

MIROSLAWA FERENS-SIECZKOWSKA, BARBARA KOSSOWSKA

## Haptoglobin Fucosylation in Ascites Fluids of Ovarian Cancer Patients

### Fukozylacja haptoglobiny w płynach wysiękowych u chorych na raka jajnika

Department of Chemistry and Immunochemistry, Wrocław Medical University, Poland

#### Abstract

**Background.** Expression of fucosylated Lewis-type antigens is considered as crucial for the metastatic potential of cancer cells. Overexpression of similar structures on circulating soluble glycoproteins was hypothesized to compete in the interaction of selectins with their sialylated and fucosylated counterparts. Anyway, fucose may be expressed in particular glycoproteins and conditions in different, not necessary Lewis-type, positions. In the current study the authors focused on the fucosylation of ascites fluid haptoglobin.

**Material and Methods.** Haptoglobin was isolated from ascites fluids of seven patients with advanced ovarian cancer and from pooled sera of healthy subjects. Fucosylation of haptoglobin and asialo-haptoglobin was analyzed in LECTIN-ELISA with three fucose-specific lectins. Expression of sialyl-Lewis<sup>x</sup> epitope was examined in ELISA tests with anti-sLe<sup>x</sup> monoclonal antibodies.

**Results.** All haptoglobins isolated from the ascites fluids showed significantly increased reactivity with *Aleuria aurantia* lectin. Reactivity with *Lotus tetragonolobus* lectin was highly elevated only in four out of seven asialo-haptoglobins, indicating for Le<sup>x</sup> (but not sialyl-Le<sup>x</sup>) expression. In one case the presence of bifucosylated Le<sup>y</sup> structure was suggested. Expression of sialyl-Le<sup>x</sup> antigen in examined haptoglobins is rather low.

**Conclusions.** Haptoglobin in ascites fluids of ovarian cancer patients is fucosylated much higher than in serum of healthy subjects. Fucose is bound with  $\alpha$ 1–6 linkage in the core region of glycans and with  $\alpha$ 1–3 linkage, as Le<sup>x</sup> antennae epitope. Weak expression of sialyl-Le<sup>x</sup> is probably due to the low amount of  $\alpha$ 2–3 bound sialic acid (**Adv Clin Exp Med 2004, 13, 4, 581–587**).

**Key words:** haptoglobin, fucosylation, ascites fluids, Lewis<sup>x</sup>.

#### Streszczenie

**Wprowadzenie.** Ekspresję fukozylowanych antygenów typu Lewis uważa się za istotną dla metastatycznego potencjału komórek nowotworowych. Nadekspresja podobnych struktur na krążących w osoczu glikoproteinach doprowadziła do sformułowania hipotezy, że mogą one konkurować w interakcjach selektyn z ich sjalowanymi i fukozylowanymi partnerami bądź receptorami. Fukoza w łańcuchach cukrowych glikoprotein może jednak być przyłączona w różnych pozycjach, nie tylko typowych dla antygenów typu Lewis. Celem pracy była analiza sposobu fukozytacji haptoglobiny obecnej w płynach wysiękowych pacjentek z zaawansowanym rakiem jajnika oraz ocena, czy jest ona związana z ekspresją antygenów Lewis<sup>x</sup>.

**Materiały i metody.** Haptoglobinę izolowano z płynów wysiękowych siedmiu pacjentek z zaawansowanym rakiem jajnika oraz z surowicy referencyjnej, przygotowanej jako pula surowic zdrowych krwiodawców. Fukozylację haptoglobiny i asjalo-haptoglobiny analizowano techniką LECTIN-ELISA z trzema fukozoswoistymi lektynami. Ekspresję epitopów sLe<sup>x</sup> badano w testach ELISA z monoklonalnym przeciwciałem anti-sLe<sup>x</sup>.

**Wyniki.** Wszystkie haptoglobiny izolowane z płynów wysiękowych wykazywały znaczący wzrost reaktywności z lektyną z *Aleuria aurantia*. Reaktywność z lektyną z *Lotus tetragonolobus* była znacznie podwyższona tylko w czterech spośród siedmiu preparatów, wskazując na wiązanie fukozy w pozycji Lewis<sup>x</sup> (ale nie sjalo-Lewis<sup>x</sup>). W jednym przypadku jest sugerowana obecność dwufukozylowanej struktury Lewis<sup>y</sup>. Ekspresja antygeny sjalo-Lewis<sup>x</sup> w analizowanych haptoglobinach okazała się niska.

