animals, human tissues did not show a detectable spot corresponding to Neu5Gc (Schauer, 1982). In keeping with this, the immune response of humans to the infusion of animal serum was shown to be partly directed against the Neu5Gc epitope on the infused animal glycoconjugates (Merrick et al., 1978; Fujii et al., 1982). Interestingly, a 1965 abstract suggested that the blood of some apes might contain Neu5Gc (Uhlenbruck and Schmitt, 1965), and another abstract suggested that Neu5Gc might be present in some human tissues.¹ It is also of note that some humans with malignancies or with certain inflammatory diseases can spontaneously develop an-

tibodies against Neu5Gc (Merrick et al., 1978; Nishimaki et al., 1979; Morito et al., 1982; Ohashi et al., 1983), and that human fetal tissues (Hirabayashi et al., 1987) and certain human tumors (Ohashi et al., 1983; Devine et al., 1991; Hirabayashi et al., 1987) are reported to contain small amounts of Neu5Gc. However, one study did not support the presence of Neu5Gc in human tumor cells (Furukawa et al., 1988). Also, most of these observations were made using mono-specific antibodies and/or TLC analysis. Analysis by TLC can be limited in sensitivity and specificity, and antibodies against carbohydrates can cross-react with other related structures. Meanwhile, despite a large amount of comparative work done in the past, to date there are no clearcut differences in the expression of gene products known between humans and the great apes. We therefore decided to explore the issue of Neu5Gc expression in humans and apes in a comprehensive fashion, using contemporary techniques.

METHODS

Extraction of biological samples for the detection and quantitation of Neu5Ac and Neu5Gc

Blood samples were collected with EDTA anticoagulant, stored and/or transported on ice for up to 24 hours, and then spun to separate the cells and plasma. The buffy coat was removed, and the erythrocytes (red blood cells) lysed in hypotonic buffer and washed to generate membrane "ghosts" (intact plasma membranes of the erythrocytes, washed free of internal contents). The blood plasma and erythrocyte ghosts were subjected to sequential lipid extraction and protease digestion to generate glycolipid and glycopeptide fractions. Chimpanzee and human tissue samples were collected at autopsy and stored and/or transported frozen prior to a similar extraction procedure (representing a simplified version of a complete tissue extraction protocol to be published elsewhere; A.E. Manzi et al., manuscript in preparation). Each fraction was then studied for the content and profile of sialic acids, by release from glycosidic linkage with mild acid treatment, followed by derivatization with DMB (1,2-diamino-4,5-methylenedioxy-

¹Schroder C, Nohle U, Shukla AK, and Schauer R. Improved methods for the isolation and structural analysis of trace amounts of new sialic acids: identification of N-glycolylneuraminic acid in man. Abstract 162 at the Seventh International Symposium on Glycoconjugates, 1983.

TABLE 1. N-Glycolyl-neuraminic acid in plasma and in erythrocyte membranes of humans and the great apes

	N-glycolyl-neuraminic acid (% of total sialic acids)			
	Plasma proteins		Erythrocyte ghosts	
	Glycopeptides	Glycolipids	Glycopeptides	Glycolipids
<i>Homo sapiens</i> (n = 8)	<0.1	<0.1	<0.1	<0.1
<i>Pan paniscus</i> (n = 5)	24	30	87	67
<i>Pan troglodytes</i> (n = 6)	28	39	79	63
<i>Gorilla gorilla</i> (n = 5)	21	27	90	69
<i>Pongo pygmaeus</i> (n = 3)	31	37	94	92

Glycolipid and glycopeptide fractions from plasma and erythrocyte ghosts were studied for the content and profile of sialic acids, as described in Methods. See Figure 2 for an example of the profiles obtained. All values are the mean of the results from the number of individuals indicated.

benzene dihydrochloride) and the detection of fluorescent adducts by reverse phase HPLC (Hara et al., 1989; Manzi et al., 1990). This method is characterized by a very high degree of specificity for the elution position of different sialic acids, as well as an excellent dynamic range (as little as 1% of one sialic acid can be detected in the presence of 99% of the other). In this study, base-labile O-acyl esters were removed by base treatment prior to DMB derivatization, to ensure complete recovery of Neu5Ac and Neu5Gc.

