Blood Group A Glycosyltransferase Occurring as Alleles with High Sequence Difference is Transiently Induced During a Nippostrongylus Brasiliensis Parasite Infection*

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Running title: Allelic blood group A transferase upon parasite infection
Neutral mucin oligosaccharides from the small intestine of control rats and rats infected with the parasite *Nippostrongylus brasiliensis* were released and analyzed by gas chromatography-mass spectrometry. Infected animals expressed seven blood group A-like structures that all were absent in the control animals. The blood group A nature of these epitopes was confirmed by blood group A reactivity of the prepared mucins of which Muc2 was one. Transferase assays and northern blotting on small intestines from infected animals showed that an \( \alpha\)-N-acetylgalactosaminyltransferase similar to the human blood group A glycosyltransferase had been induced. The expression was a transient event, with a maximum at day six of the thirteen days long infection. The rat blood group A glycosyltransferase was cloned, revealing two forms with an amino acid similarity of 95 percent. Both types had blood group A transferase activity and were probably allelic as none of twelve analyzed inbred strains carried both types. The second type was found in outbred rats and in one inbred strain. First generation offsprings of inbred rats of each type were heterozygous, further supporting the allelic hypothesis. The transient induction and the large allelic variation could suggest that glycosyltransferases are part of a dynamic system altering mucins and other glycoconjugates as a protecting mechanism against microbial challenges.
INTRODUCTION

The gastrointestinal tract is covered by a mucus layer, effectively protecting the epithelial cells from the hostile milieu including microorganisms and at the same time allowing digested nutrients and other smaller molecules to traverse. Microorganisms bind to intestinal mucus and epithelial cells of which some binding is with high specificity to glycan epitopes (1). To maintain the protective layer despite continuous damaging processes, especially by these intestinal microorganisms, a system with a continuous mucus renewal is demanded. In the intestine, a majority of the mucus is produced by the goblet cells interspersed between the enterocytes. The matrix in the mucus is made up of gel forming mucins. These are highly glycosylated proteins, where up to 80% of the mass is from O-linked oligosaccharides. Cloning and sequencing of mucin genes have revealed that the sites for glycosylation are located in domains of long stretches of protease resistant sequences rich in the amino acids serine and threonine together with proline. As these domains are typical for mucins, but also found in other proteins, they have been named mucin domains. Up until now, thirteen human mucin genes have been identified and fully or partly sequenced (2-4), in addition to mucins identified from other species. The major intestinal mucin in rat is the Muc2 mucin, and the two mucin domains of this mucin have previously been isolated as two highly glycosylated peptides, 650 kDa and 335 kDa, respectively (5,6). The rat Muc2, as well as its human homologue MUC2, belongs to a class of gel forming mucins, gaining their viscous properties by oligomerization into large mucin complexes via the formation of intermolecular disulfide bridges between mucin subunits (3,6).
The oligosaccharides clustered on the mucin domains show a remarkable diversity (7). These mucin oligosaccharides have a proximal GalNAc directly linked to serine or threonine of the peptide core. The GalNAc can be further glycosylated on both C-3 and C-6 and the extended branches often have blood group antigens as terminating structures. One of these is the blood group A antigen, Fucα1-2(GalNAcα1-3)Galβ1-, originally described as a blood group antigen present on human erythrocytes. Today it is well established that blood group A antigens also are present on other tissues, as well as in other animals as e.g. pig and rat (8). The blood group A epitope can be found both on glycosphingolipids and on various glycoproteins as N-linked or O-linked epitopes (9). Responsible for the expression of the blood group A antigen in human is the blood group A glycosyltransferase gene (10), encoding an α1-3GalNAc-transferase that acts in the final step of the biosynthesis this epitope, adding a GalNAc to the galactose of the blood group H epitope Fucα1-2Galβ1-. Most rat strains carry the blood group A determinant in the large intestine, whereas only a few do this in the small intestine (11,12). The expression of blood group A active glycosphingolipids appeared at weaning in a strain expressing this epitope in the small intestine (13).

In rats infected by the small intestinal parasite *Nippostrongylus brasiliensis*, an induced goblet cell hyperplasia has been shown, and at the time of expulsion (around day 13 of the infection) an increased amount of mucus has been found (14,15). Studies of lectin binding to tissue sections have indicated a glycosylation alteration on the secreted mucins during the infection in both normal and hypothyric rats (16,17). An increased reactivity with the Helix pomatia (HPA1) lectin, recognizing terminal GalNAc-α, was observed in the small intestine of infected rats.
In a previous study we reported glycosylation alterations among sialylated oligosaccharides on the rat Muc2 mucin during the \textit{N. brasiliensis} infection (18). Detailed structural characterization of sialylated Muc2 oligosaccharides prepared from small intestinal mucosal scrapings of infected rats at different stages of the infection revealed two events of altered glycosylation. The amount of bound NeuGc was decreased in favor for NeuAc during the infection, and a Sd\textsuperscript{a}/Cad-like terminal epitope, NeuAc/NeuGc\(\alpha\)2-3(GalNAc\(\beta\)1-4)Gal\(\beta\)1-, appeared late in the infection cycle. These events could be connected to the regulation of a NeuAc hydroxylase and a yet unidentified GalNAc-transferase. However, none of these alterations could explain the observed increased reactivity of the HPA lectin in infected rats (16,17).

In the present study we have analyzed the neutral oligosaccharides of the Muc2 mucin in the small intestine of \textit{N. brasiliensis} infected rats and found a transient induction of a blood group A-like terminal epitope due to the expression of a blood group A-type GalNAc transferase. Two alleles with a remarkably high sequence difference were found.

**EXPERIMENTAL PROCEDURES**

\textit{Infection of rats by the nematode N. brasiliensis and isolation of the small intestinal ‘insoluble’ mucin complex} – Inbred GOT-W (8,11) or outbred Sprague-Dawley rats (300-350 g, B&K Universal, Sollentuna, Sweden), both blood group A-negative in the small intestine and blood group A-positive in the large intestine, were infected subcutaneously with \textit{N. brasiliensis} third-stage larvae (19). Rats were killed by decapitation after ether anesthesia and the mucosae from the small intestine was collected and stored frozen at -80°C or immediately homogenized in 6.0 M
guanidinium chloride as described (5). The pellets collected after centrifugation were repeatedly extracted (2-4 times) in the above buffer and solubilized by reduction and alkylation (5). These samples, referred to as ‘insoluble’ mucins, were dialyzed against water and lyophilized.