**Wnioski.** Haptoglobina w płynach wysiękowych pacjentek z rakiem jajnika jest ufukozylowana w znacznie wyższym stopniu niż w surowicy ludzi zdrowych. Fukoza jest przyłączona wiązaniem  $\alpha$ 1–6 w rdzeniowej części glikanu oraz wiązaniem  $\alpha$ 1–3, jako antenowy epitop Le<sup>x</sup>. Słaba ekspresja antygeny sLe<sup>x</sup> jest prawdopodobnie związana z niewielką ilością kwasu sjalowego przyłączonego wiązaniem  $\alpha$ 2–3 (**Adv Clin Exp Med 2004, 13, 4, 581–587**).

**Słowa kluczowe:** haptoglobina, fukozylacja, płyn wysiękowy, Lewis<sup>x</sup>.

During malignant transformation, cells experience profound alterations of the glycosylation system. As the result, cancer cells bear at their surface altered oligosaccharide epitopes [1–5], which are involved in an interaction of the cells and their environmental counterparts. Among such glycans, fucosylated and sialylated Lewis type antigens play crucial role. Particular Lewis antigens differ one from the other in the type of fucose linkage to galactose or N-acetyl-glucosamine (Fig. 1). Glycoconjugates bearing sLe<sup>x</sup> and related structures are shown to be specific ligands for selectins. This interaction plays the key role in the first step of the adhesion cascade: leukocyte restraint from the bloodstream and their rolling at the endothelium surface [6]. Overexpression of sLe<sup>x</sup> and sLe<sup>a</sup> on the surface of the cancer cells is crucial for their metastatic potential, as it enables/facilitates extravasation to the matrix of the circulating cells released from the primary tumor. This step initiates secondary tumor formation [1, 7, 8]. Soluble forms of adhesion molecules and carbohydrate antigens attract attention for their potential prognostic significance in metastasis and patient survival [9–11].

Altered carbohydrate structures occur not only on the surface of cancer-transformed cells, but also in circulating glycoproteins [12–15]. For some acute phase proteins the immunomodulatory role due to their glycosylation was suggested [12]. According to that opinion, overexpression of sialyl-Lewis<sup>x</sup> tetrasaccharide in the oligosaccharides

of  $\alpha_1$ -acid glycoprotein (AGP) during inflammation supports the immune system on the way of competitive blocking of selectin interaction with their counterparts – sialylated and fucosylated glycoconjugates. Such interactions may be of considerable importance in the process of leukocyte extravasation as well as in metastasis. Overexpression of sialyl-Lewis<sup>x</sup> antigen was proved mainly for  $\alpha_1$ -acid glycoprotein [12]. In the inflammatory state, this epitope was found with substantially smaller frequency in the other positive acute phase proteins, like haptoglobin and  $\alpha_1$ -antichymotrypsin [13]. The increase of sLe<sup>x</sup> was found in serum haptoglobin of patients with breast and ovarian cancer [14].

The most common tools for studying fucosylation changes are specific lectins [13–16]. Three of them are applied most often: *Aleuria aurantia* (AAA), *Lotus tetragonolobus* (LTA) and *Ulex europaeus* (UEA) ones. All of them are specific for the  $\alpha$  fucose residue, but their ability for recognizing the direct neighborhood of this monosaccharide is substantially different. *Aleuria aurantia* lectin recognizes fucose in any possible binding position, though with different affinity; LTA is highly specific for Lewis<sup>x</sup>, and UEA for O(H) blood group determinants [17–19]. Detailed binding preferences of all three lectins are shown in Table 1.

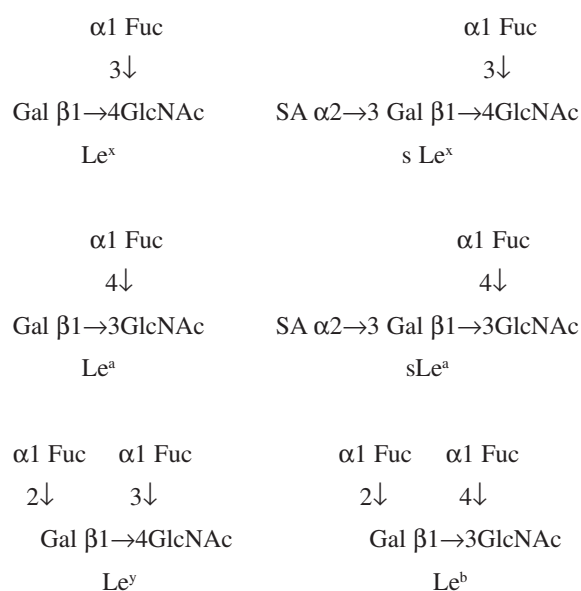
In the current work the authors have examined the alterations in fucose expression in oligosaccharides of haptoglobin isolated from ascites fluids of ovarian cancer patients. The authors have focused on elucidation of the type of fucose binding in glycan chains of this glycoprotein. Further goal was to find out if possible fucosylation changes are followed by overexpression of sialyl Lewis<sup>x</sup> epitope. Detailed characteristic of glycan structures produced by tumor cells and present in body fluids may lead to their application as prognostic factors [9–11]. Such information may also help in design of specific antimetastatic therapy.

