Glycoconjugate Journal (1998) **15:** 389–395

Sialyl Lewis^x epitopes do not occur on acute phase proteins in mice: relationship to the absence of α3-fucosyltransferase in the liver

Ellen C. Havenaar, Ruben C. Hoff, Dirk H. van den Eijnden and Willem van Dijk*

Department of Medical Chemistry, Faculty of Medicine, Vrije Universiteit, 1081 BT, Amsterdam, The Netherlands

Mice are frequently used in models for the study of immunological processes related to inflammation. Since it is known that the degree of fucosylation of human acute phase proteins (APPs) is altered as a consequence of an inflammatory response, we have undertaken this study to gain more insight into the fucosylation of acute phase proteins as it occurs in mouse liver. Mice carrying the cluster of the three genes encoding human α_1-acid glycoprotein (AGP), one of the well known APPs, were used and the fucosylation of AGP was assessed. A complete absence of fucosylation on the transgenic human AGP was found, which is in sharp contrast to AGP in human serum, of which a major proportion is normally α3-fucosylated. Remarkably, a large proportion of mouse AGP did contain fucose residues. Fucosylation was also detected on another APP, mouse protease inhibitor (PI).

α3-Fucosylation of the transgenic human AGP can be achieved *in vitro*, using an α3/4-fucosyltransferase (α3/4-FucT) isolated from human milk, showing that the glycoprotein is not intrinsically resistant to fucosylation. Upon subsequent measurement of the activities of the possible fucosyltransferases present in liver membranes of parent and transgenic mice, only an *N*-linked-core α6-FucT and no α2-, α3- or α4-FucT activity was detected. This indicates that fucose residues found on the mouse serum proteins AGP and PI, which are synthesized in the liver, are most probably in α6-linkage to the core chitobiosyl unit. Interestingly, both α6- and α3-FucT activity was detectable in human liver membranes. None of the above mentioned findings were influenced by the induction of an acute phase response by administration of bacterial lipopolysaccharide. This study shows that: (a) α6-FucT is probably a protein specific-glycosyltransferase, since mouse AGP, but not human AGP, may be used as an acceptor; (b) in contrast to human liver, mouse liver does not express any α3-FucT-activity, thereby making the mouse incapable of producing the Sialyl Lewis^x epitope on APPs, which is an important part of the inflammatory reaction in humans. This last finding indicates that the mouse is not suitable as a model for the study of those phenomena related to inflammation in humans, in which glycosylation of acute phase proteins could play a significant role.

***Keywords*: α_1-acid glycoprotein, orosomucoid, mouse, fucosyltransferase, liver, blood serum, sialyl lewis$_x$**

***Abbreviations*: AAL, *Aleuria aurantia* lectin; AGP, α_1-acid glycoprotein; PI, α_1-protease inhibitor; CAIE, crossed affino-immunoelectrophoresis; LPS, lipopolysaccharide; FucT, fucosyltransferase; LacNAc, Galβ1 → 4GlcNAc; H-type 2, Fucα1 → 2Galβ1 → 4GlcNAc; H-type 1, Fucα 1 → 2Galβ1 → 3GlcNAc; ag-GP-F2, asialo/agalacto-diantennary glycopeptide from human fibrinogen. sLe^x, sialyl Lewis x.**

Introduction

α_1-Acid glycoprotein (AGP) is an acute-phase protein with five N-linked glycans [1, 2], synthesized mainly in the liver [3, 4]. The degree of branching and α3-fucosylation of these glycans can vary during inflammatory states, and under the influence of hormones and cytokines [5–8]. It is known that during inflammation the concentration and α3-fucosylation of AGP and other acute-phase proteins in human serum is increased (see [9, 10] for a review), and that the enzyme responsible for this fucosylation is fucosyltransferase VI (Fuc-TVI, [11]). This higher fucosylation of AGP invariably coincides with increased expression of the sialyl Lewis^x structure (sLe^x, NeuAcα2 → 3Galβ1 → 4[Fucα1 → 3]GlcNAc) [5, 10], which is a counter-receptor for the selectin-type adhesion molecules involved in leukocyte extravasation [12–14]. A possible regulatory role for AGP expressing sLe^x in the inflammatory response, has been suggested [5].

