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In vitro influence of lectins and neoglycoconjugates on the growth of three human sarcoma cell lines

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Abstract ***Purpose*:** The aim of our study is to investigate the in vitro effects of plant lectins, galectins and neoglycoconjugates on the proliferation of three human sarcoma cell lines. ***Methods*:** Proliferation was assessed by means of the tetrazolium derivative reduction (MTT) assay. In addition, glycohistochemistry was used to make visible the plant-lectin-specific binding sites; the intensity of the lectin binding pattern was quantified by means of image analysis. ***Results*:** Depending on the cell lines, the staining intensity and the percentage of labelled cells were different. With respect to growth modulation, the cell lines also responded differently to the probes used. Besides a predominant inhibitory effect elicited by the probes at 50 μg/ml, dose-dependent effects, including growth stimulation, were detectable in several instances. These effects relate to the animal galectins tested and several neoglycoconjugates, e.g. the lactose- and blood-group-A-trisaccharide-bearing probes. ***Conclusions*:** Endogenous lectins and lectin-reactive cellular glycoconjugates can apparently affect the regulation of the growth of human sarcoma cells. We suggest that these results are relevant for further histopathological monitoring in correlation with prognosis and in vitro assays to reveal possible clinical applications.

Key words Soft tissue tumour · In vitro · Cell growth · Lectins · Neoglycoconjugates

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Introduction

Soft-tissue tumours constitute a large and heterogeneous group of neoplasms arising from derivatives of the embryonic mesoderm. While accounting for about 1% of all malignant neoplasms in adults, they represent over 2% of the deaths attributed to cancer (Hadju 1986). In the sarcoma group we were particularly interested in leiomyosarcomas (tumours originating from smooth muscles) and rhabdomyosarcomas (originating from striated muscles).

Little is known about the factors regulating the growth of these tumours (Remmelink et al. 1998). Growth regulation can be triggered by protein/carbohydrate interaction, which is known to influence the activity of diverse intracellular signalling pathways (Villalobo et al. 1997). Generally, plant lectins are employed in this research area as they are readily available laboratory tools, e.g. for the stimulation of immune cells. Physiologically their application supports the notion that an in situ recognitive interplay between a sugar receptor and its ligand will be part of the regulation capacity of human cells. This encouraged us to deliberately undertake the assay of animal lectins and distinct naturally occurring oligosaccharide sequences presented as glycoconjugate mimetics.

Lectin/glycan interactions have been associated with a variety of biological recognition processes (Gabius and Gabius 1991; Danguy et al. 1997). Carbohydrate-recognition mechanisms have also been involved in cancer metastasis (Gabius and Gabius 1991; Hakomori 1989;

Dennis 1992). Thus, the monitoring of both sides of a protein/sugar interaction has received considerable attention (Gabius and Gabius 1993). The conjugation of custom-made carbohydrate structures to an inert carrier yields suitable tools, termed neoglycoconjucates, that expose the pivotal glycan structures as ligands on their surface (Lee et al 1994). Their application has already proved useful in histology (Kannan and Nair 1997; Danguy et al. 1995) and pathology (Kannan and Nair 1997; Kayser et al. 1994).

Since both tumour cells and other mammalian cell types are known to express endogenous lectins (Gabius 1991; Ohannesian and Lotan 1997), it seems relevant to initiate the analysis of cell growth proliferation in vitro. Among these endolectins, galectins constitute a major group that shares an affinity for β-galactosyl residues (Barondès et al. 1994a,b).

In addressing the question of whether exolectins, galectins and neoglycoconjugates can affect the growth rate of sarcoma cells, we investigated the effects of five plant lectins (PHA-L, GSA-IA4, PNA, WGA and ConA), three animal β-galactose-binding lectins (bovine galectin-1, CL-14, and CL-16) and six carrier-immobilised glycans (PL-Lac, PL-Mel, PAA-A, PAA-B, PAA-Le^d, PAA-H) on the proliferation of three sarcoma cell lines (Hs 729, SK-UT-1 and SK-LMS-1) growing in vitro as monolayers. The proliferation level was assessed by means of the tetrazolium derivative reduction (MTT) assay (Mosman 1983). Finally, we quantified the cytochemical characterisation of some lectin-binding patterns by cell-image analysis (Goldschmidt et al. 1996; Janssen et al. 1996).

Materials and methods

Cell lines and culture medium

The three cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, Md.). They were the Hs 729 (ATCC HTB-153), of rhabdomyosarcomatous origin, the SK-UT-1 (ATCC HTB-114) (Fogh et al. 1977) and the SK-LMS-1 (ATCC HTB-88) (Fogh et al. 1977), the latter two of which were of leiomyosarcomatous origin. The cell lines were cultured as previously described (Kiss et al. 1997; Remmelink et al. 1998). Briefly, they were cultured as monolayers at a temperature of 37°C in an atmosphere containing 5% CO_2 in closed Falcon plastic dishes (Nunc, Poly Labo, Strasbourg, France). The culture medium consisted of Eagle's minimal essential medium (Gibco, Cergy Pontoise, France) supplemented with 5% fetal calf serum (FCS, Gibco) for the SK-LMS-1 and SK-UT-1, and 10% FCS (and 1% non-essential amino acids, Gibco) for the Hs 729 cell line. All the media were supplemented with a mixture of 0.6 mg/ml glutamine (Gibco), 200 IU/ml penicillin (Gibco), 200 µg/ml streptomycin (Gibco) and 0.1 mg/ml gentamicin (Gibco). The FCS was heat-inactivated for 1 h at 56°C.