## Material and Methods

### Clinical Subjects

Ascites fluids were collected from seven patients suffering from ovarian cancer of advanced, III and IV<sup>o</sup> FIGO grade, treated in the Gynaecology and Obstetrics Clinic, Wrocław Medical University. These samples were a kind gift from Professor J. Gerber. The samples were stored at –20°C before haptoglobin isolation.

Control sample was the haptoglobin isolated from pooled sera of 44 healthy volunteers of both sexes and different age (median 38 years), attend-



**Fig. 1.** Detailed structures of Lewis-type antigens. Gal – galactose, GlcNAc – N-acetyl-glucosamine, SA – sialic acid, Le – Lewis, sLe – sialyl-Lewis

**Ryc. 1.** Struktury antygenów typu Lewis, Gal – galaktoza, GlcNAc – N-acetyloglukozamina, SA – kwas sjałowy, Le – Lewis, sLe – sjalo-Lewis

ing control laboratory tests at Lower Silesia Medical Diagnostic Center Dolmed.

### Lectins, Conjugates and Antibodies

Biotinylated UEA, LTA and extravidin-alkaline phosphatase (AP) conjugate were purchased from Sigma, digoxigenin-labelled AAA, SNA (*Sambucus nigra* agglutinin), MAA (*Maackia amurensis* agglutinin) and AP-conjugated anti-digoxigenin antibodies (Fab fragments) from Boehringer Mannheim. Anti-mouse IgG and IgM AP-conjugate was purchased from Chemicon. Goat anti-human haptoglobin polyclonal antibodies were obtained in the collaboration with Dr. Stefaniak from the Department of Veterinary Prevention and Immunology, Wrocław Agriculture Academy. Anti-sialyl Lewis<sup>x</sup> antibodies (IgM class) were a kind gift from Professor D. Duś from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences.

### Haptoglobin Isolation and Determination

Haptoglobin from ascites fluids was isolated in standard procedure basing on DEAE-cellulose ion-exchange chromatography [20]. Control haptoglobin was isolated from the pooled sera in affinity chromatography on Sepharose immobilized two monoclonal anti-human-Hp antibodies [21]. The oligosaccharide structure of this control haptoglobin preparation was previously elucidated in detail [22]. All haptoglobin preparations were shown to be pure in SDS-PAGE followed by silver staining and in the Scheidegger immunoelectrophoresis.

Haptoglobin concentration in the isolated preparations was determined with Laurell's rocket immunoelectrophoresis against polyclonal anti-human Hp antibodies.

### Desialylation of Haptoglobin

One hundred micrograms of each haptoglobin was desialylated with 7.5 mU of *Clostridium perfringens* neuraminidase. The reaction was carried out for 16 hours at room temperature. The efficiency of desialylation was checked with dot-blot reaction with sialic acid specific lectins. Haptoglobin samples after neuraminidase treatment were spotted on nitrocellulose (NC) paper. After blocking with 0.1% Tween-TBS the NC was incubated with digoxigenin labelled SNA or MAA, and next with anti-digoxigenin AP-conjugate. After another extensive washing phosphatase reaction was developed with NBT and X-phosphate (Sigma) as the substrates. No positive reaction was found in desialylated samples.

### Lectin-ELISA

For studying haptoglobin – lectin interactions modified Goodarzi and Turner's [23] procedure was applied. For the reaction with AAA the microplate (Nunc MaxiSorb) wells were coated with 200 ng of haptoglobin by two hours incubation at 37°C in TBS (10 mM Tris-HCl pH 7.4 with 0.15 M NaCl). The plate was then washed 4-fold and blocked with 0.1% Tween-TBS by 1 h incubation at 37°C and overnight at 4°C. The plate was incubated with digoxigenin-labelled AAA (2 µg/ml) for 1 h at 37°C. After washing out the excess of lectin the plate was incubated at the same conditions with AP-labelled anti-digoxigenin antibodies. After another extensive washing the phosphatase reaction was developed in 0.1 M diethanolamine buffer, pH 9.8, containing 1 mM MgCl<sub>2</sub>; using 1 mg/ml pNPP as a substrate. The reaction was stopped with 1 M NaOH and the absorbance was read at 405 nm (with reference filter 630 nm) at the StatFax 2000 microplate reader.