Cultured lymphoblastoid cells were washed twice in PBS pH 7.4, resuspended in 50 mM Hepes, pH 7.4, and lysed by repeated 5-second pulses with a Polytron homogenizer (Brinkmann). After a spin at 75g for 5 minutes to remove nuclear debris, the homogenate was ultracentrifuged at 100,000g for 1 hour. The membrane pellet was studied for content and profile of sialic acids by DMB derivatization and HPLC, as described above. Cerebrospinal fluid samples were analyzed directly, without extraction.

Assay of CMP-sialic acid hydroxylase activity in lymphoblastoid cells

Cultured lymphoblastoid cells were washed twice in PBS pH 7.4, resuspended in 50 mM Hepes, pH 7.4, lysed by repeated 5-second pulses with a Polytron homogenizer (Brinkmann) and the high-speed supernatant (cytosolic fraction) assayed for CMP-Neu5Ac hydroxylase activity. Microsomal cytochrome b₅ and b₅ reductases were prepared from mouse liver as follows: after homogenization in 50 mM HEPES, pH 7.4 with 0.25 M sucrose, 1 mM EDTA and leupeptin, the liver suspension was centrifuged at 10,000g for 20 minutes, and the supernatant layered onto a 0.5 M sucrose

cushion and centrifuged at 140,000g for 3 hours. The microsomal pellet was dialyzed against 50 mM Hepes, pH 7.4 with 10 mM DTT, 0.5% Triton-X-100 and the suspension centrifuged at 100,000g for 30 minutes. The supernatant extract was used for the assay. Enzyme reactions included 1 mM NADH, 40 µM CMP-[³H]Neu5Ac (0.1 µCi), 10 mM DTT, 0.5 mM FeSO₄, 100 µg murine liver microsomal extract, and the lymphoblastoid cell cytosolic extract to be tested, brought to final volume of 100 µL with 50 mM HEPES buffer, pH 7.4. After incubation for up to 1 hour at 37°C, the reactions were quenched by adding 600 µL ice-cold ethanol. After 15 minutes on ice, the tubes were centrifuged, the supernatant evaporated to dryness, resuspended in 70% ethanol and applied to Whatman 3MM paper for separation of [³H]Neu5Gc and [³H]Neu5Ac in 1-butanol:1-propanol:0.1 N HCl (1:2:1), as previously described (Muchmore et al., 1989).

RESULTS AND DISCUSSION

Analysis of sialic acids in blood samples and cultured lymphoblastoid cells

Blood samples from adult humans and great apes, as well as from some other adult mammals, were studied for their content and profile of sialic acids as described in the legend to Table 1. We first studied the glycoconjugates derived from erythrocyte cell membranes (representing the hematopoietic system) and from the blood plasma (representing the secretory output of the liver, immune system and vascular endothelium). The HPLC system used for analysis can separate the different types of sialic acids

based on their types of modification (Hara et al., 1989; Manzi et al., 1990). This method has a very high degree of specificity for the derivatization reaction and for the elution position of different sialic acids, as well as an excellent dynamic range (as little as 1% of one sialic acid can be detected in the presence of 99% of another). To avoid pattern complexities caused by O-acetylation of sialic acids, the samples were first treated with mild base to eliminate any O-acetyl esters (Manzi et al., 1990), and the sialic acids then released, derivatized and profiled, examining the ratio between Neu5Ac and Neu5Gc. An example of the results is shown in Figure 2. In the plasma glycolipids and glycoproteins of all the apes studied, the most common sialic acid was found to be Neu5Ac (see Table 1). The same was true in samples from several other mammals (cow, dog, cat, rat and horse; data not shown). However, in all the animals studied (including the great apes), Neu5Gc was also an easily detectable major component. In contrast, this hydroxylated sialic acid was undetectable in all the human samples. Neu5Gc also proved to be a major component of erythrocyte membrane glycolipids and glycoproteins of all the great apes (in many cases exceeding the amount of Neu5Ac; see Table 1). Once again, humans were the exception, being the only mammals in which erythrocyte membranes were free of Neu5Gc.

