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Effect of cytokines on glycosylation of acute phase proteins in human hepatoma cell lines

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SUMMARY

The effects of various cytokines on synthesis and microheterogeneity of carbohydrate structure of α_1-proteinase inhibitor (PI) and alpha-fetoprotein (AFP) in the human hepatoma cell lines Hep 3B and Hep G2 were studied. In both lines, crude cytokine preparations from LPS-activated human monocytes (CM) and several cell lines led to increased PI and decreased AFP synthesis, while recombinant interleukin 1 (IL-1), recombinant tumor necrosis factor (TNF) and hepatocyte stimulating factor preparations (HSF) affected AFP but not PI production. Several of the crude cytokine preparations, but not IL-1, TNF, or HSF, caused Hep 3B cells to secrete forms of PI and AFP showing increased reactivity with Con A upon testing by affinity electrophoresis, while decreased reactivity with Con A was seen in these proteins secreted by Hep G2 cells. Determination of molecular size of PI inducing activity in CM showed a sharp peak at about 17 kD while AFP inhibiting activity was present in a very broad range of molecular size fractions maximal at 17–30 kD. Changes in patterns of glycosylation of these proteins were attributable to cytokines of about 30 kD in Hep 3B and 44 kD in Hep G2 cells. These findings demonstrate the existence of a family of glycosylation regulating cytokines, and suggest that distinct mechanisms within hepatocytes, responsive to different cytokines, may lead to increased or decreased Con A binding of glycoproteins and to altered gene expression.

Keywords cytokines glycosylation acute phase proteins α_1-proteinase inhibitor, alpha-fetoprotein

INTRODUCTION

Infection and tissue injury lead to changes in concentration of some plasma proteins, the acute phase proteins (APP), resulting from up-regulation (positive APP) and down-regulation (negative APP) of gene expression in hepatocytes (Gordon & Koj, 1985; Kushner & Mackiewicz, 1987). There is considerable heterogeneity in the effect of cytokines on expression of APP genes (Ganapathi *et al.*, 1988) and a number of different cytokines have been implicated as mediating the induction of APP, including interleukin 1 (IL-1), tumor necrosis factor α/cachectin (TNF) and hepatocyte stimulating factors (HSF), at least one of which appears to be closely related or identical to interferon β_2/IL-6 (Gauldie *et al.*, 1987; May *et al.*, 1988).

Changes of APP levels in plasma are often accompanied by changes in the pattern of binding of these proteins to the lectin Concanavalin A (Con A) reflecting alterations in structure of heteroglycan side chains. Several studies indicate that increasing degrees of binding to Con A are correlated with an increased proportion of bi-antennary side chain units, as opposed to tri- and tetra-antennary units (Vaughan, Lorier & Carrell, 1982; Bierhuizen *et al.*, 1988). In certain clinical states there is a substantial relative increase in Con A binding of plasma glycoproteins (Nicollet *et al.*, 1981; Raynes, 1982; Mackiewicz *et al.*, 1987a) while in others there is a significant relative decrease (Serbouce-Goguel Seta *et al.*, 1986; Mackiewicz *et al.*, 1987b). We have recently shown that one of these two types of change in APP glycosylation can be attributed to alterations occurring within hepatocytes as a result of regulation by a monokine(s) (Mackiewicz *et al.*, 1987), and that mechanisms involved in regulation of APP synthesis may differ from those responsible for alterations in glycosylation.

The present study was carried out to define further the mechanisms involved in regulation of changes in glycosylation of APP employing two human hepatoma cell lines, Hep 3B and Hep G2. As marker glycoproteins we used α_1-proteinase

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Table 1. Alpha-fetoprotein (AFP) and α_1-proteinase inhibitor (PI) synthesized by Hep 3B and Hep G2 cell lines after 72 h exposure to cytokines