For the reaction with UEA and LTA the wells of the plate were coated with 200 ng of Hp or asialo-Hp as described above. The following changes were introduced to the procedure: 10 mM phosphate buffered saline was used instead of TBS, and 0.5% BSA (bovine serum albumin) was applied as a blocking agent. Biotinylated UEA and LTA lectins were used at 10 µg/ml concentration; extra-vidin-AP (1 : 10 000) was a conjugate.

Detailed specificity of lectins is shown in Table 1.

**Table 1.** Specificity of lectins used for the study

**Tabela 1.** Swoistość lektyn stosowanych w badaniach

Lectin (Lektyna)	Specificity (Swoistość)	References (Piśmiennictwo)
<i>Aleuria aurantia</i>	Fuc α1–6 GlcNAc (core region) – (część rdzeniowa) >> Fuc α1–2 Gal > Fuc α1–3 GlcNAc (Le <sup>x</sup> )	[17]
<i>Lotus tetragonolobus</i>	Fucα1–3 GlcNAc (Le <sup>x</sup> ) (terminal sialic acid prevents binding) (końcowy kwas sjałowy uniemożliwia wiązanie)	[18]
<i>Ulex europaeus</i>	Fuc α1–2 Gal, O(H), (sialic acid limits binding) (kwas sjałowy ogranicza wiązanie); Fuc α1–2 Gal α1(Fucα1–3)–4 GlcNAc (Le <sup>y</sup> )	[19]

## Sialyl-Lewis<sup>x</sup> Determination

The wells of the microplate were coated with 200 ng of haptoglobin as described above. The procedure was carried out according to Kątnik et al. [14]. Briefly, 0.5% BSA and 0.1% Tween in 1mM Tris-HCl with 0.015M NaCl was used for blocking, 1 mM Tris-HCl with 0.015 M NaCl buffer for washing procedures and 0.25% BSA in 1 mM Tris-HCl with 0.015 M NaCl buffer as a solvent for anti-sLe<sup>x</sup> antibodies and anti-IgM-AP conjugate. The incubation conditions and the development of the phosphatase reaction were the same as in the lectin-ELISA procedures.

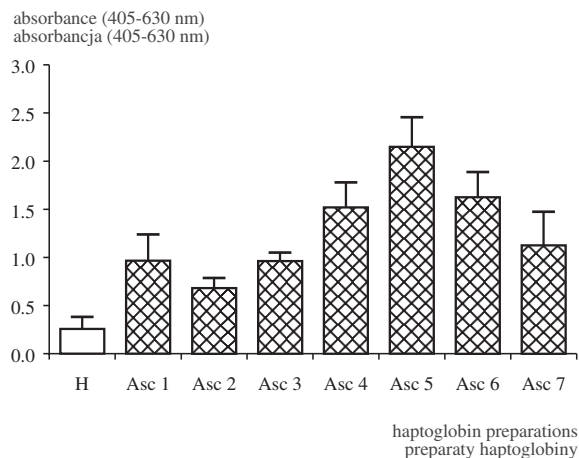
In all ELISA procedures the results were shown as the mean value of two experiments, each with triplicate sampling.

## Results

Reactivity of haptoglobin preparations with fucose specific lectins is shown on Figures 2 and 3. All seven haptoglobins isolated from the ascites fluids of the ovarian cancer patients showed significantly increased reactivity with the *Aleuria aurantia* lectin when compared to control haptoglobin of healthy donors (Fig. 2). The lowest absorbance obtained for the cancer sample was almost twice as high as the control one. In three cancer Hp preparations the absorbance value exceeded the control one trice, in another three it was 4–6 times higher.

Native haptoglobins, control as well as these of cancer origin, did not bind both *Lotus tetragonolobus* and *Ulex europaeus* lectins (Fig. 3A, B). The only exception was haptoglobin of ascites no 2, in which a weak reactivity was found for both lectins, however, it did not increase after desialylation. Haptoglobin obtained from healthy individuals after neuraminidase treatment leading to complete release of terminal sialic acid still reacted only slightly with LTA. Three out of seven cancer haptoglobins showed a weak reactivity with the lectin. In another three haptoglobins the reactivity was elevated to significant values, exceeding 5–7 times the absorbance obtained for control Hp.

Similar results were obtained for the reactivity with *Ulex europaeus* lectin. Desialylated control haptoglobin did not bind the lectin significantly. The reactivity of cancer asialo-haptoglobins was elevated in all (except Asc 2) samples, though in four out of seven it was still low (Fig. 3B), with the absorbance not exceeding 0.1 value. Three cancer haptoglobins, the same as highly reactive with LTA, showed high reactivity with UEA as well.