Previously, a mouse line transgenic for human AGP was used to study the effect of transgenic expression of AGP on the glycosylation process [15]. These studies were focused on the degree of branching of the N-linked glycans of mouse

*To whom correspondence should be addressed. Fax : 31.20.4448144; Tel: 31.20.4448159; E-mail: W.Van_Dijk.medchem@med.vu.nl.

0282–0080

and transgenic AGP. In the present study we have used the same animal model to gain insight into the fucosylation process in mouse liver, with mouse and transgenic human AGP as reporter proteins. Crossed affino immunoelectrophoresis (CAIE) with *Aleuria aurantia* lectin (AAL) as fucose-specific affinity component was used to assess the extent of fucosylation of human and mouse AGP from transgenic mice. Unexpectedly, transgenic human AGP was found to be non-fucosylated, whereas mouse AGP was fucosylated. In order to ascertain whether transgenic human AGP was intrinsically resistant to fucosylation, *in vitro* fucosylation of the non-fucosylated transgenic human AGP was attempted, using CAIE with AAL as a detection technique. Furthermore, the presence of α2-, α3-, α4- and α6-FucTs was studied in transgenic and parent mouse livers by measurement of enzyme activities in liver-membrane fractions, using specific acceptors for each of the enzymes. The results were compared with those obtained for Balb-c mouse liver and with those obtained for human liver.

Materials and methods

Materials and animals

GDP-[^{14}C]Fuc (225 Ci mol^{-1}) was obtained from Du Pont-New England Nuclear. Unlabelled GDP-Fuc was kindly donated by Drs H. Lönn and T. Nordberg (BioCarb AB, Lund, Sweden). The glycopeptide from fibrinogen (ag-GP-F2, GlcNAcβ1 → 2Manα1 → 6[GlcNAcβ1 → 2Manα1 → 3]Manβ1 → 4GlcNAcβ1 → 4GlcNAc-Asn) was obtained from human fibrinogen according to [16], by pronase digestion of the desialylated glycoprotein (0.1 M trifluoro acetic acid, 1 h at 80 °C) followed by β-galactosidase treatment of the isolated diantennary glycopeptide. Purity was checked by 400 MHz ^{1}H NMR spectroscopy. The H-type 2 (Fucα1 → 2Galβ1 → 4GlcNAcβ-O-$(CH_2)_8COOCH_3$) and H-type 1 (Fucα1 → 2Galβ1 → 3GlcNAcβ-O-$(CH_2)_8COOCH_3$) acceptors were a kind gift from Dr O. Hindsgaul (Alberta, Canada). Galactoside-β-O-*p*-nitrophenyl (Galβ-O-pNP) was obtained from Sigma, St. Louis, MD, USA. An α3/4-FucT preparation was isolated from human milk [17]. Human milk was obtained from healthy lactating mothers and stored frozen until use. A non-fucosylated AGP glycoform that was used as a standard (A0), was isolated by preparative affinity electrophoresis [18]. AGP from pooled human serum was isolated from Cohn fraction V, by the method of Hao and Wickerhauser [19]. AAL was isolated from fruiting bodies of the *Aleuria aurantia* mushroom as detailed earlier [5]. Rabbit anti human glycoprotein monospecific antisera were obtained from Dakopatts, Glostrup, Denmark. Rabbit anti mouse AGP was a kind gift from Dr Heinz Baumann, Buffalo, NY, USA. Rabbit anti mouse PI was a kind gift from Dr Peter Heegaard, Copenhagen, Denmark. The antisera were specific and did not cross react with glycoproteins from other species as revealed by immunoelectrophoresis of control mouse and human sera with all antiserum preparations. Coomassie Brilliant Blue R250 was obtained from Sigma, St Louis, USA. All other materials were of analytical grade.

Sera and livers from young adult mice transgenic for the cluster of three human AGP-genes (*ABB′*), as well as from parent mice (C3H-strain) were obtained from Dr Luciana Dente (Rome, Italy [20]), and stored at − 80 °C until use. These mice were also used in a previous study regarding the glycosylation of AGP [15]. All mice were homozygous obtained by heterozygous intercrosses as described in [20]. Inflammatory conditions had been induced in these animals by the injection of 100 μg lipopolysaccharide, as described in [20]. Young adult male Balb-c mice were obtained from Harlan CPB, Zeist, The Netherlands. A sample of human liver was a kind gift from Dr Chamuleau, Academic Medical Centre, Amsterdam, The Netherlands, and stored at − 80 °C until use.