	*n**		AFP (%)		αPI (%)	
Cytokine preparation	Hep 3B	Hep G2	Hep 3B	Hep G2	Hep 3B	Hep G2
Control	16	9	100	100	100	100
CM (10%)	16	9	22±2†	26±4†	311±31†	234±42†
COLO 16 (10%)	8	4	49±5†	55±4†	165±7†	178±16†
HUVE (10%)	2	2	101±1	101±3	101±2	98±3
HUVE+LPS (10%)	4	4	24±2†	45±2†	250±11†	241±7†
U937 (10%)	2	2	99±1	100±2	99±1	99±3
U937+PMA+LPS (10%)	2	2	45±3†	42±3†	201±5†	187±9†
IL-1β (12·5 ng/ml)	10	2	41±3†	45±2†	103±9	103±6
TNFα (250 ng/ml)	7	2	51±4†	53±3†	101±11	101±5
IL-1+TNF	2	2	32±2†	37±4†	102±2	101±3
HSF(COLO 16) (10%)	3	ND	61±3†	ND	99±3	ND
HSF(CM) (10%)	2	ND	65±2†	ND	98±4	ND

Results are expressed as percentages of the control, mean ± s.d.
* Number of experiments.
† $P < 0{\cdot}01$.
ND not done.

inhibitor (PI), a positive APP, and alpha-fetoprotein (AFP), which appears to be a negative APP in the rat (Magielska-Zero *et al.*, 1987).

MATERIALS AND METHODS

Conditioned medium (CM) was prepared as described previously (Mackiewicz *et al.*, 1987). For size fractionation, CM was concentrated 65-fold using a 10,000 mol. wt. cut-off ultrafiltration membrane (PTGC, Millipore Corporation, Bedford, MA) and the pH was adjusted to 7·2–7·4. After addition to a final concentration of 10 mM HEPES and 0·5% octyl α-D-glucopyranoside (Calbiochem, La Jolla, CA), 0·5 ml aliquots of the concentrated supernatant were applied to a Superose 12 high pressure liquid chromatography gel filtration column, and eluted with 10 mM HEPES, 0·15 M NaCl, pH 7·0 and 0·71 ml fractions were collected. The column was calibrated using protein molecular weight standards obtained from BioRad (Richmond, CA).

After subculture U937 cells were washed three times by suspending in Hank's balanced salt solution with Ca^{2+} and Mg^{2+} and centrifuging at room temperature in a swinging bucket rotor for 15 min at 1,800 rpm. The final cell pellet was resuspended in 30 ml of serum free RPMI 1640 with tobramycin (50 μg/ml) at a density of 1×10^6 cells/ml (control supernatants). Conditioned medium from differentiated induced cells was prepared by adding 5 mM phorbol 12-myristate 13-acetate (PMA) and 20 μg/ml LPS. Control and induced cultures were incubated in 75 ml Falcon tissue culture flasks for 48 h at 37°C in a humidified atmosphere (5% CO_2/95% air).

Both cell lines were maintained after subculture in 60 mm plastic Falcon dishes at 37°C, in a humidified atmosphere of 5% CO_2/95% air in RPMI 1640 supplemented with 10% fetal calf serum, dexamethasone (1 μM), insulin (0·02 U/ml) and tobramycin (50 μg/ml) for 5 days and then for 24 h in serum-free RPMI 1640 with dexamethasone and insulin. At this point (time 0),

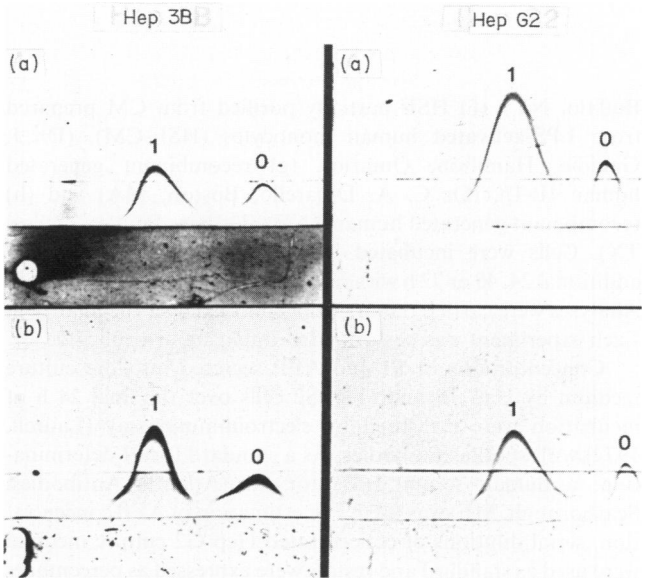

Fig. 1. Microheterogeneous forms of alphafetoprotein. Affinity electrophoresis with Con A as a ligand of AFP synthesized by human hepatoma cell lines: Hep 3B (left panel) and Hep G2 (right panel). (a) Control, (b) induction with 10% CM. Variant 0—non-reactive with Con A, variant 1—reactive with Con A. For electrophoresis in the first dimension the anode was to the right.