Lectins

Five plant lectins were used. They included *Phaseolus vulgaris* leukoagglutinin (PHA-L, which binds to *N*-acetylglucosamine-β1,2-mannose triantennary complex oligosaccharides), *Griffonia simplifolia* I-A4 agglutinin (GSA-IA4, which binds to terminal α-*N*-acetylgalactosaminyl groups), *Arachis hypogaea* agglutinin (peanut agglutinin, PNA, which binds to galactose-β1,3-*N*-acetylgalactosamine), *Triticum vulgare* agglutinin (wheat-germ agglutinin, WGA, which binds to *N*-acetylglucosamine and *N*-acetylneuraminic acid) and *Canavalia ensiformis* agglutinin (concanavalinA, ConA, which binds to mannose and glucose); the lectins were purchased from Vector Laboratories Inc. (Burlingame, Calif., USA).

We also used three animal β-galactoside-binding lectins. They included chicken 14-kDa lectin (CL-14), chicken 16-kDa lectin (CL-16) and galectin-1 isolated from the bovine heart (Bardosi et al. 1990; Schneller et al. 1995). These proteins belong to the galectin family, which interacts with portions of the galactose residue in lactose

Neoglycoconjugates

The histo-blood-group trisaccharides (A, B, H and Le^d), prepared as 3-aminopropylglycosides, were attached to poly-4-nitrophenylacrylate (PAA), as described previously (Abramenko et al. 1992; Korchagina and Bovin 1992). The glycosylation of commercially available poly-L-lysine (PL) was performed by reductive amination with sodium cyanoborohydride, and residual amino groups were blocked with *N*-hydroxysuccinimide acetate as described (Imai and Irimura 1994). We investigated poly-L-lysine with lactose (PL-Lac) and melibiose (PL-Mel), which bind to β- and α-galactoside receptors respectively.

Experimental schedule for cell growth assessments

The cell lines were plated on multiwell plates 24 h before the addition of lectin to ensure adequate plating. The cell lines were then incubated for an additional 1, 3, 5 and 7 days in either a lectin-free medium (control) or in medium supplemented with one of the lectins under study at 0.05, 0.5, 5 or 50 µg/ml. These concentrations were in accordance with other recent in vitro models (Koninnkx 1995; Kulkarni et al. 1995). Each experiment was carried out in six times.

As previously described (Camby et al. 1996; Janssen et al. 1996), cell growth was assessed by means of the MTT assay according to Mosman (1983), but with the modifications recommended by Carmichael et al. (1987). Briefly, the assessment of proliferation is based on the ability of living cells to reduce the yellow product MTT [3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma] to a blue product, formazan, by a reduction reaction occurring in the mitochondria. The number of living cells is directly proportional to the intensity of the blue colour, which is quantitatively measured by spectrophotometry on a DIAS microplate reader (Dynatech Laboratories) at 570 nm wavelength (with a reference of 630 nm). This method has been detailed previously (Kiss et al. 1997; Remmelink et al. 1998).

In view of recent criticism of the MTT method as a means of assessing cell proliferation (Rubinstein et al. 1990; Vistica et al. 1991), we ensured the accuracy of our measurements by the use of appropriate controls. Thus, in addition to the MTT assay, we also assessed cell proliferation in a separate experiment by directly counting the cell nuclei. This method was detailed in a recent issue of this journal (Remmelink et al. 1998). Briefly, it consists of culturing cells for 96 h in a factor-free medium (control) or in medium containing lectins or neoglycoconjugates (at the various concentrations described in the previous section). Cell proliferation was assessed by the direct counting of Feulgen-stained nuclei, a microscope grid (20 areas of 1.6 mm^2 each were analysed for each slide).

As reported in Results, the influence of ConA on the SK-LMS-1 and Hs 729 cell lines was evaluated by the two cell-growth assessment methods. Our purpose was to investigate whether the cell proliferation measurements carried out by direct counting and the MTT calorimetric assay corresponded.

Cytochemical characterisation of the lectin binding patterns

Aliquots of 2.5 ml solution containing 60 000 cells/ml (to correspond to the exponential growth phase, each concentration selected

was a function of the cell growth pattern peculiar to each cell line) were plated in petri dishes (Nunc, Roskilde, Denmark), which were equipped with 18 × 18-mm glass microscope coverslips at the bottom. The cells were cultured for 72 h and the glass coverslips were then immersed in buffered formalin for 20 min. Three glass coverslips were available for staining for each of the five exolectins, with one as negative control (to determine lectin positivity, see below). The cytochemical lectin staining method is described elsewhere (Kiss et al. 1997; Janssen et al. 1996). The five exolectins (PHA-L, GSA-IA4, PNA, WGA and ConA) were purchased as biotinytaled proteins for glycocytochemichal staining with kit reagents.