Crossed affinity immunoelectrophoresis (CAIE)

CAIE was performed according to the method of Bøg-Hansen [21], using 2.5 mg ml^{-1} (with a haemagglutination titre of 1024) AAL as the fucose-specific affinity component as described previously [11]. AAL is specific for fucose linked in either α2-, α3-, α4- or α6-linkages [22]. However, interactions of human AGP with AAL may be assumed to be only due to α3-linked fucose [2, 5, 23–25]. For PI it is known that not only α3-, but also α6-linked fucose are present [26], and that both these groups will result in retardation in CAIE with AAL [5, 11]. Briefly, 0.5–2.0 μl of serum was electrophoresed through a 7.5% polyacrylamide gel containing 2.5 mg ml^{-1} AAL, resulting in the separation of the differently glycosylated AGP forms. In a second, perpendicular dimension, at 90° to the first dimension the glycoprotein forms were immunoelectrophoresed against the precipitating monospecific antiserum. The resulting precipitation curves were visualized by staining with Coomassie Brilliant Blue R250. The areas under the precipitation curves indicate the relative amounts of separated glycoprotein.

Preparation of liver membrane fractions

Approximately 200 mg of mouse or human liver was homogenized in 5 ml 0.25 M sucrose on ice with a Potter-Elvehjem homogenizer, (3–4 strokes of 15 s at 1400 rpm). Membrane fractions were obtained by centrifugation $10\,000 \times \mathbf{g}_{av}$ for 60 min after removal of cell debris by centrifugation for 10 min at $1000 \times \mathbf{g}_{av}$. The final pellets were resuspended in 200 μl 0.25 M sucrose and were stored at − 20 °C until use.

Fucosyltransferase assays

The following compounds Gal-β-O-pNP, H-type 2, H-type 1 and ag-GP-F2 were used as specific acceptors for the determination of α2-, α3-, α4- and α6-fucosyltransferase

activities, respectively. Incubation-mixtures (30 µl) were composed of: 0.1 mM GDP-[^{14}C]Fuc (4.5–5 Ci mol^{-1}); 50 mM MOPS/NaOH, pH7.5; 0.4% Triton X-100; 20 mM $MnCl_2$; 4 mM ATP; 0.1 M NaCl, 1 mM acceptor and liver membrane fraction (10 µl, corresponding to 10 mg wet weight) or serum (10 µl). Incubations were carried out at 37 °C for 4 h in the case of liver membranes and 16 h in the case of serum. In all assays the reaction was stopped by the addition of 1 ml water. In the case of the acceptors used to determine α2-, α3- and α4-FucT activities, the labelled oligosaccharide products (having a hydrophobic aglycon) were separated from the radioactive GDP-Fuc according to [27]. Sep-Pak-C18 reverse-phase cartridges (Waters, Milford MA, USA) that had been conditioned immediately before use by washing with 5 ml of methanol and 10 ml of H_2O were used. After application of the sample the cartridge was washed with 40 ml of H_2O, whereupon the radiolabelled product was eluted with 5 ml of methanol. After evaporation of the methanol the radioactivity in the product was counted by liquid scintillation. The α6-FucT activity was assayed by application of the incubated sample to a column (0.7 × 56 cm) of Bio-Gel-P4 (100–200 mesh), which was equilibrated and eluted with 50 mM ammonium acetate, pH 5.2. Void volume fractions, which contain the ^{14}C-fucosylated glycopeptide product, were pooled and radioactivity was counted by liquid scintillation [28]. In all assays, values were corrected for incorporation found in the absence of added acceptors. Protein content of livers and sera were determined according to Peterson [29].

In vitro fucosylation of non-fucosylated AGP

Non-fucosylated transgenic human AGP was fucosylated *in vitro*, using an α3/4 fucosyltransferase preparation isolated from human milk [17]. The composition of the incubation mixtures was in a total volume of 25 µl: mouse serum containing 10 µg transgenic human AGP, 4 mM ATP, 20 mM $MnCl_2$, 0.2 mM GDP-fucose, 123 µU human milk fucosyltransferase, 50 mM sodium cacodylate (pH 7.2), 0.1 M NaCl, 50% glycerol, 0.05% sodium azide. The mixtures were incubated at 37 °C for 72 h. As a control, a non-fucosylated AGP-glycoform from human serum was also incubated under the same conditions, as was AGP isolated from Cohn fraction V from pooled normal human serum. Fucosylation was determined by means of CAIE using *Aleuria aurantia* lectin as the affinity component.