Release and isolation of neutral mucin oligosaccharides – Oligosaccharides were released from the ‘insoluble’ mucins (5 mg), using 0.05 M KOH containing 1.0 M NaBH₄ (5). Neutral oligosaccharides were isolated after applying the oligosaccharide mixtures to a DEAE-Sephadex A-25 column and eluting with methanol (20).

GC and GC-MS of permethylated oligosaccharides – Permethylation of the oligosaccharides was done using methyl iodide and NaOH in DMSO (21,22). GC and GC-MS were performed using 11-12 m columns (0.25 mm i.d.) with 0.02-0.04 µm of cross-linked PS264 prepared as described (20). Temperature program and parameters as described (23).

Immunochemical detection of oligosaccharide epitopes and apomucin – Reduced and alkylated small intestinal ‘insoluble’ mucins were coated onto microtiter plates and washed and blocked as described (24). The primary antibodies used (100 µl) were the MAbs anti-blood group H diluted 1:50 (DAKO, Copenhagen, Denmark), anti-blood group A diluted 1:50 (DAKO), anti-blood group A-type 2 (A005) diluted 1:50 (Monocarb, Lund, Sweden), and anti-Tn (1E3) hybridoma supernatant diluted 1:10 (25). Lectin binding assay was performed using biotinylated HPA (Sigma) in concentration 1 µg/ml. For immunochemical detection of Muc2, ‘insoluble’ mucins
(1-5 µg) were coated onto microtiter plates by a slow evaporation of a water solution at 37°C for 12 h. Alternatively, 0.5 µg of insoluble mucins (100 µl PBS) were incubated for 3 h in microtiter wells pre-coated with 1 µg HPA (Sigma). The plates for detection of Muc2 were further dried in an exicator for 2 h, and treated with gaseous HF in an HF-apparatus (Peptide Institute, Tokyo, Japan) for 18 h at room temperature. The samples were recoated in their original wells, by adding 100 µl of PBS, incubating for 24 h at 37°C. Washing and blocking was performed as described above, followed by incubating for 2 h with anti-gpA polyclonal rabbit antiserum (diluted 1/100) (26). Fluoroimmunoassays and lectin assays were performed as described (24) using secondary goat anti-rabbit antiserum, and goat anti-mouse antiserum (Jackson Immunoresearch, West Grove, PA), or streptavidin, all labeled with europium.

Measurement of blood group A transferase activity in small intestinal homogenates - A 30 cm piece was cut 20 cm proximal to the distal end of the small intestine from infected animals. Mucosal scrapings were collected and homogenized and the blood group A transferase activity was measured as described (23) using 65 mg (wet weight) of the pellet reconstituted in PBS and incubated together with LNF-1 and UDP-[14C]GalNAc. The enzyme activity was measured as the retarded fraction of radioactively labeled material from a blood group A affinity column (23).

Genomic screening and northern blot analysis – An EMBL-3 rat genomic DNA phage library (Clontech) was screened with a cDNA probe (P718), derived from the human blood group A glycosyltransferase (10) and corresponding to nucleotides 148-865 of the human cDNA sequence. Positive recombinants were purified, digested with EcoRI
and analyzed by Southern Blot using the P718 probe. Two hybridization-positive fragments (4.0 and 3.5 kb) were obtained and digested by HinfI. The products were ligated into the pUC18-vector and subjected to DNA sequencing. One clone, from the 4.0 kb fragment digest, contained an insert of 406 bp, of which a stretch was 84.4% similar to exon six of the human blood group A gene coding sequence. A 129 bp DNA probe (P129) was made by PCR amplification from rat genomic DNA with primers derived from the candidate exon sequence. mRNA was prepared as previously described (6) from a 20 cm section of the proximal part of the small intestine from infected animals, electrophoresed, blotted and probed with the \(^{32}\)P-labeled P120 DNA probe.

Both hybridization-positive fragments (4.0 and 3.5 kb) were also ligated into the pBluescript II SK- vector and subjected to DNA sequencing. All 7.5 kb were sequenced and submitted to Genbank (#AF296761). The two fragments were shown to be adjacent in the rat genome by sequencing of the uncleaved genomic clone in the junction region. Genomic DNA sequencing was performed with the Cy™ 5 Thermo Sequenase™ Dye Terminator Kit (Amersham-Pharmacia, Uppsala, Sweden). The reactions were analyzed on an ALF Express using the ReproGel™ Long Read (Amersham-Pharmacia).

cDNA cloning and construction of expression plasmids – The 7.5 kb rat genomic DNA sequence was compared with the seven exons of the human blood group A glycosyltransferase (27,28) and was found to contain regions similar to human exons 3 to 7. From this, a partial cDNA sequence of the rat transferase was proposed, containing the stop codon in exon 7. To obtain the sequence of the 5’ terminus, a 5’ RACE was performed with the SMART™ RACE cDNA Amplification Kit.
(Clontech, Palo Alto, CA), according to manufacturers instructions. The 5’ RACE primer (GH137A) was constructed from the rat genomic region similar to human exon 5. Template was small intestinal mRNA, prepared as previously described (6) from an infected outbred rat (day 6 of infection). In parallel, a second 5’ sequence was obtained from Genbank™, accession number AF264018. Two cDNA clones, denoted A(1) and A(2), were constructed by RT-PCR on mRNA from colon of inbred GOT-W rats for A(1) or on the same mRNA used for the 5’ RACE for A(2), with primer pairs GH170B/GH170R for A(1) and GH170A/GH170R for A(2). Resulting PCR fragments were cloned into the pcDNA3.1 expression vector, using the pcDNA 3.1 Directional TOPO Expression Kit (Invitrogen, Carlsbad, CA), to obtain the expression plasmids pA(1) and pA(2). Sequencing of the 5’ RACE clones and cDNA clones were done by GATC Biotech AG (Constance, Germany) or MWG-Biotech AG (Ebersberg, Germany).