Hep 3B and Hep G2 cells were exposed to a number of inducers of APP changes: (a) CM prepared from LPS-activated human monocytes, (b) medium prepared from PMA-transformed and LPS-activated histiocytic line U937 cells, known to contain HSF activity, (c) medium from human umbilical vein epithelial cells (HUVE) cultured for 48 h in the presence and absence of LPS (Dr M. Ziff, Dallas, TX), (d) medium from the keratocarcinoma cell line, Colo 16, which contains HSF activity, (e) Colo 16 derived purified HSF I (Baumann, *et al.*, 1986) (Dr H. Bauman,

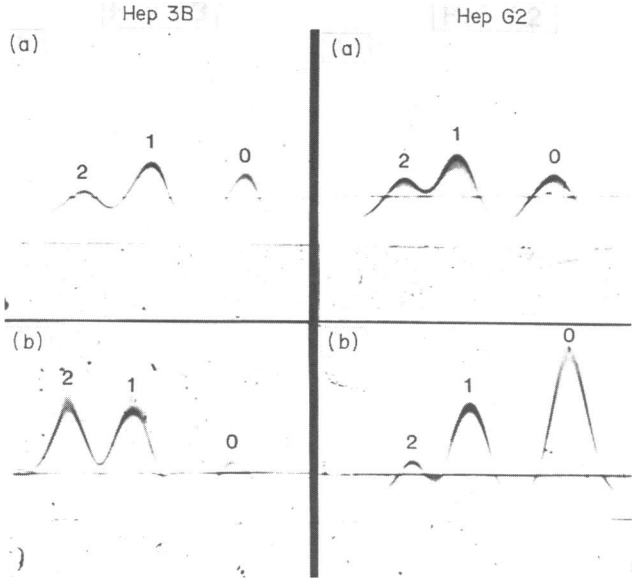

Fig. 2. Microheterogeneous forms of α_1-antiproteinase. Affinity electrophoresis with Con A as a ligand of αPI synthesized by Hep 3B cells (left panel) and Hep G2 (right panel). (a) Control, (b) induction with 10% CM. Variant 0—non-reactive with Con A, variant 1—weakly reactive and variant 2—strongly reactive with Con A.

Buffalo, NY), (f) HSF partially purified from CM prepared from LPS-activated human monocytes (HSF-CM), (Dr J. Gauldie, Hamilton, Ontario), (g) recombinant generated human IL-1β, (Dr C. A. Dinarello, Boston, MA) and (h) recombinant generated human TNFα (Dr B.A. Beutler, Dallas, TX). Cells were incubated with these preparations for an additional 24, 48 or 72 h with replacement of medium every 24 h. Analyses were carried out in media collected over the final 24 h. Each experiment was performed in duplicate or triplicate

Concentrations of PI and AFP secreted into the culture medium by Hep 3B and Hep G2 cells over the final 24 h of incubation were measured by electroimmunoassay (Laurell, 1972) with specific antibodies. As a standard for PI determination, a human serum calibrator kit (Atlantic Antibodies, Scarborough, ME) was used. For estimation of AFP concentration, serial dilutions of concentrated Hep G2 culture medium were used as standard and results were expressed as percentages of the control. Because we have previously shown (Ganapathi *et al.*, 1988) that increased accumulation of PI in Hep 3B medium was parallel to increase of newly synthesized ^{35}S-methionine labelled PI and increase in its intracellular mRNA concentration, indicating regulation at a pre-translational level, the terms accumulation and synthesis will be used interchangeably throughout this paper.