Results and discussion

In this study the fucosylation of transgenic human AGP, as well as the fucosylation of mouse AGP and PI, was used for the study of the capacity of mouse liver to fucosylate glycoproteins, since this is an important phenomenon in the human acute phase response. It became apparent that transgenic human AGP in mice is not fucosylated, in contrast to mouse AGP and PI. Furthermore we showed that mouse liver possesses a different panel of fucosyltransferases than human liver.

Fucosylation of mouse and (transgenic) human AGP

The extent of fucosylation of mouse- and transgenic human-AGP in mice serum, was determined using CAIE with AAL as the affinity component. By this method, fucosylated AGP-glycoforms are retarded in an AAL-containing gel, resulting in fractionation of AGP into a non-reactive glycoform (A0) containing no fucose, weakly reactive (Aw) and strongly reactive (As) glycoforms, containing increasing amounts of fucose, as is shown for normal human serum AGP in Figure 1d. In contrast to human serum AGP, the transgenic human AGP was not retarded at all in an AAL-containing gel (Figure 1b), displaying a pattern identical to that observed for the non-fucosylated human AGP standard (A0) that was used as a control (Figure 1c). Remarkably, a substantial amount of mouse AGP present in the same transgenic mouse was retarded in CAIE with AAL (Figure 1a). Therefore it can be concluded that mouse AGP is fucosylated, and transgenic human AGP in the same mouse is not.

Fucosylation of mouse PI

The extent of fucosylation of mouse PI was also studied with CAIE (Figure 2c). Human serum PI is known to possess both α3-, and α6-linked fucose [11, 26] and will therefore also be retarded by AAL, which is specific for fucose linked in either α2-, α3-, α4- and α6-linkages [22]. The relative distribution of weakly and strongly reactive glycoforms of mouse PI with AAL (Figure 2c) was comparable to that found previously for human PI from individuals with a mutation in the *FUT6*-gene, lacking α3-fucosylation of the serum proteins [11] (Figure 2b). The virtual absence of the As peak in PI from mouse and PI from individuals with the *FUT6* mutation, and its presence in PI from normal human serum (Figure 2a), indicates that the PI of the first two sources contains only α6-linked and no α3-linked fucose.

The fucosylation found for mouse AGP and PI does not lead to the expression of the sLe^x-epitope, since we were unable to detect specific staining of mouse plasma components in either ABB′- or balb-c mouse serum (not shown) with the monoclonal antibody CSLEX-1, which is directed towards sLe^x-groups [5].

In vitro fucosylation of AGP

In order to determine whether the absence of fucosylation of transgenic human AGP was due to an intrinsic resistance of this glycoprotein to fucosylation, *in vitro* fucosylation was attempted and detected by means of CAIE with AAL. This has previously been shown to be a very sensitive technique for the monitoring of *in vitro* fucosylation [11]. Figure 1g