**DNA transfection and immunostaining** – The expression plasmids pA(1) and pA(2) were transiently transfected into HeLa cells, using the Geneporter transfection system (Gene Therapy Systems, San Diego, CA) according to manufacturer’s protocol. Seventy-two hours after transfection, the cells were immunostained for blood group A epitopes on their cell surface. Cells were fixed with 2 % paraformaldehyde in PBS, blocked with tissue culture medium containing 10 % fetal bovine serum, incubated for 2 h in RT with or without monoclonal anti-blood group A antibody A003 (Monocarb, Lund, Sweden) diluted 1:5 in serum-containing medium, washed and incubated for 1 h in RT with FITC-conjugated rabbit anti-mouse antibody (DAKO) diluted 1:100 in serum-containing medium. After the staining, cover slips were mounted with Pro-Long antifade (Molecular Probes Inc., Eugene, OR) for fluorescence microscopy.
Preparation of genomic DNA from mucosal scrapings – To obtain genomic DNA from the same rats as mRNA, a method was developed to prepare genomic DNA by extraction from previously collected intestinal mucosal scrapings stored at -80°C. Approximately 250 µl of mucosal scrapings were dissolved without homogenization in 10 ml of guanidinium thiocyanate with 1 % mercaptoethanol. After incubation at RT for 4 h, 0.5 ml of 10 % laurylsarcosinate was added to the mixture, followed by 2 M KAc pH 5.5 (0.5 ml) and 1 M HAc (0.8 ml). Room temperature 99.5 % ethanol was added while shaking and the DNA was precipitated in -20°C for a minimum of two hours and then centrifuged at 10000×g, +4°C, 20 min. The pellet was dissolved in 5 ml 7.5 M GuHCl with 10 mM DTT added. DNA was precipitated again by incubation in -20°C after the addition of 2 M KAc pH 5.5 (0.25 ml) and RT 99.5 % ethanol (12.5 ml). Centrifugation and precipitation was repeated once. After an additional centrifugation, the DNA was dissolved in 1.4 ml of DNA extraction buffer (100 mM NaCl, 1 % SDS, 100 mM EDTA, 50 mM Tris-HCl, pH 8.0) with proteinase K added (0.5 µg/µl). Samples were incubated at 56°C over night and DNA was precipitated by the addition of one volume of isopropanol. DNA was spooled around a pipet tip, transferred to a new tube and dissolved in 10 mM Tris-HCl pH 8.0.

PCR analysis of relationship between rat A(1) and A(2) glycosyltransferases - Primer pairs specific for rat A(1) (GH158C/GH183C) or rat A(2) (GH158A/GH183A) were constructed. PCR was performed on the expression plasmids pA(1) and pA(2), on genomic DNA prepared from small intestinal mucosal scrapings from the same rats, on genomic DNA prepared from inbred rat strains BN/Mol, COP/Mol, DA/Mol,
F344/Mol, GK/Mol, LE/Mol, LEW/Mol, NEDH/Mol, PVG/Mol, SPRD-Cu3/Han, WF/Mol and WKY/Mol, and on genomic DNA from an animal from an F1 cross between DA and LEW. The GH158C/GH183C and GH158A/GH183A primer pairs were also used in combination with primers for β-actin in a multiplex PCR on cDNA libraries derived from small and large intestinal mRNA of infected rats. The β-actin primers were TGG CCT TAG GGT GCA GGG GG (3’ oligo) and GTG GGC CGC TCT AGG CAC CA (5’ oligo) with an expected amplified fragment length of 270 bp. For PCR, an annealing temperature of 70°C was used, the Mg²⁺ concentration was 2 mM and PCR was performed over 25 or 30 cycles on a GeneAmp PCR system 2400 machine (Perkin Elmer, Foster City, CA).

RESULTS

The effect of the N. brasiliensis infection on the expression of intestinal mucins – Rats were infected with the intestinal dwelling parasite N. brasiliensis. The two rat types used, the inbred GOT-W strain (Sprague-Dawley type) and outbred Sprague-Dawley rats, are blood group A negative in the small intestine and blood group A positive in the large intestine. The parasite was introduced subcutaneously, and migrated via the lungs to the small intestine, where it remained until expulsion after twelve or thirteen days of infection. Mucosal scrapings from Sprague-Dawley rats were collected at different stages of the infection, and the ‘insoluble’ mucin complex (5) was isolated after repeated extraction with guanidinium chloride. As described before, the amount of collected ‘insoluble’ mucins increased up to day 12-13 and then quickly reverted to normal after parasite expulsion (18).
Analysis of released neutral oligosaccharides by GC and GC-MS – Oligosaccharides were released from the ‘insoluble’ mucins collected at different stages of the infection. The neutral oligosaccharides were isolated as the non-retarded fraction from an anion exchange column. After permethylation, the oligosaccharides were analyzed by high temperature GC and GC-MS. Infected Sprague-Dawley rats showed not only the normally expressed compounds, but also seven additional oligosaccharides appearing transiently during the infection. In Fig. 1, the gas chromatograms of the permethylated oligosaccharides from day 0 and day 7 are shown. Structural characterization by GC-MS (Table I) revealed that the seven induced oligosaccharides all had a terminal trisaccharide epitope similar to the blood group A determinant GalNAcα1-3(Fucα1-2)Galβ1-. The general fragmentation features of permethylated oligosaccharides are described by the mass spectrum of the blood group A-type structure 5.2 (Fig. 2 and Table I), deduced to be a branched pentasaccharide containing an A-type 1 structure by the fragment ions at m/z 189 (Fuc-), m/z 260 (HexNAc-), m/z 638 (Fuc-(HexNAc-)Hex-) and at m/z 883 (Fuc-(HexNAc-)Hex-HexNAc-). The fragment ion at m/z 693 is due to further fragmentation of the ion m/z 883 by the loss of a fucose unit (m/z 189) and an additional proton. This type of fragmentation is indicative for type 1 chains (Galβ1-3GlcNAcβ1-) as is the lack of a fragment ion at m/z 182 (29).