Quantitative glycocytochemical assessment was carried out with a 20× magnification lens by means of a Samba 2005 cell image processor (Alcatel-TITN, Grenoble, France) coupled to a JVC (KY15) colour camera and a Leitz (Diaplan) microscope. This method is detailed elsewhere (Kiss et al. 1997; Janssen et al. 1996). The quantitative extent of the reaction was determined on 200 cells/slide, i.e. 600 cells/cell line. Lectin positivity was defined by integrated optical values in excess of the mean + 2 SD of the corresponding negative control slide. We determined two parameters by means of this technique, i.e. the labelling index (LI) and the mean absorbance. LI is obtained by calculating the percentage of positive cells out of the 200 analysed for any given coverslip. The mean absorbance is related to the glycocytochemical staining intensity and is obtained by dividing the integrated absorbance value of the lectin staining by the area occupied by a cell on the glass coverslip. A "quick score" (QS) was also assessed, with QS = LI × mean absorbance. The QS index takes into account both the proportion of positive cells and the intensity of the cytochemical staining.

To ascertain the significant binding of the lectins, each lectin-specific carbohydrate was added to the culture media to a concentration of 10–50 nM.

Statistical analyses

Cell growth assessments, glycocytochemical staining intensity and labelling index values are reported as means ± SEM and statistically compared by means of the Fisher *F*-test (one-way analysis of variance). The equality of variance was checked by the Bartlett test and the normal-distribution fit of the data by the χ^2-test. If these parametric conditions were not satisfied, the non-parametric Mann-Whitney *U*-test was used.

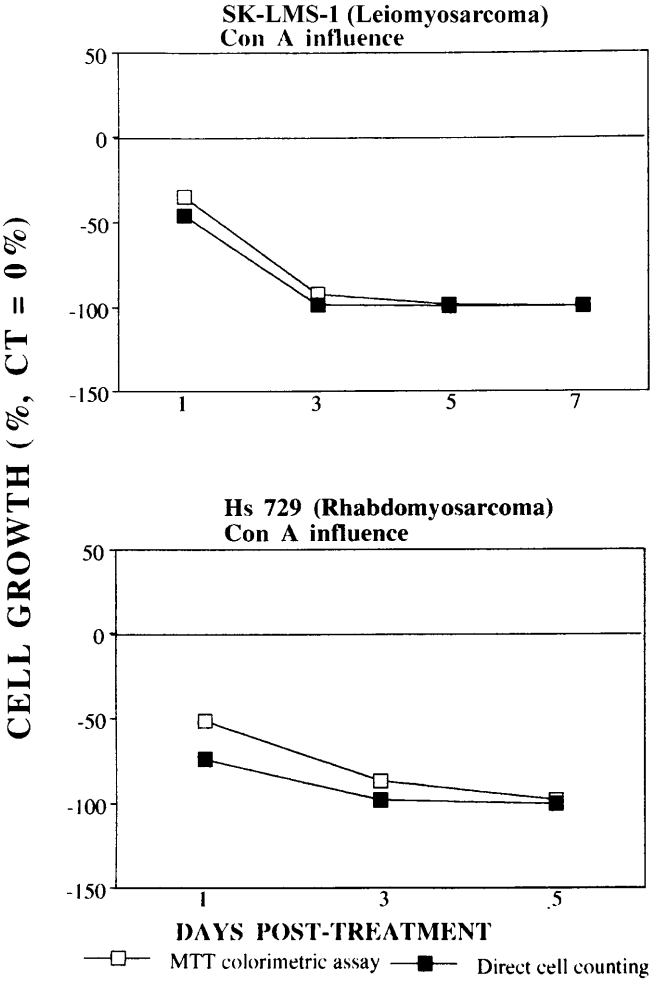

Fig. 1 Determination of the action of concanavalinA (*ConA*, at 50 μg/ml concentration) on the growth of the SK-LMS-1 and Hs 729 cell lines by two different techniques: the MTT colorimetric assay and direct cell counting, as described in Materials and methods. ■ Direct cell counting, □ the MTT colorimetric assay. *CT* control

Results

Validation of the methodology

Figure 1 illustrates the influence of ConA on the growth of the SK-LMS-1 and Hs 729 cell lines. Cell growth was assessed either by means of the MTT colorimetric assay (open squares) or by directly counting the nuclei (black squares). The data show that identical results were obtained with the two tests. Thus the MTT colorimetric assay is a valuable method for evaluating cell growth in our experimental conditions.

Cytochemical characterization of lectin-binding patterns

Figure 2 shows the quantitative glycocytochemical assessment mean mean absorbances and LI values obtained for the ConA, WGA, GSA-IA4, PHA-L and PNA lectins (at a concentration of 50 μg/ml). This figure shows that each of the three cell lines exhibited significant binding to the five lectins, but with different binding patterns. Hs 729 was the cell line that was the least marked in terms of the labelling index. PHA-L was the lectin least effective in terms of the labelling index for two cell lines of leiomyosarcomatous origin (SK-UT-1 and SK-LMS-1). For the Hs 729 line, GSA-IA4 was the least expressed in terms of LI. In terms of mean absorbance values, the number of binding sites for GSA-IA4 was lowest for the SK-LMS-1 and SK-UT-1 cell lines. The Hs 729 cells almost completely lacked any binding of PNA.