Microheterogeneity of PI and AFP was studied by agarose affinity-electrophoresis (AFF-EP) with free Con A as a ligand (Mackiewicz & Mackiewicz, 1986; Mackiewicz *et al.*, 1987). This method has been shown to reflect findings obtained when mixtures of proteins are passed over columns of immobilized Con A (Bog-Hansen, 1973). The area under the precipitate was measured by planimetry and the relative amounts of the various microheterogeneous forms were expressed as percentages of the total. For each sample, a reactivity coefficient (RC) was calculated according to the formula: sum of Con A reactive variants/Con A non-reactive variant. All statistical analyses were carried out by the Mann–Whitney test.

RESULTS

As shown in Table 1, changes in accumulation of APP and αPI induced by cytokine preparations were consistently parallel in the two cell lines studied. Incubation of both lines with CM, medium from Colo 16, HUVE plus LPS and U937 plus PMA plus LPS caused reduction in synthesis of AFP and increased synthesis of PI. In addition, IL-1β and TNF, separately and in combination, caused decreased accumulation of AFP in both cell lines but did not affect PI synthesis. Similarly, two HSF preparations tested only in Hep 3B cells decreased AFP accumulation, but did not affect PI synthesis. Medium from non-stimulated HUVE cells and from non-transformed and non-stimulated U937 cells had no effect on AFP or PI accumulation in either cell line.

In contrast, cytokine preparations caused opposite changes in patterns of glycosylation of AFP and PI in the two cell lines. Two forms of AFP were found in media obtained from both Hep 3B and Hep G2 lines: one which was non-reactive with Con A (variant 0) and another which was reactive with Con A (variant 1) (Fig. 1). Three microheterogeneous forms of PI secreted into media from both lines were seen (Fig. 2): one which was non-reactive with Con A (variant 0) and two others which were weakly reactive (variant 1) or strongly reactive with Con A (variant 2).

Exposure of Hep 3B cells to CM and medium from Colo 16 and HUVE plus LPS caused a relative increase in Con A-reactive forms of AFP and PI (Figs. 1 and 2), while other preparations tested had no effect (Table 2). In contrast, incubation of Hep G2 cells with CM or HUVE plus LPS caused a relative decrease of reactivity of both AFP and PI with Con A (Figs. 1 and 2). Neither Colo 16 nor the other inducers used affected Con A reactivity of AFP or PI in medium secreted by this cell line (Table 2).

To determine the molecular size of the active cytokines, CM was fractionated by gel permeation chromatography on a Superose 12 column. AFP-inhibiting activity eluted in fractions ranging in size from 10 to 158 kD with peak activity in fractions between 17–30 kD (Fig. 3) in both Hep 3B and Hep G2 cells. The molecular size range of PI inducing activity was much narrower than for AFP, with a sharp peak at about 17 kD for both cell lines (Fig. 4). In contrast to the wide range of AFP inhibiting activity, the size range of AFP glycosylation regulating activity was narrow, with a sharp peak at about 30 kD in Hep 3B cells and at about 44 kD in Hep G2 cells (Fig. 3). Similarly, maximum PI glycosylation regulating activity was observed in fractions of about 30 kD for Hep 3B and 44 kD for Hep G2 cells (Fig. 4).

We evaluated the possibility that monocyte-derived PI (Perlmutter *et al.*, 1985) might contribute to the pattern seen in CM-treated hepatoma cells. No PI was detected by the electroimmunoassay employed in this study. CM concentrated 100-fold revealed only trace amounts of PI which did not reveal the pattern seen on affinity electrophoresis in CM-treated Hep 3B or Hep G2 cultures.

Table 2. Glycosylation patterns of alpha-fetoprotein (AFP) and α_1-proteinase inhibitor (PI) synthesized by Hep 3B and Hep G2 cell lines after 72 h exposure to cytokines