Many prior studies using less sensitive methods have mentioned the apparent lack of Neu5Gc in human samples and its immunogenicity has been noted in many humans. However, to ensure that the lack of Neu5Gc seen with the current more sensitive methods was not simply an intrinsic human polymorphism, we examined 60 additional blood samples obtained from humans representing a wide variety of ethnic and national origins. All samples were found to be deficient in Neu5Gc, making it very likely that this is a condition characteristic of all humans. Since we (and others in the past) have found Neu5Gc to be expressed in a wide variety of other mammals, it appears that the human deficiency is a derived condition of recent evolutionary origin. Thus, we did not attempt to obtain further data concerning the different subspecies of great apes.

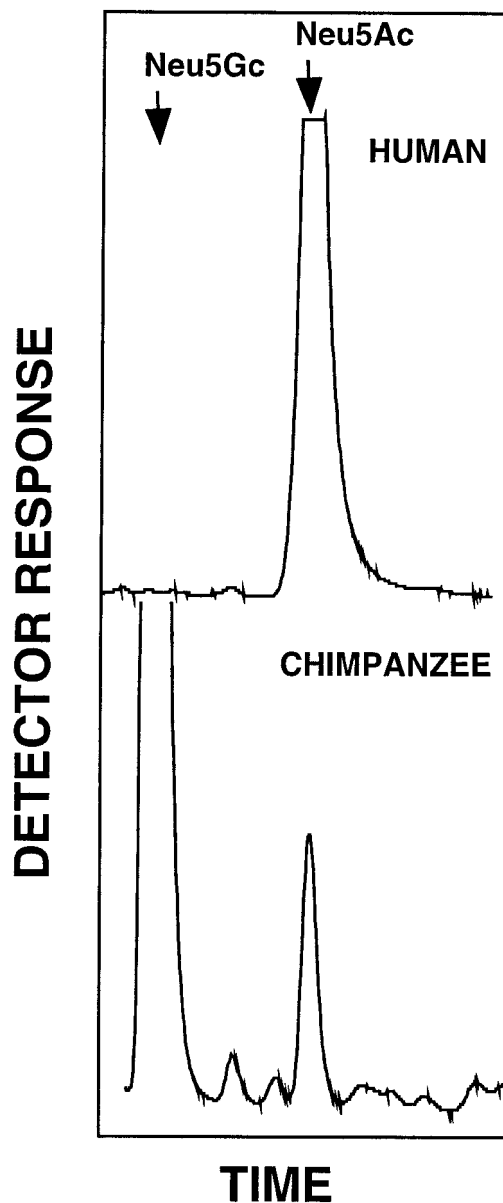


Fig. 2. HPLC detection and separation of Neu5Ac and Neu5Gc in glycoprotein fractions of human and chimpanzee erythrocyte membranes. Glycolipid and glycopeptide fractions from plasma and erythrocyte ghosts were studied for the content and profile of sialic acids, as described in Methods. Some peaks are off-scale because the detector sensitivity was deliberately adjusted to allow identification of low levels of Neu5Gc. See Table 1 for the results obtained with other related samples.

EBV-transformed lymphoblastoid cell lines derived from humans and great apes were also studied for the profile of membrane-bound sialic acids. All of the great ape cells

showed significant amounts of Neu5Gc expression (data not shown). The human cells also had low levels of detectable membrane-bound Neu5Gc, when cultured in fetal bovine serum (see Table 2). However, prior studies have indicated that human cells in culture are capable of incorporating sialic acids from fetal bovine proteins (Furukawa et al., 1988; Muchmore et al., 1989; Hubbard et al., 1994). Indeed, as shown in Table 2, continuous culture in serum-free media resulted in a complete loss of Neu5Gc expression from the human lymphoblasts, while the amount of Neu5Gc in the cells from chimpanzees actually increased under these growth conditions.