**Fig. 2.** *Aleuria aurantia* reactivity of haptoglobins.

H – haptoglobin isolated from healthy subjects, Asc – haptoglobin isolated from the ascites fluids

**Ryc. 2.** Reaktywność haptoglobiny z lektyną z *Aleuria aurantia*. H – haptoglobina izolowana z surowicy osób zdrowych, Asc – haptoglobina izolowana z poszczególnych płynów wysiękowych

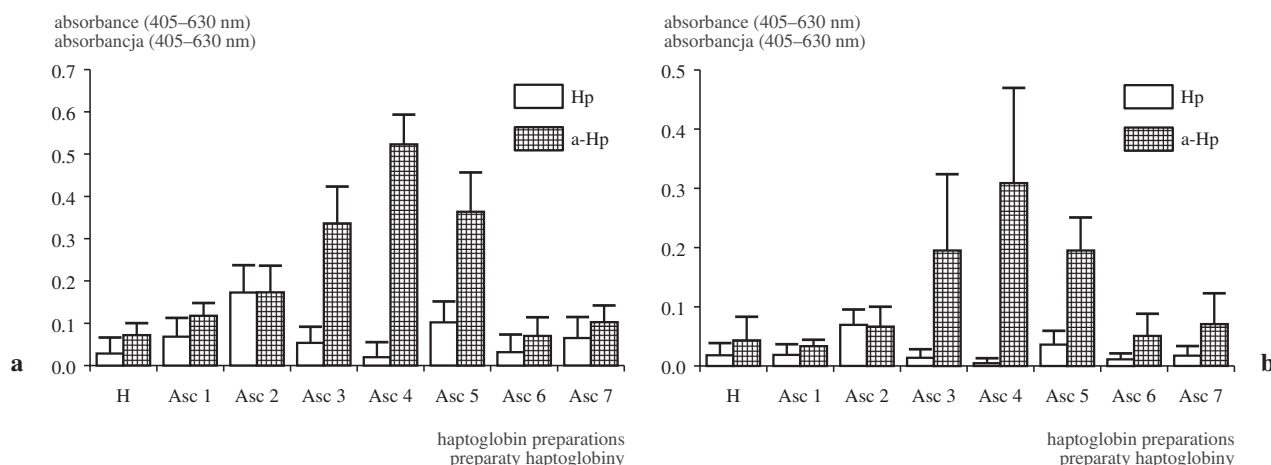
Significantly elevated reactivity with sialyl-Lewis<sup>x</sup> antibodies was found in only three out of seven cancer haptoglobins (Fig. 4). The increase of reactivity reached 40 to 60% of the absorbance in ELISA test. Only one Hp preparation, Asc 5, showed the increase of both LTA and anti-sLe<sup>x</sup> activity, Asc 3 and Asc 4 haptoglobins reacted strongly with LTA but not with anti-sLe<sup>x</sup>, in Asc 6 and Asc 7 strong binding of anti sLe<sup>x</sup> but not LTA was found (Fig. 3, 4).

## Discussion

Haptoglobin, containing about 20% of carbohydrate moiety, is not only an important acute phase marker, but also an indicator of the glycosylation process. Haptoglobin of ovarian cancer ascites fluids, though partly of serum origin, may also considerably reflect the functional state of tumour cells glycosylation pathway. For the purpose of this study, the authors decided to purify haptoglobin for the detailed fucosylation analysis to avoid interference of sugar moieties with the other soluble glycoconjugates. As the isolation procedures need a significant amount of the material, haptoglobin obtained from pooled sera and characterized in detail was applied as a reference instead of its purification from individual control samples.

Fucosylated glycoconjugates on the cell surface are engaged in cell migration and colonization, including cancer cell invasiveness [7, 15, 24]. Inhibition of selectin-mediated extravasation by





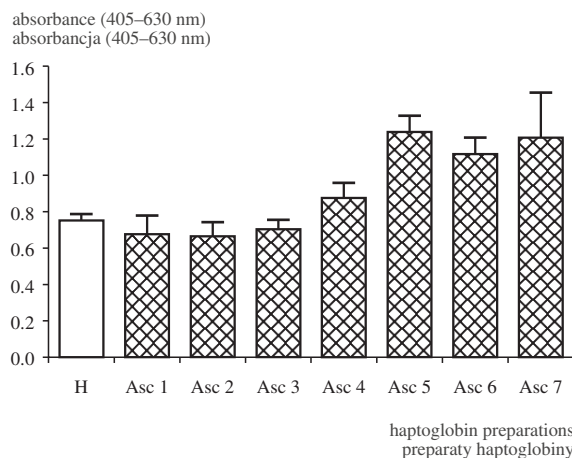
**Fig. 3.** Reactivity of haptoglobin (Hp) and asialo-haptoglobin (a-Hp) with *Lotus tetragonolobus* and *Ulex europaeus* lectins: **a** – LTA, **b** – UEA. H – haptoglobin from healthy subjects, Asc – haptoglobin from ascites fluids