	*n**		AFP (RC)		αPI (RC)	
Cytokine preparation	Hep 3B	Hep G2	Hep 3B	Hep G2	Hep 3B	Hep G2
Control	16	9	2·1±0·2	4·9±0·6	1·7±0·2	2·0±0·2
CM (10%)	16	9	3·4±0·3†	2·3±0·3†	3·6±0·1†	0·7±0·1†
COLO 16 (10%)	8	4	3·0±0·2†	5·0±0·1	2·9±0·2†	2·3±0·3
HUVE (10%)	2	2	2·1±0·2	5·1±0·1	1·8±0·2	2·2±0·1
HUVE+LPS (10%)	4	4	4·2±0·2†	3·4±0·3†	4·9±0·1†	1·1±0·1†
U937 (10%)	2	2	2·0±0·1	4·8±0·4	1·7±0·1	2·1±0·1
U937+PMA+LPS (10%)	2	2	2·1±0·1	4·9±0·3	1·6±0·1	1·9±0·3
IL-1β (12·5 ng/ml)	10	2	2·0±0·1	4·9±0·2	1·7±0·1	2·0±0·2
TNFα (250 ng/ml)	7	2	2·0±0·1	4·7±6·1	1·2±0·1	1·9±0·1
IL-1+TNF	2	2	2·0±0·1	4·9±0·3	1·8±0·1	2·1±0·3
HSF(COLO 16) (10%)	3	ND	2·3±0·1	ND	1·8±0·1	ND
HSF(CM) (10%)	2	ND	1·9±0·1	ND	1·5±0·2	ND

Results are expressed as reactivity coefficient (RC), mean±s.d.
* Number of experiments.
† $P<0·01$.
ND not done.

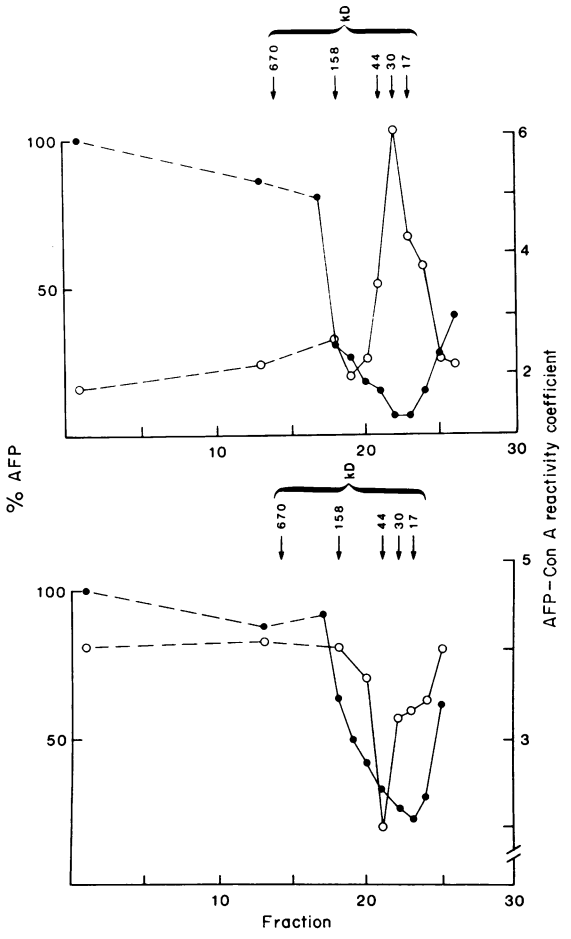

Fig. 3. Fractionation of conditioned medium: effect on alpha-fetoprotein. Effect of fractionated CM on AFP synthesis (●) and Con A reactivity (○) by Hep 3B cells (top) and Hep G2 cells (bottom).