Characterisation of the lectin-induced modification of cell growth

Figures 3–6 show selected lectin- and neoglycoconjugate-induced effects on cell proliferation for each of the three cell lines under study. In all the figures the mean absorbance value measured in the control is set to the baseline value of 0%, so enabling any inhibitory or

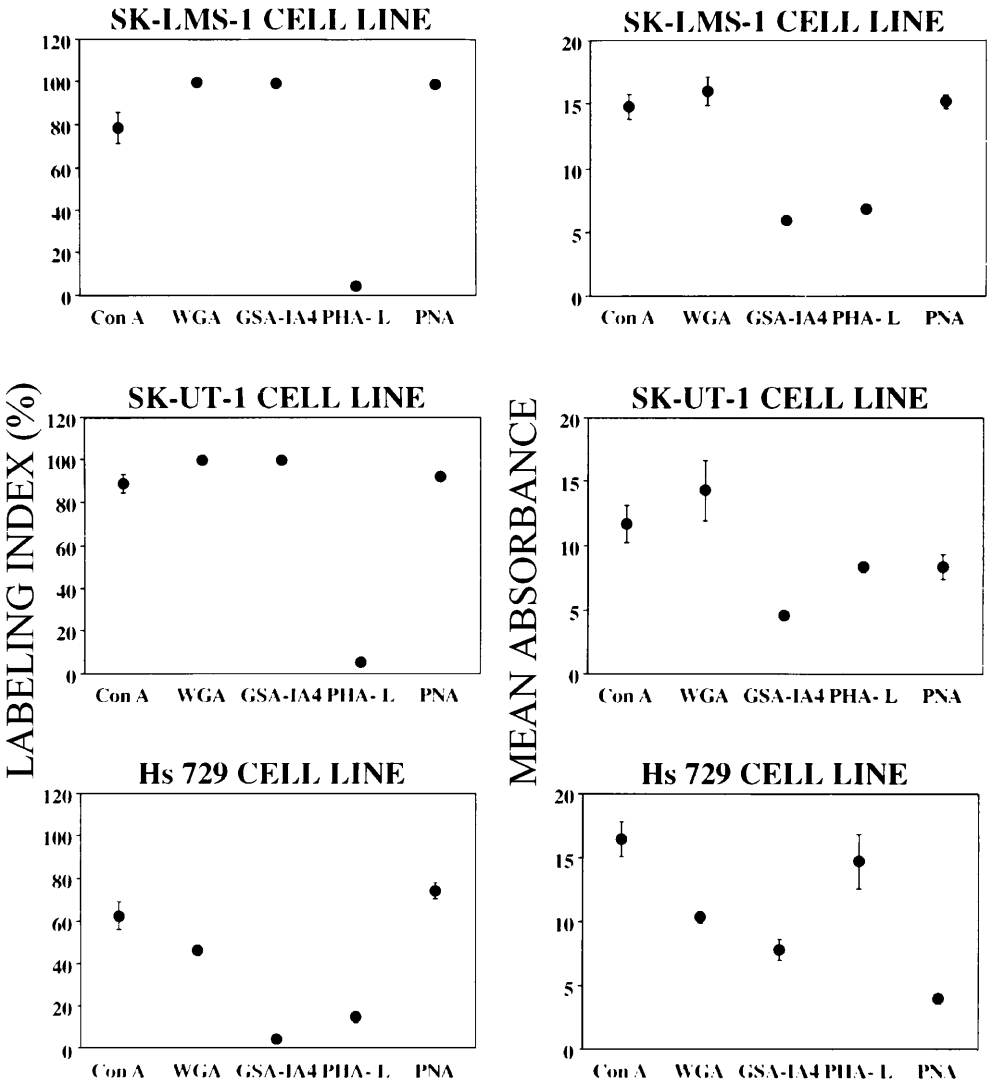

Fig. 2 Determination of the labelling index (*left-hand side*), and the mean absorbance (*right-hand side*) for the five exolectins under study (50 μg/ml). *Bars* represent the mean ± SEM

stimulatory influence to be conveniently described as a deviation from the dark central horizontal line.

Exogenous lectins

a. Peanut agglutinin (PNA). As illustrated in Figs. 3–5, PNA slightly stimulated the growth rate in the three sarcoma cell lines except at the highest concentration (50 μg/ml). This concentration inhibited ($P < 0.01$) SK-UT-1 cell growth; the same concentration significantly ($P < 0.01$) increased the growth of the SK-LMS-1 cell line (Figs. 4, 5).

b. Griffonia simplicifolia *I-A4 agglutinin (GSA-IA4).* GSA-IA4 was the most active exolectin among those studied here. At high concentrations (50 μg/ml and 5 μg/ml) it was highly inhibitory ($P < 0.001$) to SK-UT-1 and SK-LMS-1 growth (Figs. 4, 5). The 50-μg/ml concentration of GSA-IA4 significantly ($P < 0.001$) inhibited Hs 729 cell growth (Fig. 3). The two lowest concentrations (0.5 μg/ml and 0.05 μg/ml) had no effect on the growth of the Hs 729 cell line, but slightly inhibited ($P < 0.01$) SK-LMS-1 growth. At the 0.05-μg/ml concentration the growth of the SK-UT-1 cell line was ($P < 0.001$) inhibited markedly.

c. Concanavalin A agglutinin (ConA). As illustrated in Figs. 3–5, the high ConA concentration (50 μg/ml) consistently inhibited growth in the three cell lines under study. The other concentrations had an overall stimulatory effect, especially the weakest concentration (0.05 μg/ml), though this did not apply to the SK-UT-1 cell line, where we did not observe any effect (Fig. 5).

d. Wheat-germ agglutinin (WGA). WGA had no effect on the proliferation of the three sarcoma cell lines, even at the highest concentration (50 μg/ml). Only on the 7th day after culture was the proliferation of the SK-LMS-1 cell line significantly ($P < 0.001$) inhibited at the 50 μg/ml concentration (Fig. 4), and the growth of the SK-UT-1 line was moderately ($P < 0.01$) inhibited at the