**Ryc. 3.** Reaktywność haptoglobiny (Hp) i asialo-haptoglobiny (a-Hp) z lektynami z *Lotus tetragonolobus* i *Ulex europaeus*: **a** – LTA, **b** – UEA. H – haptoglobina osób zdrowych, Asc – haptoglobina z płynów wysiękowych

supplementation of exogenous oligosaccharides could be helpful in preventing metastasis [6, 8, 26, 27]. Endogenous sugar structures, expressed on the circulating glycoproteins, probably can also mimic Lewis structures and interfere in recognition process. Such immunomodulatory role was already suggested for inflammatory AGP [12].

The structure of fucose containing oligosaccharides is variable [28–30]. The occurrence and importance of both 1–3 and 1–6 fucose links for the development of cancer was suggested so far [2, 14, 28, 29]. Serum  $\alpha$ -fetoprotein (AFP) of patients with hepatocarcinoma and cancer haptoglobin were reported to contain  $\alpha$ 1–6 bound fucose [15, 29]. Although sLe<sup>x</sup> or Le<sup>a</sup> overexpression of cancer cells enables or facilitates extravasation, the possible role of  $\alpha$ 1–6 fucosylation is still not clear [2, 27, 28].

Haptoglobin isolated from ovarian cancer ascites fluids is highly fucosylated, when compared to serum haptoglobin of healthy subjects. The increase of AAA reactivity, highly variable regarding its value, was significant in all examined subjects. *Aleuria aurantia* lectin recognizes all possible types of fucose linkages. In AGP there is no  $\alpha$ 1–6 fucosylation, so AAA-reactivity is actually associated with Le<sup>x</sup> type glycans in this particular glycoprotein, but not necessarily in Hp. Native ovarian cancer haptoglobins did not react with LTA and UEA, indicating the absence of Le<sup>x</sup> or Le<sup>y</sup> (without terminal sialic acid) structures. In one case, Asc 2, native haptoglobin reacted slightly with both LTA and UEA (Fig 2); in this only case desialylation was not followed by the increase of lectin reactivity. This suggested the existence of bifucosylated Le<sup>y</sup>, in which the sec-



**Fig. 4.** Haptoglobin reactivity with anti-sialyl Lewis<sup>x</sup> antibodies. H – haptoglobin isolated from healthy subjects, Asc – haptoglobin isolated from the ascites fluids

**Ryc. 4.** Reaktywność haptoglobiny z przeciwciałami anty-sialoLewis<sup>x</sup>. H – haptoglobina osób zdrowych, Asc – haptoglobina z płynów wysiękowych

ond fucose molecule is  $\alpha$ 1–2 bound to galactose. Desialylation uncovered epitopes reactive to LTA and UEA in cancer, but not in control reference asialo-Hp, which is in good accordance with the earlier findings [22]. In three out of seven ascites fluids haptoglobins the elevation of  $\alpha$ 1–3 fucose content was extremely high. Similarity of LTA and UEA reactivity resulted rather from the less selective specificity of the latter lectin than the possible abundance of 1–2 linkage.

Comparison of the reactivity of ascites fluids haptoglobins with AAA and both LTA and UEA indicates that apart from  $\alpha$ 1–3, high content of

$\alpha$ 1–6 fucose linkage is present in cancer Hp glyco-cans. This suggests glycosylation different from inflammatory AGP, but similar to cancer AFP. The questions open, if  $\alpha$ 1–6 fucosylation could be more specific for cancer transformation than  $\alpha$ 1–3 one and sLe<sup>x</sup> appearance, or is this feature rather due to different glycosylation mechanism of particular glycoproteins.

Alpha 1–3 fucosylation of haptoglobins from ascites fluids contributed to sLe<sup>x</sup> expression only to the limited extent. The high reactivity of haptoglobin with anti sLe<sup>x</sup> antibodies concomitant with high LTA binding was found only in two out of seven samples (Asc 4 and Asc 5). This indicates the role of another monosaccharide forming sLe<sup>x</sup> epitope – terminal sialic acid (SA) linked  $\alpha$ 2–3 to galactose. The occurrence of  $\alpha$ 2–3 bound SA is limited in normal Hp [22] and probably only

slightly increases in examined cancer haptoglobins. So haptoglobin seems to be a weaker carrier of terminal  $\alpha$ 2–3 SA than AGP, in which sLe<sup>x</sup> structures tend to overexpress. Probably the linkage of SA and not fucosylation is responsible for the lesser expression of sLe<sup>x</sup> in haptoglobin.