Induction of the blood group A-epitope on the rat Muc2 mucin – To establish that the induced oligosaccharides found by GC-MS were connected to the blood group A-activity of these samples, the corresponding ‘insoluble’ mucins were coated into microtiter wells and tested for their reactivity with blood group A specific antibodies (Fig. 3A). The binding of the HPA lectin (GalNAc-α binding) was found to coincide
with the blood group A-activity (Fig. 3A) and the appearance of blood group A-type oligosaccharides in the gas chromatograms. No obvious change of the amount of GalNAcα1 was found in the GC-MS and no reactivity with a MAb against the Tn-antigen (GalNAcα1-polypeptide) was detected (Fig. 3A), thus it was less likely that the increased HPA-binding was due to the alteration of Tn-antigen level. When the experiment was repeated by infecting a second set of rats (Infection 2), the induction of blood group A-activity occurred slightly later (Fig. 3B).

To show that the Muc2 mucin carried the induced HPA reactive epitopes, the lectin was used as a specific catcher. After HF-deglycosylation, bound material reacted strongly with the anti-gpA antiserum, which reacts to rat Muc2 (6), on days with high HPA-activity (Fig. 3C). Furthermore, staining of tissues from the small intestine of an infected animal using the anti-blood group A antibody showed that the Muc2 secreting goblet cells were strongly stained, as were the enterocytes suggesting that not only Muc2 carried the induced blood group A epitopes (not shown).

**Induction of blood group A glycosyltransferase** – To analyze the enzymatic background of the induced blood group A-activity, an enzyme assay was performed. Mucosal scrapings were homogenized and incubated with the blood group H substance LNF-1 and UDP-[14C]GalNAc. The enzyme activity was measured as the retarded fraction of radioactive material on a blood group A affinity column (23). As expected, small intestinal homogenates from infected animals (days 3-12) produced radioactive blood group A containing oligosaccharides while non-infected animals (day 0) did not (Fig. 4). However, the highest enzyme activity was found four days before the maximum blood group A-activity (compare Fig. 3B and Fig. 4), indicating an accumulation of blood group A-substances during this period.
To identify the induced glycosyltransferase, and to study the expression of the gene, a northern blot analysis was performed with mRNA prepared from the small intestine of infected rats. The mRNA blot was probed with a 129 bp DNA probe (P129), PCR amplified from rat genomic DNA. This sequence of rat genomic DNA could code for a sequence of 44 amino acids with 90.9% homology to the human blood group A glycosyltransferase (10). The northern blot (Fig. 5) showed two transcripts, with estimated sizes of 3.1 kb and 1.8 kb, that were transiently up-regulated during the infection. The expression coincided with the enzyme activities in the tissue homogenates (compare Figs. 4 and 5), indicating that the northern blot revealed the enzyme responsible for the blood group A transferase activity. The control, small intestinal mRNA isolated from an inbred rat strain (GOT-BW) constitutively expressing blood group A-antigens in the small intestine, showed the same two transcripts. The presence of two mRNA bands is relatively common for glycosyltransferases and also observed for the human blood group A glycosyltransferase (10).

Cloning of rat blood group A glycosyltransferases – To further study the induced blood group A glycosyltransferase the gene was cloned. However, during the cloning process not only one but two cDNA sequences containing a P129 similar sequence were found (Fig. 6). These cDNAs, denoted rat blood group A glycosyltransferases 1 and 2, or shortly A(1) and A(2), had a sequence similarity of 94.9% at the nucleotide and 95.1% at the amino acid level and were about equally similar to the human blood group A glycosyltransferase (Fig. 7). The sequences were submitted to the Genbank database (accession numbers AF469945 and AF469946). A(1) was almost identical
with the rat sequence AF264018, already in Genbank\textsuperscript{TM}, the only difference being a mutation A110G, giving rise to the amino acid exchange Q37R.

The exon structure of the rat blood group A glycosyltransferase (Fig. 6) was determined by comparing the A(1) cDNA sequence with A(1) containing rat genomic DNA (Genbank\textsuperscript{TM} accession numbers AF296761, AF469947 and AF469948). All exon-intron boundaries conformed to the GT-AG consensus rule (not shown), and their number and localization were similar to the human blood group A glycosyltransferase gene (27,28).

\textit{Confirmation of blood group A transferase activity} – To analyze if both cloned genes encoded functional blood group A glycosyltransferases, expression plasmids were constructed for the A(1) and A(2) transferases. These constructs were transiently transfected into HeLa cells, normally expressing blood group H epitopes on their cell surface. Transfected cells expressed blood group A epitopes as revealed by immunostaining with an anti-blood group A antibody (Fig. 8). This was observed for both A(1) and A(2). No reactivity was observed on non-transfected HeLa cells (Fig. 8) or on transfected cells stained with secondary antibody alone (not shown). From this it can be concluded that both A(1) and A(2) are functional blood group A glycosyltransferases. This conclusion is further supported by the \textit{in vivo} observation that expression of either form in rat small or large intestines results in blood group A expression on Muc2.

\textit{The two blood group A glycosyltransferase cDNAs represent two allelic forms of one gene} – To elucidate if A(1) and A(2) represented two different genes – as suggested by the relatively large difference in amino acid sequence (4.9 \%) between the A(1) and
A(2) transferases – or two alleles of one gene, PCR primers were constructed that were specific for A(1) and A(2), respectively. To obtain specificity, the primers were designed from regions of A(1) and A(2) showing several nucleotide substitutions, and to have the most 3’ nucleotide different. The specificities of the primer pairs GH158C/GH183C for A(1) and GH158A/GH183A for A(2) were confirmed in a PCR-experiment where both pairs were used on the pA(1) and pA(2) expression plasmids (Figs. 6 and 9A). The A(1)/A(2) specific primer pairs were used in RT-PCR on mRNA from the small and large intestine of outbred Sprague-Dawley rats six days after infection with *N. brasiliensis* (large intestine is shown in Fig. 9B). That the same allelic forms are expressed in the small and large intestine has been shown several times. One rat was found to express both A(1) and A(2), while the other one only expressed A(2). To investigate if this difference was also reflected at the genomic level, PCR was performed on genomic DNA from these rats (Fig. 9C). Both A(1) and A(2) were detected in DNA from the rat expressing both transferases, while only A(2) was detected in the DNA from the rat only expressing A(2). These results suggest that A(1) and A(2) are alleles of one gene. To further support this, a survey of inbred rat strains was performed. The A(1)/A(2) specific PCR was performed on genomic DNA from twelve available rat strains. Only one, the DA strain, was A(2)-type, while all the others were A(1)-type (Fig. 9D). No inbred strain carried both A(1)- and A(2)-type transferases, which is consistent with the allele hypothesis. Support for this was also obtained when an animal from an F1 cross between DA (A(2)-type) and LEW (A(1)-type) was shown to be heterozygous (Fig. 9E).
DISCUSSION

We have detected a qualitative modification of the terminal epitopes of the neutral oligosaccharides expressed on the Muc2 mucin in the small intestine of rats infected with the parasitic nematode *Nippostrongylus brasiliensis*. The enzyme responsible for this modification, the rat blood group A glycosyltransferase, was identified and subsequently cloned.