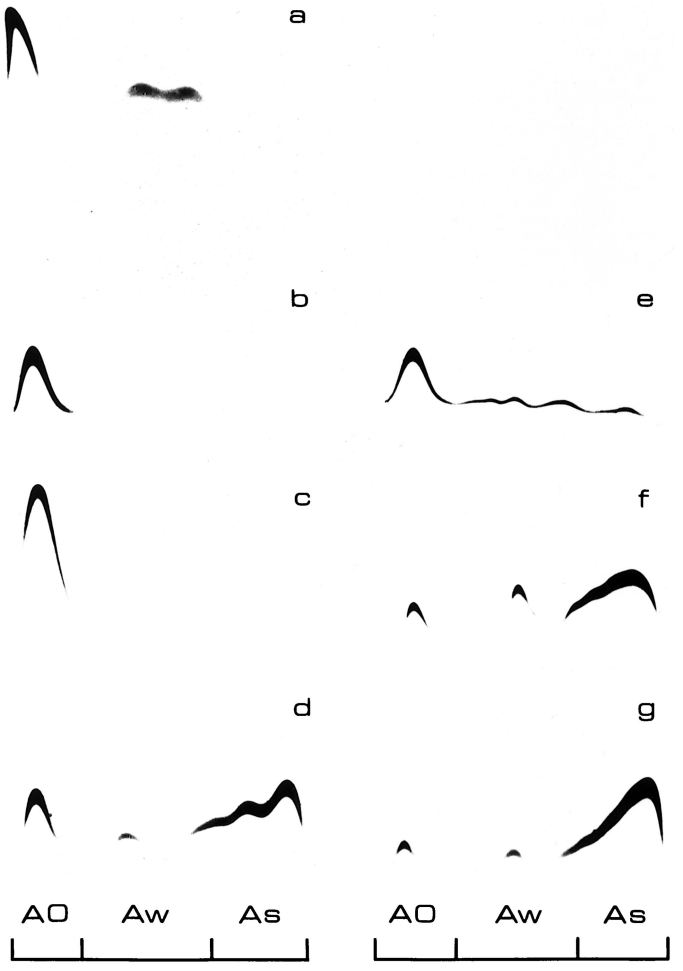

Figure 1. Reactivity of AGP with AAL before (a–d) and after (e–g) *in vitro* fucosylation: (a) mouse AGP; (b, e) transgenic human AGP; (c, f) non-fucosylated human AGP standard; (d, g) AGP from normal human serum. Sera were subjected to CAIE as described in the Materials and methods. Only the second dimension gels are shown. The lower right corner of each pattern corresponds to the site of application in the first dimension gel. Electrophoresis was performed from right to left in the first dimension, and from bottom to top for the second dimension. A0: AGP-glycoforms non-reactive with AAL; Aw: AGP-glycoforms weakly reactive with AAL; As: AGP-glycoforms strongly reactive with AAL. No fractionation was obtained when samples were analysed in the absence of AAL, resulting in recovery of all AGP-protein at the position of A0.

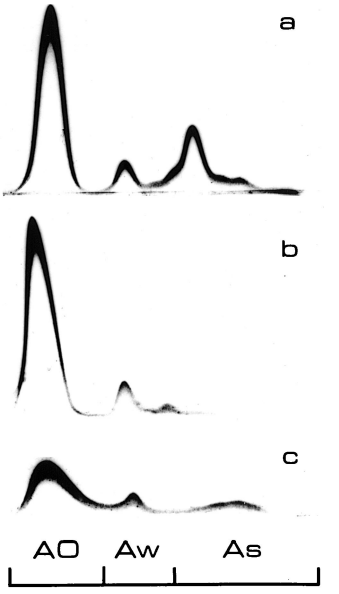

Figure 2. Reactivity of PI with AAL. (a) normal human serum PI; (b) PI from an individual with a mutation in the *FUT6*-gene [11]; (c) mouse PI (balb-c mouse). Sera were subjected to CAIE as described in the Materials and methods, see also Figure 1. A0: PI-glycoforms non-reactive with AAL; Aw: PI-glycoforms weakly reactive with AAL; As: PI-glycoforms strongly reactive with AAL. No fractionation was obtained when samples were analysed in the absence of AAL, resulting in recovery of all PI-protein at the position of A0.

shows an increase in retardation of normal human serum AGP by AAL, after 72 h *in vitro* fucosylation at 37 °C. As expected the non-fucosylated human-AGP standard was highly susceptible to fucosylation, also resulting in a high amount of strongly reactive glycoforms (Figure 1f). Transgenic human AGP was not intrinsically resistant to fucosylation (Figure 1e), although the degree of fucosylation was much lower than for the non-fucosylated human AGP standard. The reason for this much lower susceptibility to fucosylation may be found in the type of sialylation. Previously, we have shown that the diantennary glycan-content of transgenic human AGP, as well as mouse AGP, in the transgenic mouse strain, was very much higher than for normal human AGP [15]. It is known that diantennary glycans are preferentially α6-sialylated [30] which will result in a relatively low degree of α3-fucosylation of the diantennary glycans, since α6-sialyltransferase and α3-FucT show a mutually exclusive action [31, 32]. This might explain why the degree of *in vitro* fucosylation of transgenic human AGP could not be as high as the fucosylation achieved for the normal human non-fucosylated AGP standard, containing a low amount of diantennary glycans [18]. This is further corroborated by the finding that a desialylated form of the transgenic AGP could be fucosylated to a much higher degree than the sialylated form (results not shown).