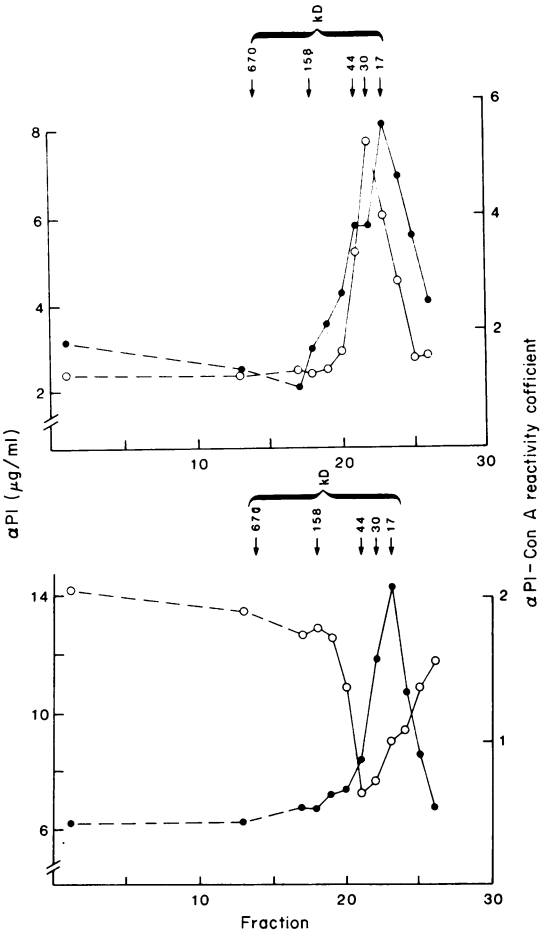

Fig. 4. Fractionation of conditioned medium: effect on α_1-anti-proteinase. Effect of fractionated CM on αPI synthesis (●) and Con A reactivity (○) by Hep 3B cells (top) and Hep G2 cells (bottom).

DISCUSSION

Carbohydrate moieties of human plasma glycoproteins of hepatic origin are composed of *N*-linked glycans presenting a range of branching structures (Hatton, Marz & Regoeczi, 1983). Microheterogeneity of plasma glycoproteins is observed, due in large part to structural variations in bi-, tri- and tetra-antennary heteroglycan side-chains. AFF-EP with the lectin Concanavalin A (Con A) as a ligand has been used widely for the detection of different microheterogeneous forms of APP. The affinity electrophoresis method employed in these studies permits estimation of the relative proportions of the microheterogenous forms of a glycoportein in solution, which differ in their degrees of binding to Con A (Mackiewicz & Mackiewicz, 1986). This simple and convenient method, while not providing a complete description of the precise changes in the glycoproteins under study, does reveal reproducible patterns of change which reflect the patterns of glycosylation of these glycoproteins, and permits evaluation of the effects of cytokines on this process.

The observations that glycoproteins in sera from patients with different inflammatory diseases differ in their patterns of binding to Con A, and that there was no correlation between changes in AGP concentration and changes in AGP Con A reactivity in patients with lupus erythematosus (Mackiewicz *et al.*, 1987a) suggest that changes in patterns of glycosylation are regulated by mechanisms different from those regulating simple induction of acute phase protein synthesis. Initial studies in Hep 3B cells were consistent with this view (Mackiewicz *et al.*, 1987). IL-1, TNFα and HSF preparations led to increased synthesis of ceruloplasmin, but did not alter its pattern of glycosylation, while CM did. In this study we found again that change in rate of synthesis of proteins induced by cytokine preparations was not always accompanied by an altered pattern of glycosylation. In addition, we found that patterns of glycosylation induced by CM and activated HUVE were opposite in the two lines, and that activities were attributable to fractions of apparent different molecular sizes.

Several studies have indicated that human hepatoma cell lines may demonstrate abnormal postsynthetic processing of glycoproteins (Carlson *et al.*, 1984; Goldberger *et al.*, 1984). In our current study, we took advantage of this tranformation-induced 'experiment of nature' to delineate further the mechanisms by which cytokines may regulate alterations in glycosylation of acute phase proteins, reasoning that findings in different cell lines might reveal differential intracellular effects of cytokines which might not be apparent in native hepatocytes. The two human hepatoma cell lines we studied, Hep 3B and Hep G2, were selected because patterns of glycosylation of PI were found to be similar in both lines when cultured under unstimulated conditions, as were glycosylation patterns of AFP.

There are several possible explanations for the effects of cytokines upon glycosylation of these glycoproteins, including effects on one or more glycosylating enzymes or on route or kinetics of intracellular transport. Similarly, the difference between the two hepatoma cell lines in the alterations of glycosylation exhibited undoubtedly reflects differences between these transformed lines in the intracellular mechanisms involved in transduction of extracellular signals regulating glycosylation. Intact pathways of glycosylation regulation are presumably expressed in liver cells of healthy subjects and may be altered by different cytokines in different inflammatory states, alternatively leading to increased or decreased Con A binding.