The *N. brasiliensis* infection is a model for studies of mucosal dynamics, as both quantitative and qualitative changes of the mucus occur during the infection. The detailed characterization of sialylated Muc2 oligosaccharides from *N. brasiliensis* infected rats in a previous study revealed two events of an altered glycosylation (18), but it did not explain the reported increased HPA lectin reactivity in the small intestine of infected rats (16,17). When neutral mucin oligosaccharides were released and characterized, seven additional oligosaccharides were found in the infected animals, besides the normally expressed ones. These seven oligosaccharides were all terminated by the HPA-reactive blood group A-type terminal epitope, GalNAcα1-3(Fuc1-3)Galβ1-. The appearance of this epitope was a transient event as revealed by anti-blood group A antibody reactivity and HPA-binding. Moreover, the anti-blood group A reactivity and the HPA reactivity matched perfectly over the time-course of the infection. This, together with the observation from the structural characterization and the lack of Tn-reactivity, is supporting that the blood group A epitope is the sole HPA epitope appearing during this infection.

The maximum level of HPA binding mucins has previously been found at the time of parasite expulsion (16,17). However, here it was observed that this peak in expression could vary in time between different infections. Since the infectability of
the parasite can vary and the amount of injected larvae was only roughly estimated, one can suggest that the times for the maximal expression of blood group A epitopes could depend on the dosage of infectious larvae. Earlier studies (16,17) have indicated that the qualitative changes in mucin glycosylation might be critical for the expulsion of the worms, although their lectin binding studies only offered limited information regarding the structural basis for the observed glycosylation alterations. The refined structural and temporal characterization described here suggests that the blood group A epitopes are not directly involved in the expulsion. The blood group A activity varied in time between different experiments with the same expulsion time, and the blood group A activity had already disappeared at the time for parasite expulsion. Furthermore, just as the GOT-W rats and the Sprague-Dawley rats studied here recovered from the infection after thirteen days, so did an inbred strain (GOT-BW) constitutively expressing blood group A-antigens in the small intestine (23), as well as an inbred strain (WKY) where the blood group A epitopes were not induced by the *N. brasiliensis* infection (not shown). Rather than being directly involved in the parasite expulsion, one can speculate that the induction of this terminal epitope is one step in a pre-programmed mechanism, perhaps unspecific to the parasite, to change the oligosaccharide pattern as a response to a pathogen in the intestine.

The appearance of blood group A epitopes during the infection can be explained by the addition of an \(\alpha_1-3\)-linked GalNAc to the Fuc1-3Gal\(\beta_1\)-carrying oligosaccharides normally expressed. In the human, the only enzyme known to catalyze this reaction is the blood group A glycosyltransferase (10). An enzyme assay using homogenized small intestinal cell scrapings showed a transient appearance of an activity as for a glycosyltransferase carrying out the same reaction as the human blood group A transferase. The maximal enzymatic activity preceded by three to four days
the maximal level of mucin blood group A epitopes. The reason for the delayed appearance of the products is probably the slow turnover and the storage of the Muc2 mucin in goblet cells (30). A similar delay between mRNA regulation and its effect on Muc2 in the mucosae was observed in a previous study of sialylated Muc2 oligosaccharides from *N. brasiliensis* infected rats. (18).

The cloning strategy for the induced glycosyltransferase was based on the assumption that the activated enzyme was homologous to the human blood group A glycosyltransferase. Probing with a part of the human cDNA a rat genomic library was screened and a 7.5 kb fragment was isolated. Sequencing showed that this genomic sequence contained regions similar to the exons 3 to 7 of the human transferase. To confirm that the gene was responsible for the blood group A transferase activity during the infection, a short piece, chosen for its similarity to human exon 6, of the gene was used to probe a northern blot of small intestinal mRNA from infected rats. The transcripts were found to be transiently expressed at the same time as the blood group A transferase activity was observed, suggesting that the probe corresponded to the blood group A glycosyltransferase. During the cloning process, two different cDNA sequences (named A(1) and A(2)) were found and shown to encode active blood group A glycosyltransferases.

In human, allelic polymorphism of the blood group A glycosyltransferase alters its donor specificity, a phenomenon that is the mechanistic background to the ABO blood group system. Four amino acid substitutions changes the specificity for donor nucleotide from GalNAc (making blood group A) to Gal (making blood group B), converting the enzyme into a blood group B glycosyltransferase (31). In mouse, the corresponding transferase was recently cloned and reported to have *cis-AB* activity, i.e. accepting both GalNAc and Gal as donor (32). These examples imply that
the donor specificity of the enzyme is sensitive to small changes of certain amino acids, and the positions 266 and 268 in the human gene have been suggested to be critical (33). Comparing sequences of different members of the ABO glycosyltransferase family suggests that substitution of Gly (A) or Ala (B) at position 268 of the human transferase is particularly important for the donor specificity (Fig. 7B). A Gly seems to give an absolute GalNAc specificity, while an Ala in most cases gives Gal specificity, sometimes with a degree of GalNAc tolerance. The mouse gene is more similar to the Gal specific human blood group B transferase at these positions (32), suggesting that the mouse enzyme could be regarded as a blood group B transferase with A activity. In rats, both blood group A and B epitopes are found (8,12,34), but their expression and tissue distribution do not indicate an allelic system similar to the human. No blood group B epitopes were found on small intestinal Muc2 from rats expressing the blood group A glycosyltransferase, suggesting that this transferase has no in vivo blood group B activity. Further arguments for this is found in the amino acid sequence of the rat transferase. Three of the four amino acid positions, including the important Gly (human position 268) that differ between the human blood group A and B transferases, is conserved between rat A(1), rat A(2) and the human blood group A enzyme. Although the rat transferase is overall more homologous to the mouse transferase, it still seems to be more similar to the human A transferase regarding its function, most probably due to these important amino acids.