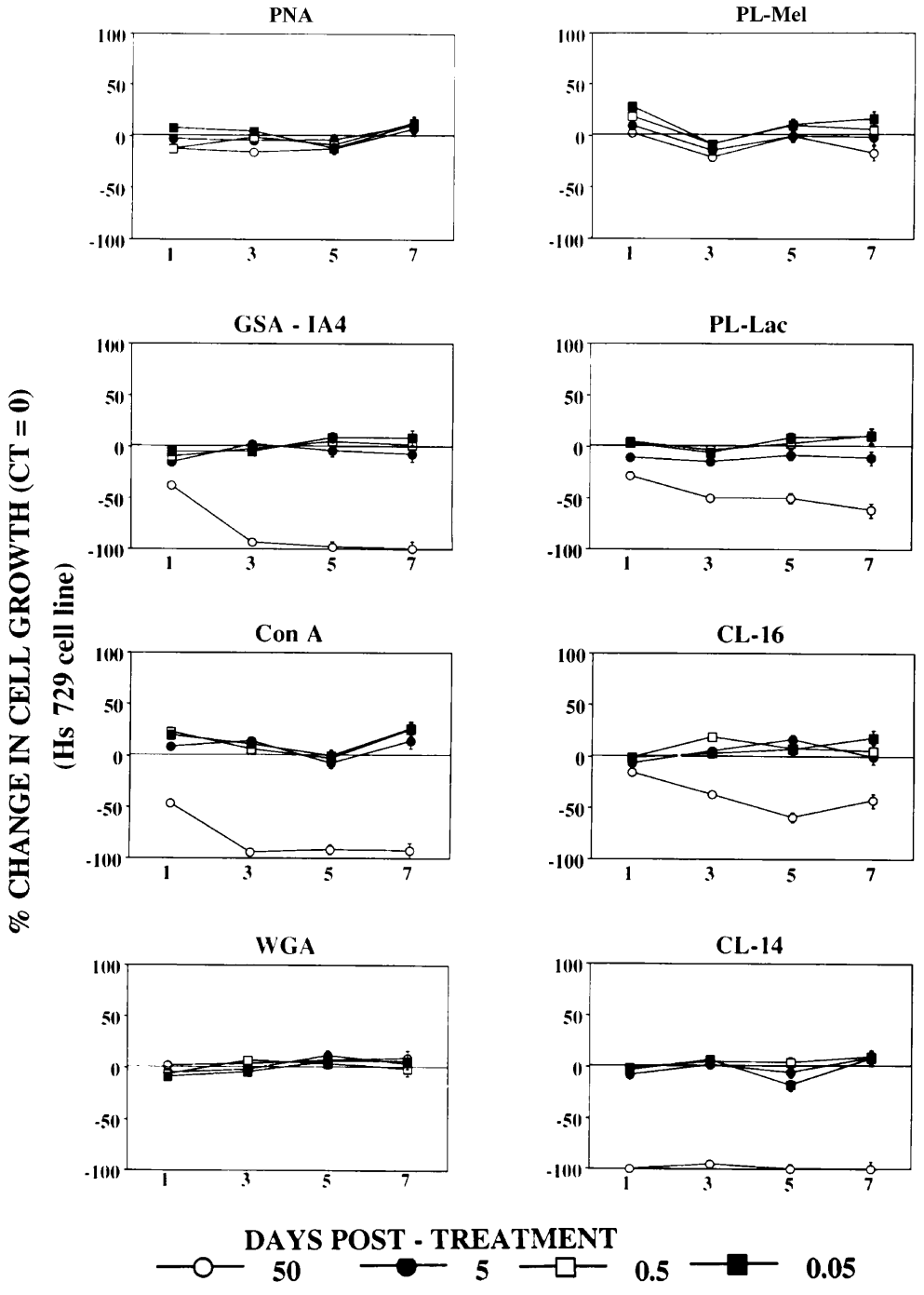

Fig. 3 Determination of the influence of the PNA, GSA-Ia4, ConA, WGA, PL-Mel, PL-Lac, CL-16 and CL-14 lectins on the growth of the Hs 729 cell line by means of the MTT colorimetric assay: ○ 50 μg/ml, ● 5 μg/ml, □ 0.5 μg/ml, ■ 0.05 μg/ml. *CT* control

same concentration (Fig. 5). WGA had no effect on the Hs 729 line (Fig. 3).

e. Phaseolus vulgaris *leukoagglutinin (PHA-L)*. At the highest concentration (data not shown) the PHA-L lectin had a significant inhibitory effect ($P < 0.001$) on cell proliferation in the three cell lines, the Hs 729 line being the most affected. It must be pointed out that, for the smallest concentration (0.05 μg/ml), a weak but significant ($P < 0.05$) stimulatory effect on cell proliferation was observed in the case of the SK-UT-1 and Hs 729 cell lines at the end of the culture. The elicitation of inhibitory and stimulatory responses by the same lectin is thus possible as a function of the concentration.