Fucosyltransferase activities in liver and serum

To further understand the absence of fucosylation on transgenic human AGP, the various FucT activities were assayed in livers and sera. It was found that in mouse liver only α6-FucT activity is present; α2-, α3- or α4-FucT activities were below detectable levels. This is in contrast to human liver, where not only both α3- and α6-FucT activities were detectable (Table 1), but the α3-FucT activity in human liver is also three-fold higher than the α6-FucT activity. The sole presence of α6-FucT activity in mouse supports our conclusion that only α6-linked fucose is present on mouse AGP and PI. LacNAc-R was also tested as an acceptor, but this did not result in a detectable incorporation of fucose by

Table 1. Fucosyltransferase activity in liver membrane fractions.

Acceptor	*FucT*	*Fucosyltransferase activity (pmol h^{-1} mg protein^{-1})*					
		C3H		*ABB′*		*Balb-c*	*Human*
		−	+	−	+		
Galβ-O-pNP[a]	α2	ND[e]	ND	ND	ND	ND	ND
Fucα1 → 2Galβ1 → 4GlcNAcβ-O-R[b]	α3	ND	ND	ND	ND	ND	229.0 ± 64.9
Galβ1 → 4GlcNAcβ-O-R[b]	α2/3	–[d]	–	13.1 ± 1.8	–	–	67.8 ± 20.3
Fucα1 → 2Galβ1 → 3GlcNAcβ-O-R[b]	α4	ND	ND	ND	ND	ND	ND
ag-GP-F2[c]	α6	156.7 ± 51.1	105.0 ± 0.4	439.9 ± 22.4	77.7 ± 1.7	40.2 ± 8.9	73.4 ± 12.4

Membranes were obtained from parent (C3H) and transgenic (ABB′) mouse liver before (−) and after (+) LPS treatment, and from Balb-c mouse and human liver.
[a]pNP, *p*-nitrophenyl.
[b]R = $(CH_2)_8COOCH_3$.
[c]asialo/agalacto-diantennary glycopeptide from human fibrinogen;
[c](–), not determined.
[e]ND, below detectable levels.
Samples were measured at two separate instances, in duplicate.

mouse liver membranes (Table 1). This excludes the presence of an α3-FucT in mouse liver with a high preference for LacNAc over H-type 2. LPS was unable to induce α3-FucT-activity in mouse liver, since fucosylation of transgenic human AGP did not occur, nor could α3-FucT enzyme activity be detected after treatment of mice with LPS.

We have shown that the fucose residues found on mouse AGP (and PI) are not in α3-linkage, and must be in α6-linkage (glycoproteins containing either, or both, linkages are retarded by AAL [22]), since only α6-FucT activity is present in mouse liver. Although only the porcine α6-FucT [33] and human α6-FucT [34] have been cloned, these studies indicate that the enzyme must be present and functional in mice, and is probably a protein-specific glycosyltransferase.

In humans, the major source for serum α3-FucT activity appears to be the liver [11, 35]. In view of the absence of α3-FucT activity in mouse liver, the very small amounts of α3-FucT activity found in (transgenic) mouse serum, relative to serum from healthy human individuals (Table 2), must have another tissue origin. Possible candidates are leukocytes or kidney [36], or perhaps even the gastrointestinal tract [37]. This is corroborated by the total absence of α3-FucT activity in plasma, of individuals lacking the *FUT6*-gene in all tissues, Table 2 [11]. It should be noted however, that the level of activity in serum is very low in mice. Possibly the small increase in serum α3-FucT

Table 2. Fucosyltransferase activity in mouse-serum compared to human serum.

Acceptor	*FucT*	*Fucosyltransferase activity (pmol h^{-1} mg protein^{-1})*						
		C3H		*ABB′*		*Balb-c*	*Human*	
		−	+	−	+		*Normal*	*FUT6-def.*
Galβ-O-pNP[a]	α2	ND[e]	ND	ND	ND	ND	–[d]	–
Fucα1 → 2Galβ1 → 4GlcNAcβ-O-R[b]	α3	ND	10.9	15.6 ± 0.0	5.3 ± 0.6	9.7 ± 4.6	33 ± 19	ND
Fucα1 → 2Galβ1 → 3GlcNAcβ-O-R[b]	α4	ND	ND	ND	ND	ND	–	–
ag-GP-F2[c]	α6	ND	ND	ND	ND	ND	–	–

Serum was obtained from parent (C3H) and transgenic (ABB′) mice before (−) and after (+) LPS treatment, and from Balb-c mice. Values for human sera were measured earlier in our group under the same experimental conditions, and are adapted from [11].
[a]pNP, *p*-nitrophenyl.
[b]R = $(CH_2)_8COOCH_3$.
[c]asialo/agalacto-diantennary glycopeptide from human fibrinogen.
[d](−), not determined.
[e]ND, below detectable levels.
Mouse samples were measured at two separate instances, in duplicate. For humans: normal ($n = 12$), for FUT6-deficient ($n = 8$).

activity in transgenic mice compared to parent mice, might be the result of activation of an endogenous α3-FucT-gene due to transgenesis. Interestingly, such an activation has been described earlier for CHO-cells after transfection with human DNA [38]. Furthermore, we have previously indicated that the introduction of the *AGP*-genes has an effect on the glycosylation process in the transgenic mouse liver [15].