The relatively large difference in amino acid sequence between the rat A(1) and A(2) glycosyltransferases suggested that these could be two different genes. However, when PCR was performed with A(1)/A(2) specific primers on mRNA and genomic DNA from infected outbred rats, the results were in line with those of two allelic types of one gene. The A(1)/A(2) profiles at the mRNA and genomic levels of
individual rats were identical, and not all rats carried both types of transferases in their genome. Furthermore, none of the twelve investigated inbred strains were typed for both A(1) and A(2), and when an A(1)-typed strain (LEW) was crossed with an A(2)-typed strain (DA), the offspring was heterozygous. These results suggest that A(1) and A(2) are allelic forms of one gene. A second alternative, that A(1) and A(2) are different genes that are absent in certain individuals due to gene deletions, is less likely as then the same gene deletion (A(2)) should have occurred in eleven out of twelve inbred strains, whereas the A(1) gene should have been deleted in the only strain carrying the A(2) gene.

Comparing the two allelic forms of the rat blood group A glycosyltransferase, they differ at sixteen out of 348 amino acids, more than expected for alleles. Studying the localization of the differing amino acids it is observed that half of them are clustered in a small region apart from the catalytically active domain, less conserved between species. Despite the differences in amino acid sequence, no striking functional difference is observed either in vivo in the rats or in their capacity of converting HeLa cells into blood group A active ones. Both alleles are induced in the same way during the infection, indicating similar regulatory promotor regions. The relatively large difference between the two allelic forms indicates that the gene is subjected to a low selection pressure for conservation or maybe even that there is a pressure for variation in the sequence. One can argue for some rational in this, as the much shorter generation time of the microbes challenging us at our epithelial surfaces favors a defense system based on structural variability. As the glycosyltransferases are important in assembling the molecules covering the mucosal surfaces, a high diversity between them might be important for achieving this structural variability. One can speculate that the development of an allele with sixteen out of 348 amino acids
substituted, only found in one out of twelve inbred rat strains, was due to a rapid diversification in line with the hypothesis of Gagneux and Varki (35).

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REFERENCES

1. Karlsson, K. A. (1989) *Annu. Rev. Biochem.* **58**, 309-350

2. Moniaux, N., Escande, F., Porchet, N., Aubert, J. P., and Batra, S. K. (2001) *Front. Biosci.* **6**, D1192-1206

3. Perez-Vilar, J., and Hill, R. L. (1999) *J. Biol. Chem.* **274**, 31751-31754

4. Gendler, S. J., and Spicer, A. P. (1995) *Annu. Rev. Physiol.* **57**, 607-634

5. Carlstedt, I., Herrmann, A., Karlsson, H., Sheehan, J., Fransson, L. Å., and Hansson, G. C. (1993) *J. Biol. Chem.* **268**, 18771-18781

6. Karlsson, N. G., Johansson, M. E., Asker, N., Karlsson, H., Gendler, S. J., Carlstedt, I., and Hansson, G. C. (1996) *Glycoconj. J.* **13**, 823-831

7. Hounsell, E. F., Davies, M. J., and Renouf, D. V. (1996) *Glycoconj. J.* **13**, 19-26

8. Hansson, G. C. (1988) *Adv. Exp. Med. Biol.* **228**, 465-494

9. Race, R. R., and Sanger, R. (1975) *Blood groups in man*, Plenum Press, New York

10. Yamamoto, F., Marken, J., Tsuji, T., White, T., Clausen, H., and Hakomori, S. (1990) *J. Biol. Chem.* **265**, 1146-1151

11. Breimer, M. E., Hansson, G. C., Karlsson, K. A., and Leffler, H. (1980) *FEBS Lett.* **114**, 51-56

12. Bouhours, D., Hansson, G. C., and Bouhours, J. F. (1995) *Biochim. Biophys. Acta* **1255**, 131-140

13. Bouhours, J. F., Bouhours, D., and Hansson, G. C. (1987) *J. Biol. Chem.* **262**, 16370-16375

14. Miller, H. R., and Nawa, Y. (1979) *Exp. Parasitol.* **47**, 81-90
15. Miller, H. R., Huntley, J. F., and Dawson, A. (1981) in *Current Topics in Veterinary Medicine and Animal Science* (Bourne, F. J., ed) Vol. 12, pp. 402-440, Nijhoff, The Hague

16. Ishikawa, N., Horii, Y., and Nawa, Y. (1993) *Immunology* 78, 303-307

17. Ishikawa, N., Horii, Y., Oinuma, T., Suganuma, T., and Nawa, Y. (1994) *Immunology* 81, 480-486

18. Karlsson, N. G., Olson, F. J., Jovall, P. Å., Andersch, Y., Enerbäck, L., and Hansson, G. C. (2000) *Biochem. J.* 350, 805-814

19. Wingren, U., Enerbäck, L., Ahlman, H., Allenmark, S., and Dahlström, A. (1983) *Histochemistry* 77, 145-158

20. Karlsson, N. G., Karlsson, H., and Hansson, G. C. (1995) *Glycoconj. J.* 12, 69-76

21. Ciucanu, I., and Kerek, F. (1984) *Carbohydr. Res.* 131, 209-218

22. Larson, G., Karlsson, H., Hansson, G. C., and Pimlott, W. (1987) *Carbohydr. Res.* 161, 281-290

23. Karlsson, N. G., Herrmann, A., Karlsson, H., Johansson, M. E., Carlstedt, I., and Hansson, G. C. (1997) *J. Biol. Chem.* 272, 27025-27034

24. Baeckström, D., Hansson, G. C., Nilsson, O., Johansson, C., Gendler, S. J., and Lindholm, L. (1991) *J. Biol. Chem.* 266, 21537-21547