Animal lectins

a. Chicken liver lectin (CL-16). CL-16, at 50 μg/ml, exerted an inhibitory effect ($P < 0.001$) on the growth of the three cell lines under study, but this effect was

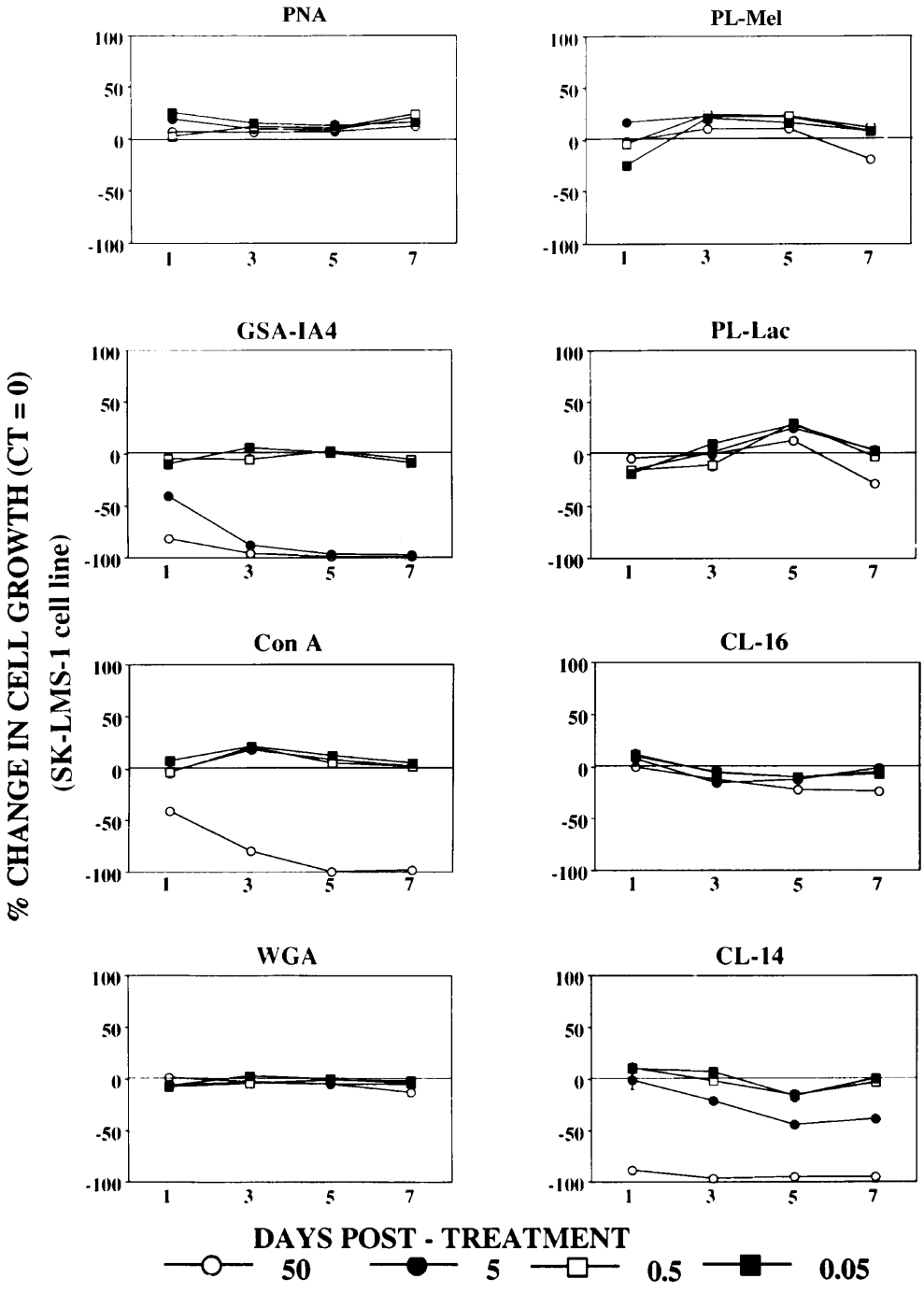

Fig. 4 Determination of the influence of the PNA, GSA-IA4, ConA, WGA, PL-Mel, PL-Lac, CL-16 and CL-14 lectins on the growth of the SK-LMS-1 cell line. Details as in Fig. 4

more pronounced for the SK-UT-1 and Hs 729 lines. At 0.05 μg/ml, the growth of the SK-UT-1 and Hs 729 lines was marked in the former case and stimulated moderately in the latter (Figs. 3–5).

b. Chicken intestine lectin (CL-14). The CL-14 lectin exerted a marked inhibitory effect on the growth of the three cell lines under study (Figs. 3–5). The highest concentration (50 μg/ml) completely inhibited cell growth, an effect starting on day 1 of treatment. For the SK-UT-1 and SK-LMS-1 lines, this inhibitory effect was also observed at 5 μg/ml; on the other hand, the Hs 729 line was not affected by this concentration. On the first day of treatment the growth of SK-UT-1 cells was markedly inhibited ($P < 0.001$) at the 5 μg/ml dose, while the growth of SK-LMS-1 was not modified ($P < 0.5$). It must be pointed out that the lowest concentration (0.05 μg/ml) clearly stimulated ($P < 0.5$) the growth of the SK-UT-1 cell line.

c. Bovine galectin-1. Bovine galectin-1 had a marked ($P < 0.001$) inhibitory effect on the growth of the SK-UT-1 cell line, but only at the highest concentration