Comparison of sialic acids between other tissues of humans and chimpanzees

To see if this marked difference in Neu5Gc expression is a generalized feature and not just confined to blood cells and plasma, a variety of adult human and chimpanzee tissues were analyzed in a similar manner, preparing glycopeptides and glycolipids and studying the sialic acids released by mild acid hydrolysis. Again, Neu5Gc proved to be a major component in all the chimpanzee tissues (Table 3), and was very low or undetectable in most human tissues. Possible exceptions might be the human liver, spleen, heart and testis (Table 3), where small peaks corresponding to Neu5Gc comprised about 1-3% of the Neu5Ac peak area. One possible explanation for this peak in organs like the heart and liver is that it is as a derivative from ingested animal foods (Nohle et al., 1982). This matter needs to be investigated further, perhaps by comparing human omnivores to strict vegans. However, this may not explain the low levels of the Neu5Gc peak in organs like the testis. An alternative explanation is that humans retain the capacity to express small amounts of Neu5Gc. This would also fit prior reports of traces of Neu5Gc in malignant or fetal human tissue. Regardless, there is clearly a marked loss of overall Neu5Gc expression in humans, and this must have occurred after the time when humans shared their last common ancestor with the African great apes.

TABLE 2. *N-glycolyl-neuraminic acid content in cultured lymphoblastoid cells derived from Homo sapiens and Pan troglodytes*

	N-glycolyl-neuraminic acid (% of membrane sialic acids)	
	Growth in serum	Growth without serum
<i>Homo sapiens</i> 1	1.3	<0.2
<i>Homo sapiens</i> 2	1.0	<0.2
<i>Pan troglodytes</i> 1	39	50
<i>Pan troglodytes</i> 2	54	76

Membrane pellets from cultured lymphoblastoid cells were analyzed for the content and profile of sialic acids as described in Methods. Values represent two to five separate experiments.

TABLE 3. *N-glycolyl-neuraminic acid in tissues of Homo sapiens and Pan troglodytes*

Tissue	N-glycolyl-neuraminic acid (% of total sialic acids)			
	<i>Homo sapiens</i>		<i>Pan troglodytes</i>	
	Glycopep- tides	Glyco- lipids	Glycopep- tides	Glyco- lipids
Liver (n = 2)	<1	3	35	37
Heart (n = 2)	2	<1	35	43
Spleen (n = 2)	1	1	44	74
Testes (n = 1)	1	<1	26	22
Skin (n = 1)	<1	<1	45	52
Kidney (n = 2)	<1	1	38	38

Glycolipid and glycopeptide fractions from each tissue were studied for the content and profile of sialic acids, as described in Methods. All values are the mean of the individual determinations. In some human tissues, small peaks close to the limit of detection (~1-3%) were seen in the region expected for the Neu5Gc adduct.

Analysis of CMP-Neu5Ac hydroxylase activity in cultured lymphoblastoid cells

The primary donors for the addition of sialic acids to glycolipids and glycoproteins are the CMP-sialic acids. To explore the biochemical basis for this difference in Neu5Gc expression between humans and apes, we studied the activity of the CMP-Neu5Ac hydroxylase which converts CMP-Neu5Ac to CMP-Neu5Gc. Because of limitations in routinely obtaining fresh human and ape tissue samples for enzyme analysis, we used the EBV-transformed lymphoblast lines as a reliable source of cellular material for assaying the CMP-Neu5Ac hydroxylase. As shown in Figure 3, the hydroxylase activity was clearly detectable in extracts from the chimpanzee cells, but not in the human cells. This finding provides the most direct