25. David, L., Nesland, J. M., Clausen, H., Carneiro, F., and Sobrinho-Simoes, M. (1992) *APMIS Suppl.* 27, 162-172

26. Hansson, G. C., Baeckström, D., Carlstedt, I., and Klinga-Levan, K. (1994) *Biochem. Biophys. Res. Commun.* 198, 181-190

27. Bennett, E. P., Steffensen, R., Clausen, H., Weghuis, D. O., and van Kessel, A. G. (1995) *Biochem. Biophys. Res. Commun.* 206, 318-325
28. Yamamoto, F., McNeill, P. D., and Hakomori, S. (1995) *Glycobiology* **5**, 51-58

29. Smith, E. L., McKibbin, J. M., Karlsson, K. A., Pascher, I., and Samuelsson, B. E. (1975) *Biochemistry* **14**, 2120-2124

30. Sheehan, J. K., Thornton, D. J., Howard, M., Carlstedt, I., Corfield, A. P., and Paraskeva, C. (1996) *Biochem. J.* **315**, 1055-1060

31. Yamamoto, F., Clausen, H., White, T., Marken, J., and Hakomori, S. (1990) *Nature* **345**, 229-233

32. Yamamoto, M., Lin, X. H., Kominato, Y., Hata, Y., Noda, R., Saitou, N., and Yamamoto, F. (2001) *J. Biol. Chem.* **276**, 13701-13708

33. Yamamoto, F., and Hakomori, S. (1990) *J. Biol. Chem.* **265**, 19257-19262

34. Ångstrom, J., Falk, P., Hansson, G. C., Holgersson, J., Karlsson, H., Karlsson, K. A., Strömberg, N., and Thurin, J. (1987) *Biochim. Biophys. Acta* **926**, 79-86

35. Gagneux, P., and Varki, A. (1999) *Glycobiology* **9**, 747-755
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The abbreviations used are: HPA, Helix Pomatia agglutinin; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; MAb, monoclonal antibody; HexNAc, N-acetylc hexosamine; Hex, hexose; HexNAcol, N-acetylhexosaminol; GalNAcol, N-acetylgalactosaminol; PBS, phosphate-buffered saline.
FIGURE LEGENDS

Fig. 1. Gas chromatogram of released and permethylated neutral oligosaccharides from ‘insoluble’ small intestinal mucins from different stages of the Nippostrongylus brasiliensis infection. The released and permethylated neutral oligosaccharides were injected into a 10 m × 0.25 mm column coated with 0.04 µm stationary phase at 70°C (1 min) followed by a linear temperature program with 10°C/min up to 395°C (5 min). Panel A shows oligosaccharides from uninfected rats (day 0). Panel B shows oligosaccharides from infected rats (Infection 1, day 7). The numbers refer to structures interpreted by GC-MS (Table I).

Fig. 2. Mass spectrum of the permethylated induced blood group A-type pentasaccharide. From GC-MS of oligosaccharides from parasite infected rats (Infection 1, day 7). The electron impact spectrum, from the average of 14 scans (background subtracted) from GC-MS, corresponding to the component detected at 25.7 min in the GC chromatogram of Fig. 1B (component 5.2 in Table I).

Fig. 3. Blood group A-expression during the progress of the parasite infection. Sprague-Dawley rats were infected (Infection 1 and Infection 2) with an estimated amount of 6,000 N. brasiliensis third stage larvae. A and B, the ‘insoluble’ mucins from the small intestine was collected and coated into microtiter plates (50 ng) and incubated with the biotinylated lectin HPA (●), and the MAbs anti-blood group A (○), anti-blood group A, type 2 (▼), and anti-Tn (▲). The values are given relative the reactivity with the MAb anti-blood group H, after subtracting the background (the
mean of two duplicates). Bound antibodies or lectins were detected with europium-labeled streptavidin or goat anti-mouse antibodies. C, 0.5 µg of ‘insoluble’ mucins were incubated in microtiter wells pre-coated with HPA blocked by BSA and HF-deglycosylated. The amount of bound anti-Muc2 antiserum was detected with europium-labeled goat anti-rabbit antibodies (the mean of two experiments). Values are given after subtracting the signal from ‘insoluble’ mucins incubated in microtiter wells blocked only with BSA and HF-deglycosylated.

Fig. 4. Activity of a blood group A glycosyltransferase in the small intestine during the parasite infection. Small intestinal homogenates collected at different stages of the infection were incubated with LNF-1 (a blood group H type oligosaccharide) and UDP-[\(^{14}\)C]GalNAc. The material was subjected to a blood group A affinity column, and the radioactivity of the eluted fractions were measured (Panel A for days 0 and 6 of Infection 2). The total amount of radioactivity of the blood group A containing fractions was designated A. The total amount of radioactivity of unbound UDP-[\(^{14}\)C]GalNAc in the void volume was designated V. In panel B, the enzyme activity throughout the infection is shown as the ratio A:V of each sample.

Fig. 5. Expression of the putative rat blood group A glycosyltransferase in the small intestine during the parasite infection. A northern blot of rat small intestinal mRNA (from 50 µg of total RNA) isolated during the progress of the parasite infection (Infection 2), was probed with a 129 bp DNA probe (P129, described in text), homologous to the corresponding part of exon 6 of human blood group A glycosyltransferase. Two bands of estimated sizes 3.1 kb and 1.8 kb were detected.
The right lane is small intestinal mRNA from the GOT-BW rat strain with constitutive blood group A expression in the small intestine.

Fig. 6. Alignment of cDNA sequences of rat A(1) and A(2) glycosyltransferases. The full length cDNA sequences of the A(1) and A(2) alleles of rat blood group A glycosyltransferase are compared. Asterisks indicate positions with nucleotide substitutions. The seven exons of the gene, determined for A(1), are indicated by numbers above the sequences. PCR primers used in this study are shown in the figure. GH137A was used in the 5’ RACE PCR. GH158C/GH183C and GH158A/GH183C were used as A(1)/A(2) specific primers. GH170B/GH170R and GH170A/GH170R were used to clone full-length cDNA of A(1) and A(2). The P129 probe used in the northern blot is marked by a box.