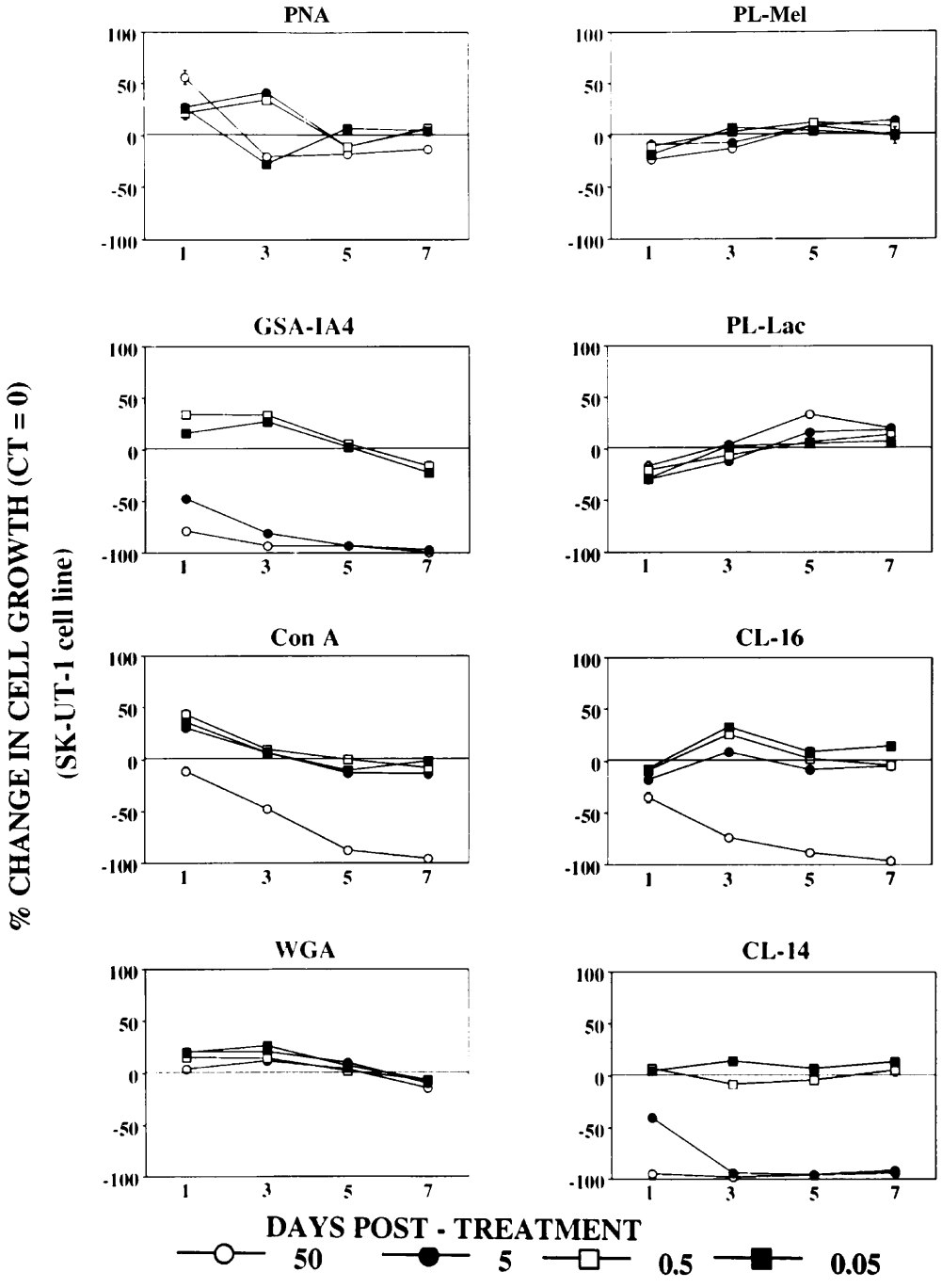

Fig. 5 Determination of the influence of the PNA, GSA-IA4, ConA, WGA, PL-Mel, PL-Lac, CL-16 and CL-14 lectins on the growth of the SK-UT-1 cell line. Details as in Fig. 4

(50 μg/ml). The growth of the SK-LMS-1 cell line was inhibited at all four concentrations: strongly ($P < 0.001$) for 50 μg/ml and 0.05 μg/ml, moderately ($P < 0.001$) at 5 μg/ml and weakly ($P < 0.1$) at 0.5 μg/ml. The growth of the Hs 729 cell line was moderately (at 50 μg/ml) or weakly (at 0.05 μg/ml) inhibited by bovine galectin-1 (data not shown).

Neoglycoconjugates

*a. Poly-*L*-lysine with lactose (PL-Lac) and melibiose (PL-Mel) as an attached ligand.* At 50 μg/ml PL-Mel had a marked inhibitory effect ($P < 0.001$) on the Hs 729 cell growth and a moderately inhibitory effect ($P < 0.01$) on the SK-LMS-1 growth. At the lowest concentration (0.05 μg/ml), the growth of the Hs 729 cell line was still markedly stimulated ($P < 0.001$) (Figs. 3–5).