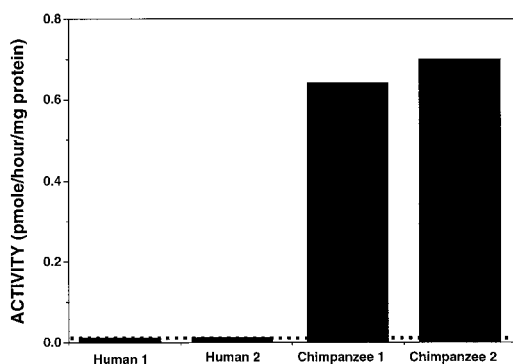


Fig. 3. Detection of CMP-sialic acid hydroxylase activity in lymphoblastoid cells of chimpanzees but not humans. Cells were extracted and the enzyme assays performed as reported in Methods. The dotted line indicates the lower limit of detection for the assay (0.05 pmole/min/mg protein). Values shown are representative of several experiments (mean values for chimpanzee 1 (n of 5) = 0.39, chimpanzee 2 (n of 5) = 0.58, humans 1 and 2 (n of 3) < 0.05).

explanation for the absence of Neu5Gc in human cells and tissues.

The genetic basis of this difference needs to be explored. A cDNA encoding the murine CMP-Neu5Ac hydroxylase has recently been cloned, and there is cross-hybridization of this murine cDNA to Southern blots of human genomic DNA (Kawano et al., 1995). Probing Northern blots with a 3' fragment of the mouse cDNA shows very low but detectable levels of messenger RNA in both the chimpanzee and human lymphoblastoid cells (unpublished observations). However, as with the mouse (Kawano et al., 1995), there appears to be substantial complexity in the number and sizes of the hybridizing transcripts. To elucidate the genetic basis for the difference in enzyme expression, it will be necessary to isolate and fully sequence all of the possible human and chimpanzee hydroxylase cDNAs, as well as the relevant regions of their genomes. Such work is currently underway in our laboratories. It is also necessary to examine the many other factors known to be involved in regulating the activity of the hydroxylase (Kozutsumi et al., 1991; Schlenzka et al., 1996; Kawano et al., 1993; Takematsu et al., 1994; Kozutsumi et al., 1990).

The difference in Neu5Gc expression is also seen in the nervous system

One of the areas of difference between humans and chimpanzees is in higher brain

functions. In this regard, it is interesting to note that the brain has the highest known concentration of sialic acids in mammalian tissues, and several roles have been suggested for sialic acids in brain development and function, including the modulation of cell membrane structure and growth factor function by gangliosides (Nagai, 1995), and the alteration of neuronal plasticity by polysialic acids (Rutishauser and Landmesser, 1996). However, some prior studies have claimed that Neu5Gc is not a component of the vertebrate brain, even in animals known to express this sialic acid in other tissues (Schauer, 1982). We therefore studied brain samples from adult human and chimpanzee brains using the approach described above. We did not detect Neu5Gc in the glycolipid fraction of the cerebral cortical samples of either species (data not shown; this probably explains prior reports describing the lack of Neu5Gc in the brain, which have all focused on the glycolipid fraction). In contrast, a significant amount of Neu5Gc (~3%) is present in the glycoproteins of the chimpanzee brain, but not human brain (data not shown). This Neu5Gc cannot have originated from cells or plasma trapped in the blood vessels of the chimpanzee brain sample, since such a contamination should have given an approximately equal amount of Neu5Gc in both the glycolipid and the glycoprotein fraction (see Table 1). However, because of the rarity of chimpanzee autopsies, we have only been able so far to study one specimen of previously frozen, unfixed chimpanzee brain. We therefore obtained multiple fresh samples of cerebrospinal fluid (CSF), the clear liquid that surrounds the brain. Indeed, we found that while chimpanzee CSF had easily detectable Neu5Gc (range of 9-16% in 10 different specimens; see examples in Figure 4), this molecule was undetectable in human CSF. Thus, the central nervous system of the chimpanzee does contain some Neu5Gc.