Fig. 7. Comparison of the amino acid sequences of the rat blood group A glycosyltransferases to related glycosyltransferases. A, the amino acid sequences of rat A(1) and rat A(2) glycosyltransferases aligned with the mouse cis-AB glycosyltransferase (accession number BAB20560) and the human blood group A glycosyltransferase (accession number P16442). Black boxes indicate diverging amino acids, and grey boxes two different pairs of amino acids. The four amino acid differences between the human blood group A and B glycosyltransferases are indicated with the amino acid of the B transferase. B, alignment of the region important for donor nucleotide specificity for some glycosyltransferases. The following sequences in Genbank™ were used: pig blood group A transferase (AAC68840), dog Forssman synthetase (AAC48667), human cis-AB glycosyltransferase (AAD26580), and mouse GalT (AAA37711). The arrows point to
the two positions reported to be most important for donor nucleotide specificity. A Gly at the second of these two positions seems to give an absolute GalNAc specificity. An Ala at this position can render either Gal specificity, a combination of GalNAc and Gal specificity, or GalNAc specificity, probably depending on the surrounding amino acids.

Fig. 8. Immunostaining for blood group A epitopes on HeLa cells transfected with rat A(1) or A(2) glycosyltransferases. Blood group H expressing HeLa cells were transiently transfected with the expression plasmids pA(1) and pA(2). After 72 hours, the cells were immunostained with an anti-blood group A antibody and visualized with a FITC-labeled secondary antibody. A, blood group A reactivity, visualized by fluorescence microscopy. B, cells visualized by phase-contrast microscopy. Observe that not all cells were transfected.

Fig. 9. Allelic relationship between A(1) and A(2) glycosyltransferases. PCR reactions were performed with the primer pairs GH158C/GH183C and GH158A/GH183A, specific for A(1) and A(2) respectively. A, expression plasmids pA(1) and pA(2) were used as template showing the specificity of the primer pairs. B, multiplex RT-PCR on large intestinal mRNA from rats sacrificed on day six of the N. brasiliensis infection. β-actin was used as internal control. C, genomic PCR on the same rats as in B. D, genomic PCR on twelve inbred rat strains. E, genomic PCR on inbred DA and LEW rats, and on an animal from an F1 cross between DA and LEW.
TABLE I.
Structures of small intestinal neutral oligosaccharides derived from the ‘insoluble’ mucin complex of Sprague-Dawley rats uninfected and infected with *Nippostrongylus brasiliensis*

| No. | Neutral oligosaccharides<sup>a</sup> | Structures found in uninfected animals (day 0)<sup>b</sup> | Additional structures induced by infection (day 2-8)<sup>b</sup> |
|-----|-----------------------------------|-------------------------------------------------|-------------------------------------------------|
| 1   | GalNAcol                          | x                                               |                                                 |
| 2.1 | Gal-3GalNAcol                     | x                                               |                                                 |
| 2.2 | GlcNAc-3GalNAcol                  | x                                               |                                                 |
| 3.1 | Fuc-2Gal-3GalNAcol                | x                                               |                                                 |
| 3.2 | Gal-3(GlcNAc-6)GalNAcol           | x                                               |                                                 |
| 3.3 | GlcNAc-3(GlcNAc-6)GalNAcol        | x                                               |                                                 |
| 4.1 | Fuc-2Gal-3GlcNAc-3GalNAcol        | x                                               |                                                 |
| 4.2 | GalNAc-3(Fuc-2)Gal-3GalNAcol      | x                                               | x                                               |
| 4.3 | Fuc-2Gal-3(GlcNAc-6)GalNAcol      | x                                               |                                                 |
| 5.1 | Fuc-2Gal-GlcNAc-3(GlcNAc-6)GalNAcol | x                       |                                                 |
| 5.2 | GalNAc-3(Fuc-2)Gal-3GlcNAc-3GalNAcol | x                   | x                                               |
| 5.3 | GlcNAc-3(Fuc-2Gal-GlcNAc-6)GalNAcol | x                     | x                                               |
| 5.4 | GalNAc-3(Fuc-2)Gal-3(GlcNAc-6)GalNAcol | x           | x                                               |
| 6.1 | Fuc-2Gal-3(Fuc-2Gal-4GlcNAc-6)GalNAcol | x                   | x                                               |
| 6.2 | Fuc-2Gal-3(Fuc-2Gal-3GlcNAc-6)GalNAcol | x                   |                                                 |
| 6.3 | GalNAc-3(Fuc-2)Gal-GlcNAc-3(GlcNAc-6)GalNAcol | x               | x                                               |
| 6.4 | GlcNAc-3(GalNAc-3(Fuc-2)Gal-GlcNAc-6)GalNAcol | x           | x                                               |
| 7.1 | Fuc-2Gal-3GlcNAc-3(Fuc-2Gal-3GlcNAc-6)GalNAcol | x               | x                                               |
| 7.2 | Fuc-2Gal-4GlcNAc-3(Fuc-2Gal-4GlcNAc-6)GalNAcol | x               | x                                               |
| 7.3 | Fuc-2Gal-3(GalNAc-3(Fuc-2)Gal-3GlcNAc-6)GalNAcol | x         | x                                               |
| 7.4 | Fuc-2Gal-3(GalNAc-3(Fuc-2)Gal-4GlcNAc-6)GalNAcol | x               | x                                               |

<sup>a</sup>Structures are based on GC-MS of permethylated oligosaccharides. HexNAc residues are usually assumed to be GlcNAc based on the monosaccharide composition. GalNAc is assumed to be present only as a terminal blood group A-determinant, Hex residues to be Gal and deoxyhexoses to be Fuc-2, similar to the linkage in H- and A-determinants, HexNAcol residues are assumed to be GalNAcol. The prediction of type 1 chains (Galβ1-3GlcNAc) or type 2 chains (Galβ1-4GlcNAc) are based on the presence of the fragment ions m/z 228 and m/z 182, respectively. Substitution on the C-6 branch of the GalNAcol are marked by bold figures.

<sup>b</sup>Results from Infection 1.
Figure 3
Figure 4
Figure 6
Figure 5
Blood group A glycosyltransferase occurring as Alleles with high sequence difference is transiently induced during a Nippostrongylus Brasiliensis parasite infection

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