This study indicates that fucosylation of cancer originated haptoglobin is high, though not necessary followed by sLe<sup>x</sup> expression. The contribution of core  $\alpha$ 1–6 linkage and possibly also  $\alpha$ 1–2 Gal bond forming Le<sup>y</sup> is considerable. It should be concluded that to obtain more information on glycosylation changes of soluble glycoproteins, accompanying cancer transformation, fucosylation and sialylation should be examined as independent features. Further study on extended clinical material would be advisable, to evaluate glycans alterations as diagnostic or prognostic markers.

## References

- [1] Ørntoft TF, Vestergaard EM: Clinical aspects of altered glycosylation in cancer. *Electrophoresis* 1999, 20, 362–371.
- [2] Scanlin TF, Glick MC: Terminal glycosylation and disease: Influence on cancer and cystic fibrosis. *Glycoconj J* 2000, 17, 617–626.
- [3] Ivanova R, Soares P, Castro P, Sobrinho-Simões M: Diffuse (or multinodular) follicular variant of papillary thyroid carcinoma: a clinicopathologic and immunohistochemical analysis of ten cases of aggressive form of differentiated thyroid carcinoma. *Virchows Arch* 2002, 440, 418–424.
- [4] Nakagoe T, Sawai T, Tsuji T, Jibiki M-A, Nanashima A, Yamaguchi H, Yasutake T, Ayabe H, Arisawa K, Ishikawa H: Predictive factors for preoperative serum levels of sialyl Lewis<sup>x</sup>, sialyl Lewis<sup>a</sup> and sialyl Tn antigens in gastric cancer patients. *Anticancer Res* 2002, 22, 451–458.
- [5] Sumikura S, Ishigami S, Natsugoe S, Miyazono F, Tokuda K, Nakajo A, Okumura H, Matsumoto M, Hokita S, Aikou T: Disseminated cancer cells in the blood and expression of sialylated antigen in gastric cancer. *Cancer Let* 2003, 200, 77–83.
- [6] Sharar SR, Winn RK, Harlan JM: The adhesion cascade and anti-adhesion therapy: an overview. *Springer Semin Immunopathol* 1995, 16, 359–378.
- [7] Bironaite D, Nesland JM, Dalen H, Risberg B, Bryne M: N-Glycans influence the in vitro adhesive and invasive behaviour of three metastatic cell lines. *Tumor Biol* 2000, 21, 165–175.
- [8] Ravindranath NMH, Nishimoto K, Chu K, Shuler C: Cell-surface expression of complement restriction factors and sialyl Lewis antigens in oral carcinoma: relevance to chemo-immunotherapy. *Anticancer Res* 2000, 20, 21–26.
- [9] Milović M, Popov I, Jelić S: Tumor markers in metastatic disease from cancer of unknown primary origin. *Med Sci Monit* 2002, 8, MT25–30.
- [10] Wołowicz D, Frydecka I, Kapelko-Słowik K, Potoczek S, Urbaniak-Kujda D, Kuliczowski K: Blood serum levels of soluble forms of adhesion molecules ICAM I (sICAM-1) and selectin E (s-selectin E) in patients with various phases of non-Hodgins lymphomas. *Med Sci Monit* 2002, BR175–178.
- [11] Brown JR, Fuster MM, Whisenant T, Esko JD: Expression patterns of  $\alpha$ 2,3-sialyltransferases and  $\alpha$ 1,3 fucosyltransferases determine the mode of sialyl Lewis X inhibition by disaccharide decoys. *J Biol Chem* 2003, 278, 23352–23359.
- [12] Van Dijk W, Brinkman-Van der Linden EMC, Havenaar EC: Glycosylation of  $\alpha$ <sub>1</sub>-acid glycoprotein (orosomucoid) in health and disease: occurrence, regulation and possible functional implications. *Trends Glycosci Glycotechnol* 1998, 10, 235–245.
- [13] Brinkman-Van der Linden EMC, de Haan PF, Havenaar EC, Van Dijk W: Inflammation induced expression of sialyl Lewis<sup>x</sup> is not restricted to  $\alpha$ <sub>1</sub>-acid glycoprotein but also occurs to a lesser extent on  $\alpha$ <sub>1</sub>-antichymotrypsin and haptoglobin. *Glycoconj J* 1998, 15, 177–182.
- [14] Kałnik I, Goodarzi MT, Turner GA: An improved ELISA for the determination of sialyl Lewis<sup>x</sup> structures on purified glycoconjugates. *Glycoconj J* 1996, 13, 1043–1047.
- [15] Dargan E, Thompson S, Cantwell BMJ, Wilson RG, Turner GA: Changes in the fucose content of haptoglobin in breast and ovarian cancer: association with disease progression. *Glycosyl & Dis* 1994, 1, 37–43.
- [16] Haselhorst T, Weimar T, Peters T: Molecular recognition of sialyl Lewis(x) and related saccharides by two lectins. *J Amer Chem Soc* 2001, 123, 10705–10714.