PI possesses three *N*-linked heteroglycan side chains, while AFP possesses one (Vaughan *et al.*, 1982; Mega, Lujan & Yoshida, 1980). The heteroglycans of human serum PI are composed of bi- and tri-antennary oligosaccharides (Mega *et al.*, 1980). Con A-Sepharose chromatography studies have indicated that the strongly reactive variant of PI is composed of three bi-antennary structures, the weakly reactive variant of two bi- and one tri-antennary unit and the non-reactive variant of one bi- and two tri-antennary units (Vaughan *et al.*, 1982). Thus, the changes in reactivity of PI with Con A observed in the present studies may reflect relative increase (Hep 3B) or decrease (Hep G2) of bi-antennary structures. The Con A reactive form of human AFP (Yoshima *et al.*, 1980; Tsuchida *et al.*, 1984) is composed of a bi-antennary structure while the Con A non-reactive variant contains a bi-antennary structure with bisecting *N*-acetylglucosamine (GlcNAc). Thus, the changes of AFP-Con A reactivity shown here may represent alterations in relative proportions of polypeptide chains whose bi-antennary units have bisecting GlcNAc, which has been found to inhibit Con A binding (Narasimhan, Freed & Schacter, 1986).

The observation that increased Con A binding of AFP occurred in Hep 3B cells incubated with CM while accumulation of this protein decreased clearly demonstrates that altered glycosylation is not dependent on increased synthesis. The observation that two different patterns of change in glycosylation of PI both accompanied increase in PI synthesis provides additional evidence for this view.

The two different altered glycosylation patterns induced by crude preparations of cytokines in Hep 3B and Hep G2 cells resemble the two different types of change found in patients' sera during different inflammatory states (v.s.), suggesting that these changes in serum result from the effects of cytokines on hepatocytes, and that the two types of glycosylation changes may be regulated by separate intracellular mechanisms.

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REFERENCES

BAUMANN, H., HILL, R.E., SAUDER, D.N. & JAHREIS, G.P. (1986) Regulation of major acute-phase plasma proteins by hepatocyte-stimulating factors of human squamous carcinoma cells. *J. Cell Biol.* **102,** 370.

BIERHUIZEN, M.F.A., DE WIT, M., GOVERS, C.A.R.L., FERWERDA, W., KOELMAN, C., POS, O. & VAN DIJK, W. (1988) Glycosylation of three molecular forms of human α_1-acid glycoprotein having different interactions with Concanavalin A. *Eur. J. Biochem.* in press

BOG-HANSEN, T.C. (1973) Crossed immuno-affinoelectrophoresis. *Anal. Biochem.* **56,** 480.

CARLSON, JN., ERIKSSON, S., ALM, R. & KJELLSTRÖM, T. (1984) Biosynthesis of abnormally glycosylated alpha 1-antitrypsin by a human hepatoma cell line. *Hepatology,* **4,** 235.

GANAPATHI, M.K., SCHULTZ, D., MACKIEWICZ, A., SAMOLS, D., HU, S.-I., BRABENEC, A., MACINTYRE, S.S. & KUSHNER, I. (1988) Heterogenous nature of the acute phase response. *J. Immunol.* **141,** 564–569.

GAULDIE, J., RICHARDS, C., HARNISH, D., LANSDORP, P. & BAUMANN, H. (1987) Interferon β_2 B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc. natl. Acad. Sci. USA*, **84,** 7251.

GOLDBERGER, G., ARNAOUT, M.A., ADEN, D., KAY, R., RITS, M. & COLTEN, H.R. (1984) Biosynthesis and postsynthetic processing of human C3b/C4b inactivator (factor I) in three hepatoma cell lines. *J. Biol. Chem.* **259,** 6492.

GORDON, A.H. & KOJ, A. (eds.) (1985) *The Acute Phase Response to Injury and Infection.* Elsevier, Amsterdam and New York.

HATTON, M.W., MARZ, L. & REGOECZI, E. (1983) On the significance of heterogeneity of plasma glycoproteins possessing *N*-glycans of the complex type—a perspective. *Trends Biochem. Sci.* **8,** 287.

KUSHNER, I. & MACKIEWICZ, A. (1987) Acute phase proteins as disease markers. *Dis. Markers*, **5,** 1.

LAURELL, C.B. (1972) Electroimmunoassay. *Scand. J. Clin. Lab. Invest. (Suppl.)* **124,** 21.

MACKIEWICZ, A. & MACKIEWICZ, S. (1986) Determination of lectin-sugar dissociation constants by agarose affinity electrophoresis. *Anal. Biochem.* **156,** 481.