General conclusions

We have recently shown that the liver α3-FucT (FucT VI) is responsible for the α3-fucosylation of serum proteins such as AGP, PI and α_1-antichymotrypsin in humans [11]. We have shown here that mouse liver does not express any α3-FucT, since human AGP is not fucosylated in transgenic mice, and no α3-FucT activity could be detected in transgenic- or Balb-c mouse liver. This is in agreement with recent findings that only one inactive pseudogene was found in an attempt to clone mouse fucosyltransferases using the same homology cloning strategy that was used to clone the human *FUT3*, *FUT5* and *FUT6* genes [39]. From our studies it can also be concluded that the mouse FUT4 [39,40] and FUT7 [41] gene products, which have been cloned and are able to synthesize α3-fucosylated structures in mice, do not take over the α3-fucosylation function in mouse liver. Furthermore, this study shows that the mouse liver possesses a different panel of fucosyltransferases compared to human liver, leading to a significantly different glycosylation of acute phase proteins in mice compared to humans. As a result the mouse liver is incapable of producing the sLe^x epitope on APPs, which is an important part of the human acute phase response [11,42]. Therefore, the mouse is not suitable as a model for the study of those processes related to inflammation in humans, in which the glycosylation of acute phase proteins such as AGP, PI and α_1-antichymotrypsin could play a significant role.

Acknowledgements

The authors thank Dr Theodora de Vries for her helpful discussions, and Wietske Schiphorst for the preparation of the α3/4-fucosyltransferase from human milk, and the ag-GP-F2 diantennary glycopeptide from human fibrinogen. We also thank Dr Luciana Dente (Rome, Italy) for the samples from transgenic and parent mice, and Dr O. Hindsgaul for the H-type 1, H-type 2 and LacNAc acceptors. This work was supported by the Netherlands Organisation for Scientific Research (NWO), grant nr. 900-512-164.