- [17] Yamashita K, Kochibe N, Ohkura T, Ueda I, Kobata A: Fractionation of L-fucose containing oligosaccharides on immobilized *Aleuria aurantia* lectin. J Biol Chem 1985, 260, 4688–4693.
- [18] Yan L, Wilkins PP, Alvarez-Manilla G, Do SI, Smith DF: Immobilized *Lotus tetragonolobus* agglutinin binds oligosaccharides containing the Le<sup>x</sup> determinant. Glycoconj J 1997, 14, 45–55.
- [19] Loris R, De Greve H, Dao-Thi MH, Messens J, Imberty A, Wyns L: Structural basis of carbohydrate recognition by lectin II from *Ulex europaeus*, a protein with a promiscuous carbohydrate-binding site. J Mol Biol 2000, 301, 987–1002.
- [20] Dobryszczyka W, Lisowska E: Effect of degradation on the chemical and biological properties of haptoglobin. I. Product of trypsin digestion. Biochim Biophys Acta 1966, 121, 42–49.
- [21] Kątnik I, Jadach J, Krotkiewski H, Gerber J: Investigating the glycosylation of normal and ovarian cancer haptoglobins using digoxigenin-labelled lectins. Glycosyl & Dis 1994, 1, 97–104.
- [22] Ferens-Sieczkowska M, Olczak M: Carbohydrate structures of haptoglobin in sera of healthy people and a patient with congenital disorder of glycosylation. Z Naturforsch 2001, 56c, 122–131.
- [23] Goodarzi MT, Turner GA: A lectin-binding assay for the rapid characterization of the glycosylation of purified glycoproteins. In: The protein protocols handbook. Ed.: Walker JM, Humana Press Inc. Totowa, New Jersey 1996, 619–625.
- [24] Yamashita K, Koide N, Endo T, Iwaki Y, Kobata A: Altered glycosylation of serum transferrin of patients with hepatocellular carcinoma. J Biol Chem 1989, 264, 2415–2423.
- [25] Kaila N, Thomas BE IV, Thakker P, Alvarez JC, Camphausen RT, Crommie D: Design and synthesis of sialyl Lewis x mimics as E-selectin inhibitors. Bioorg Med Chem Let 2001, 11, 151–155.
- [26] Thoma G, Bänthoma R, Jahnke W, Magnani JL, Patton JT: A readily available, highly potent E-selectin antagonist. Angew Chem Int Ed 2001, 40, 3644–3647.
- [27] Miyoshi E, Yukihiro K, Yamaguchi Y: The  $\alpha$ 1–6 fucosyltransferase gene and its biological significance. Biochim Biophys Acta 1999, 1473, 9–20.
- [28] Mita Y, Aoyagi Y, Suda T, Asakura H: Plasma fucosyltransferase activity in patients with hepatocellular carcinoma, with special reference to correlation with fucosylated species of alpha-fetoprotein. J Hepatol 2000, 32, 946–954.
- [29] de Vries T, Knegtel RMA, Holmes EH, Macher BA: Fucosyltransferases: structure/function studies. Glycobiol 2001, 11, 119R–128R.
- [30] Kijima H, Kashiwagi H, Dowaki S: Stromal sialyl Le<sup>a</sup> expression is correlated with vascular invasion of human gallbladder adenocarcinoma. Int J Oncol 2000, 17, 55–60.

### Address for correspondence:

Mirosława Ferens-Sieczkowska  
Department of Chemistry and Immunochemistry, Wrocław Medical University  
Bujwida 44A  
50-345 Wrocław  
e-mail: mferens@immchem.am.wroc.pl

Received: 23.02.2004

Revised: 25.03.2004

Accepted: 25.03.2004

Praca wpłynęła do Redakcji: 23.02.2004 r.

Po recenzji: 25.03.2004 r.

Zaakceptowano do druku: 25.03.2004 r.