MACKIEWICZ, A., GANAPATHI, M.K., SCHULTZ, D. & KUSHNER, I. (1987) Monokines regulate glycosylation of acute-phase proteins. *J. exp. Med.* **166,** 253.

MACKIEWICZ, A., MARCINKOWSKA-PIETA, R., BALLOU, S. MACKIEWICZ, S. & KUSHNER, I. (1987) Microheterogeneity of alpha 1-acid glycoprotein in the detection of intercurrent infection in syystemic lupus erythematosus. *Arthritis Rheum.* **30,** 513.

MACKIEWICZ, A., PAWLOWSKI, T. MACKIEWICZ-PAWLOWSKA, A., WIKTOROWICZ, K. & MACKIEWICZ, S. (1987) Microheterogeneity forms of alpha 1-acid glycoprotein in assessment of rheumatoid arthritis activity. *Clin. Chim. Acta*, **163,** 185.

MAGIELSKA-ZERO, D., ROKITA, H., CIESZKA, K., KURDOWSKA, A., KOJ, A., SIPE, J.D. & GAULDIE, J. (1987) Comparison of the acute phase response of cultured Morris hepatoma 7777 cells and of rat hepatocytes. *Br. J. exp. Path.* **68,** 485.

MAY, L.T., GHRAYEB, J., SANTHANAM, U., TATTER, S.B., STHOEGER, Z., HELFGOTT, D.C., CHIORAZZI, N., GRIENINGER, G. & SEHGAL, P.B. (1988) Synthesis and secretion of multiple forms of β_2-interferon/B-cell differentiation factor 2/hepatocyte-stimulating factor by human fibroblasts and monocytes. *J. Biol. Chem.* **263,** 7760.

MEGA, T., LUJAN, E. & YOSHIDA, A. (1980) Studies on the oligosaccharide chains of human alpha 1-protease inhibitor. I. Isolation of glycopeptides. *J. Biol. Chem.* **225,** 4053.

NARASIMHAN, S., FREED, J.C. & SCHACHTER, H. (1986) The effect of a 'bisecting' *N*-acetylglucosaminyl group on the binding of biantennary, complex oligosaccharides to concanavalin A, *Phaseolus vulgaris* erythroagglutin (E-PHA), and *Ricinus communis* agglutinin (RCA-120) immobilized on agarose. *Carbohydr. Res.* **149,** 65.

NICOLLET, I., LEBRETON, J.P., FOUNTANE, M. & HIROU, M. (1981) Evidence for alpha-1-acid glycoprotein propulations of different pI values after concanavalin A affinity chromatography. Study of their evolution during inflammation in man. *Biochem. Biophys. Acta*, **668,** 235.

PERLMUTTER, D.H., COLE, F.S., KILBRIDGE, P., ROSSING, T.H. & COLTEN, H.R. (1985) Expression of the alpha 1-proteinase inhibitor gene in human monocytes and macrophages. *Proc. natl. Acad. Sci. USA.* **82,** 795.

RAYNES, J. (1982) Variations in the relative proportions of microheterogeneous forms of plasma glycoproteins in pregnancy and disease. *Biomed. Pharmacother.* **36,** 77.

SERBOUCE-GOGUEL SETA, N., DURAND, G., CORBIC, M., AGNERAY, J. & FEGAR, J. (1986) Alterations in relative proportions of microheterogeneous forms of human alpha 1-acid glycoprotein in liver disease. *J. Hepatol.* **2,** 245.

TSUCHIDA, Y., YAMASHITA, K., KOBATA, A., NISHI, S. ENDO, Y. & SAITO, S. (1984) Structure of the sugar chain of alphafetoprotein purified from a human yolk sac tumor and its reactivity with concanavalin A. *Tumor Biol.* **5,** 33.

VAUGHAN, L., LORIER, M.A. & CARRELL, R.W. (1982) Alpha 1-antitrypsin microheterogeneity. Isolation and physiological significance of isoforms. *Biochem. Biophys. Acta*, **701,** 339.

YOSHIMA, H., MIZUOCHI, T., ISHII, M. & KOBATA, A. (1980) Structure of the asparagine-linked sugar chains of alpha-fetoprotein purified from human ascites fluid. *Cancer Res.* **40,** 4276.