References

1 Schmid K, Nimberg RB, Kimura A, Yamaguchi H, Binette JP (1977) *Biochim Biophys Acta* **492**: 291–302.
2 Yoshima H, Matsumoto A, Mizuochi T, Kawasaki T, Kobata A (1981) *J Biol Chem* **256**: 8476–84.
3 Pos O, Moshage HJ, Yap SH, Snieders JPM, Aarden LA, Van Gool J, Boers W, Brugman AM, Van Dijk W (1989) *Inflammation* **16**: 197–203.
4 Baumann H, Gauldie J (1994) *Immunol Today* **15**: 74–80.
5 De Graaf TW, van der Stelt ME, Anbergen MG, van Dijk W (1993) *J Exp Med* **177**: 657–66.
6 Baumann H, Held WA (1981) *J Biol Chem* **256**: 10145–55.
7 Pos O, Van der Stelt ME, Wolbink GJ, Nijsten MWN, Van der Tempel GL, Van Dijk W (1990) *Clin Exp Immunol* **82**: 579–82.
8 Brinkman-Van der Linden ECM, Havenaar EC, Van Ommen ECR, Van Kamp GJ, Gooren LJG, Van Dijk W (1996) *Glycobiology* **6**: 407–12.
9 Van Dijk W, Turner GA, Mackiewicz A (1994) *Glycosylation and Disease* **1**: 5–14.
10 Van Dijk, W, Havenaar EC, Brinkman-Van der Linden ECM (1995) *Glycoconjugate J* **12**: 227–33.
11 Brinkman-Van der Linden ECM, Mollicone R, Oriol R, Larson G, Van den Eijnden DH, Van Dijk W (1996) *J Biol Chem* **273**: 14492–5.
12 Springer TA, Lasky LA (1991) *Nature* **349**: 196–7.
13 Feizi T (1992) *Biochem Soc Trans* **20**: 274–8.
14 Crocker PR, Feizi T (1996) *Curr Opin Struct Biol* **6**: 679–91.
15 Van Dijk, W, Van der Stelt ME, Salera A, Dente L (1991) *Eur J Cell Biol* **55**: 143–8.
16 Nemansky M, Van den Eijnden DH (1993) *Glycoconjugate J* **10**: 99–108.
17 De Vries T, Norberg TH, Lönn H, Van den Eijnden DH (1993) *Eur J Biochem* **216**: 769–77.
18 Van der Linden ECM, De Graaf TW, Anbergen MG, Dekker RM, Van Ommen ECR, Van den Eijnden DH, Van Dijk W (1994) *Glycosylation and Disease* **1**: 5–52.
19 Hao YL, Wickerhauser M (1973) *Biochim Biophys Acta* **322**: 99–108.
20 Dente LR, Ruther U, Tripodi M, Wagner EF, Cortese R (1988) *Genes Dev* **2**: 259–266.
21 Bøg-Hansen TC (1973) *Anal Biochem* **56**: 480–8.
22 Yamashita K, Kochibe N, Okhura T, Ueda I, Kobata A (1985) *J Biol Chem* **260**: 4688–93.
23 Fournet B, Montreuil J, Strecker G, Dorland L, Haverkamp J, Vliegenthart JFG, Binette JP, Schmid K (1978) *Biochemistry* **17**: 5206–14.
24 Wieruszeski JM, Fournet B, Konan D, Biou D, Durand G (1988) *FEBS Lett* **238**: 390–4.
25 Bierhuizen MFA, De Wit M, Govers CARL, Ferwerda W, Koeleman C, Pos O, Van Dijk W (1988) *Eur J Biochem* **175**: 387–94.
26 Saitoh A, Aoyagi Y, Asakura H (1993) *Arch Biochem Biophys* **303**: 281–7.
27 Palcic MM, Heerze LD, Pierce M, Hindsgaul O (1988) *Glycoconjugate J* **5**: 49–63.
28 Easton EW, Bolscher JGM, Van den Eijnden DH (1991) *J Biol Chem* **266**: 21674–80.
29 Peterson GL (1977) *Anal Biochem* **83**: 346–56.
30 Nemansky M, Schiphorst WECM, Van den Eijnden DH (1995) *FEBS Lett* **363**: 280–4.

31 Skacel PO, Edwards AJ, Harrison CT, Watkins WM (1991) *Blood* **78**: 1452–60.
32 Paulson JC, Prieels J-P, Glasgow LR, Hill RL (1978) *J Biol Chem* **253**: 5617–24.
33 Uozumi N, Yanagidani S, Miyoshi E, Ihara Y, Sakuma T, Gao CX, Teshima T, Fujii S, Shiba T, Taniguchi N (1996) *J Biol Chem* **271**: 27810–17.
34 Yanagidani S, Uozumi N, Ihara Y, Miyoshi E, Yamaguchi N, Taniguchi N (1997) *J Biochem* **121**: 626–32.
35 Johnson PH, Donald ASR, Clarke JL, Watkins WM (1995) *Glycoconjugate J* **12**: 879–93.
36 Caillard T, Le Pendu J, Ventura M, Mada M, Rault G, Mannoni P, Oriol R (1988) *Exp Clin Immunogenet* **5**: 15–23.
37 Karaivanova V, Mookerjea S, Hunt D, Nagpurkar A (1996) *Int J Cell Biol* **28**: 165–74.
38 Potvin B, Kumar R, Howard DR, Stanley P (1990) *J Biol Chem* **265**: 1615–22.
39 Gersten KM, Natsuka S, Trinchera M, Petryniak B, Kelly RJ, Hiraiwa N, Jenkins NA, Gilbert DJ, Copeland NG, Lowe JB (1995) *J Biol Chem* **270**: 25047–56.
40 Ozawa M, Muramatsu T (1996) *J Biochem* **119**: 302–8.
41 Smith PL, Gersten KM, Petryniak B, Kelly RJ, Rogers C, Natsuka Y, Alford JA, 3rd, Scheidegger EP, Natsuka S, Lowe JB (1996) *J Biol Chem* **271**: 8250–9.
42 Brinkman-Van der Linden ECM, De Haan PF, Havenaar EC, Van Dijk W (1997) *Glycoconjugate J*: in press.

Received 3 July 1997, revised 15 August 1997, accepted 1 September 1997