Conclusions and perspectives

It is reasonable to predict that humans have lost or gained some genetic and biochemical traits during the several million years of our unique evolutionary history. Given the nearly 99% identity of chimpanzee genome to that of the human, we suggest

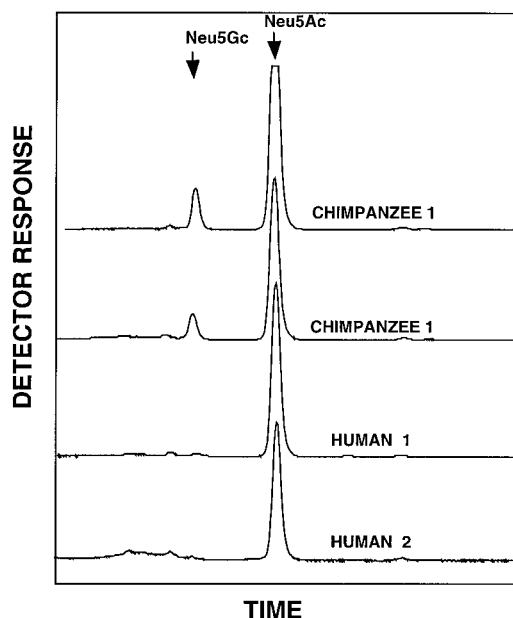


Fig. 4. Presence of Neu5Gc in the cerebrospinal fluid of chimpanzees, but not humans. Examples of profiles obtained from CSF samples subjected to direct analysis of sialic acids by DMB-HPLC, as described in Methods. Similar results were obtained with a total of six human and 10 chimpanzee samples.

that the number of significant differences are relatively few. Among the estimated 50-100,000 gene products derived from the genome, <1% of the amino acids are likely to be altered. Of these, the great majority are likely to be conservative amino acid substitutions which result in minor or qualitative functional changes. Thus, the actual frequency of major changes in gene structure and gene product expression/function is likely to be rather small. While we cannot place an upper bound on this frequency, we suggest that it is small enough that the present finding is of interest. Indeed, despite a large body of comparative work done to date, this represents, to our knowledge, the first report of such a clearcut difference in the expression of a gene product between humans and the great apes.

The question arises as to whether this represents a random enzymatic loss of limited biological consequence, or a change of major functional significance. In this regard, it is interesting to note that in support of the earlier observations utilizing monospecific

antibodies, Neu5Gc has recently been chemically demonstrated by mass spectrometry in certain human cancers (e.g., breast carcinomas) (Kawai et al., 1991; Devine et al., 1991; Marquina et al., 1996). Taken together with our current findings, and the prior indications for Neu5Gc expression in human fetuses (Hirabayashi et al., 1987), the picture emerges that the capacity to express this modification during adult life might have been specifically suppressed (rather than randomly eliminated) sometime after the existence of the last common ancestor of humans and apes. The precise genetic basis for this enzymatic and structural difference needs to be explored.

It also remains to be seen if this loss of expression of a common gene product can explain any of the major changes that occurred during hominid evolution, and thus any of the morphological and functional differences between humans and great apes. In this regard, it is worth mentioning the already known biological contexts in which lack of expression of Neu5Gc should make a specific functional difference to humans. There is no doubt that the presence or absence of Neu5Gc can markedly affect the interactions of several microbial pathogens with mammals. Thus, Influenza A and B viruses show distinct species preferences based upon Neu5Gc expression (Higa et al., 1985; Zimmer et al., 1992; Klotz et al., 1992; Ito et al., 1997; Suzuki et al., 1997; Schauer et al., 1988), and the lack of this sialic acid in humans could be one mechanism limiting the rate at which such viruses undergo interspecies transfer and reassortment (Matrosovich et al., 1997; Fitch et al., 1997; García et al., 1997). Likewise, humans appear to be immune to infection by *E. coli* K99, an intestinal pathogen of other animals that specifically binds a glycolipid containing Neu5Gc (N-glycolyl-G_{M3}) (Isobe et al., 1996; Ouadia et al., 1992; Kyogashima et al., 1989; Willemsen and de Graaf, 1993; Lanne et al., 1995). Since sialic acid residues on erythrocytes are critical for the binding and entry of *Plasmodium falciparum* merozoites, it is reasonable to suggest that the lack of high level transmission of this malaria agent between humans and chimpanzees (Ollomo et al., 1997) and the parallel

evolution of uniquely human and chimpanzee malaria agents (Escalante et al., 1995) might be due to the large difference in erythrocyte cell surface Neu5Gc expression. There are of course many other infectious agents that utilize cell surface sialic acids as specific components of their binding sites on mammalian cells (Karlsson, 1995; Varki, 1997), including major pathogens such as *Helicobacter pylori* (the proximate cause of most peptic ulcer disease and some gastric cancers). In most such instances, direct comparisons of the consequences of having Neu5Gc vs. Neu5Ac have yet to be done.

Apart from altering interactions with extrinsic microbial agents, can the loss of Neu5Gc explain intrinsic differences between humans and the great apes? The recent realization that sialic acids can constitute ligands for a variety of endogenous vertebrate lectins (Varki, 1994; Powell and Varki, 1995; Varki, 1997) suggests that the biological situation resulting from the loss of sialic acid hydroxylation may be very complex, and could affect the growth, development and function of multiple systems. For example, it is known that CD22 on B lymphocytes plays a critical role in the humoral immune response, and specifically binds sialic acid containing ligands. In this regard, it is interesting to note that mouse CD22 strongly prefers Neu5Gc, while human CD22 does not (Kelm et al., 1994). Studies of great ape CD22 are needed to ascertain if this change in binding preference was recent, and could explain the differences in the immune response of humans and apes to diseases such as hepatitis B and HIV (Krawczynski et al., 1979; Shouval et al., 1980; Shikata et al., 1980; Novembre et al., 1997). Another sialic acid-binding lectin functioning in the hematopoietic and immune system is sialoadhesin, which is found on the macrophages of the spleen, bone marrow and lymph nodes, and appears to be involved in specific interactions with certain leukocyte populations. It is known that despite the mouse having large amounts of endogenous Neu5Gc, mouse sialoadhesin specifically prefers Neu5Ac as a binding site (Kelm et al., 1994). If this binding specificity is similar in humans, there should be a dramatically increased frequency of sialoadhesin ligands

on human leukocytes. If so, this could also explain the known difference in blood leukocyte counts between humans and great apes (McClure et al., 1972a, 1972b), and/or differences in the human immune response. Perhaps the most interesting case of an endogenous sialic acid binding lectin with potential significance for human/ape differences is Myelin-Associated Glycoprotein (MAG), found on neuronal axon myelin sheaths. MAG belongs to the same class of I-type lectins as CD22 and sialoadhesin (the siglec family) and specifically recognizes sialic acids containing ligands, strongly preferring Neu5Ac over Neu5Gc (Collins et al., 1997b). Thus, the loss of Neu5Gc in the human nervous system is potentially associated with an increased level of ligands for MAG in the brain. It is possible that this can contribute to differential effects on the myelination rates and/or organization of the human and great ape brain. In this regard, it would also be interesting to identify which specific glycoproteins in the chimpanzee brain carry Neu5Gc.

Another area of interest is the reported occurrence of Neu5Gc re-expression in a variety of human cancers (Ohashi et al., 1983; Devine et al., 1991; Hirabayashi et al., 1987). Such re-expression is thought to explain the spontaneous occurrence of serum antibodies directed against Neu5Gc-containing epitopes that sometimes occurs in cancer patients. The molecular basis for this re-expression remains unexplained. Meanwhile, many previous studies have commented on the apparently low rate of neoplasms in many Old World primates in general and the great apes in particular (Schmidt, 1975; McClure, 1973). One hypothesis is that the expression of Neu5Gc has an unexplained protective effect against carcinoma progression in the great apes.

All of the above represent reasonable inferences and hypotheses about the consequences of Neu5Gc loss in humans, and each of course needs to be explored further by specific data-gathering. Since the biology of sialic acids is itself a relatively unexplored area, it is reasonable to predict that more such consequences will emerge in the future. From a more general point of view, it is noteworthy that most of the classic work on

the comparative biochemistry of humans and the great apes was done decades ago, when many modern tools and sensitive methods were not available. We suggest that it is time to revisit this general area with more vigor, searching for the specific biochemical changes that differentiate us from the great apes. Such information could then be correlated with the wealth of genetic data currently emerging from the Human Genome Project. This combined approach is necessary if we are to eventually understand the molecular basis for the evolution of some of the differences between ourselves and our closest evolutionary cousins.

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