The highest concentration of PL-Lac (50 μg/ml) markedly inhibited ($P < 0.001$) the Hs 729 and SK-LMS-1 cell lines; on the other hand, this dose had a stimulatory effect ($P < 0.01$) on the SK-UT-1 line. The Hs 729 line was the most sensitive to this probe: cell growth was weakly stimulated ($P < 0.1$) at the 0.05 μg/

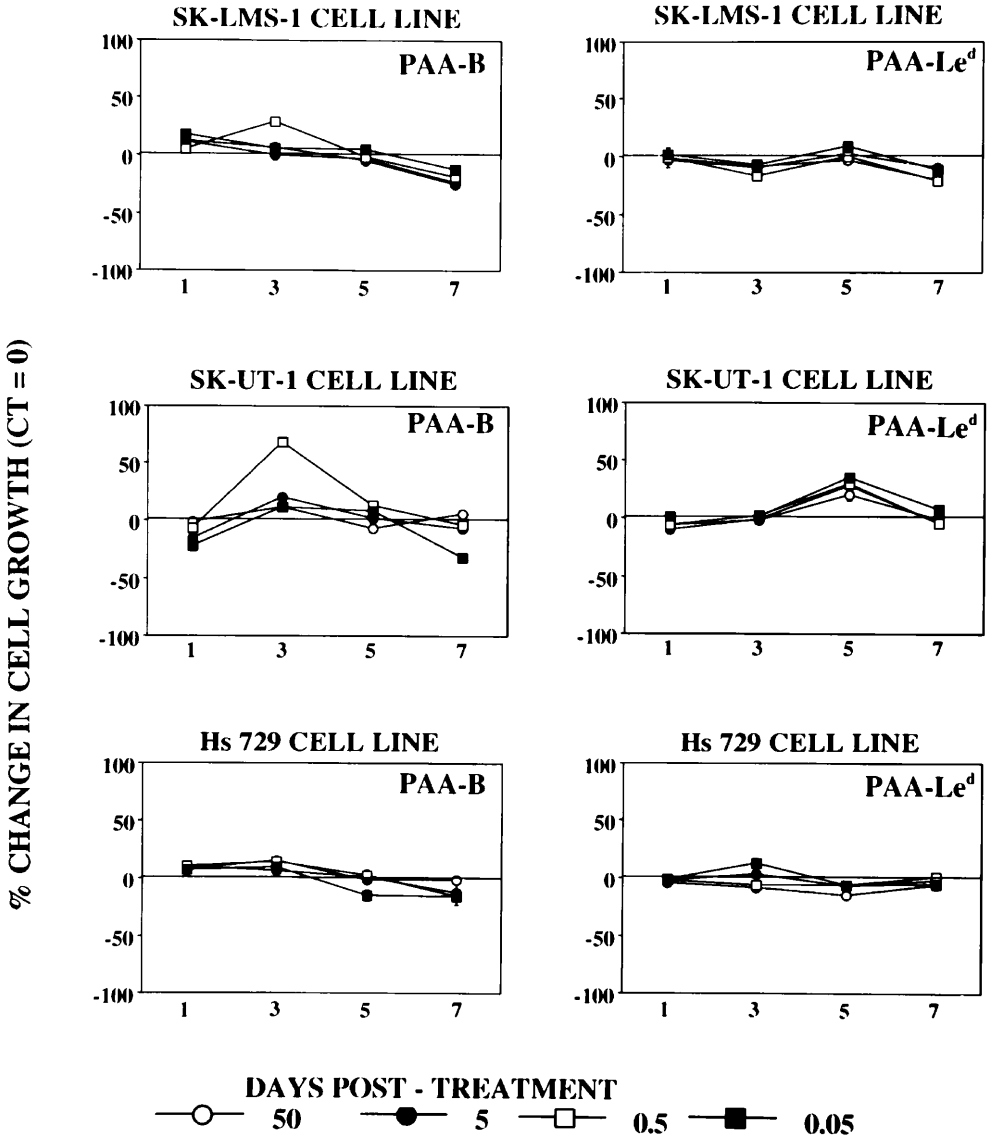

Fig. 6 Determination of the influence of the PAA-B and PAA-Le^d lectins on the growth of the SK-LMS-1 (*top*), SK-UT-1 (*middle*) and Hs 729 (*bottom*) cell lines. Details as in Fig. 4

ml and 5 μg/ml concentrations, while at 0.5 μg/ml it was moderately inhibited ($P < 0.01$).

b. Polyacrylamide-bound histo-blood-group trisaccharides. At the four concentrations tested (50, 5, 0.5 and 0.05 μg/ml), PAA-B and PAA-Le^d had a marked inhibitory effect on the growth of the SK-LMS-1 cell line (Fig. 6). At 0.05 μg/ml, PAA-B clearly inhibited ($P < 0.001$) the growth of the SK-UT-1 and the Hs 729 lines. The growth of the Hs 729 line was inhibited at 0.5 μg/ml ($P < 0.001$) and at 5 μg/ml ($P < 0.01$). The growth of this cell line was not influenced by PAA-Le^d, unlike the SK-LMS-1 cell line, the growth of which was markedly inhibited at each concentration tested; the growth of the SK-UT-1 cell line was weakly inhibited at 0.05 μg/ml and at 5 μg/ml (Fig. 7). PAA-A lectin at 50 μg/ml moderately inhibited ($P < 0.01$) the growth of the SK-LMS-1 cell line, had no effect on the Hs 729 cell line, and distinctly stimulated the growth of the SK-UT-1 line. The growth of the SK-LMS-1 line was clearly inhibited ($P < 0.001$) at 5, 0.5 and 0.05 μg/ml concentrations. Hs 729 was not sensitive to any concentration of this probe. At 50 μg/ml, PAA-H had a moderately inhibitory effect on the growth of the SK-UT-1 cell line, but no effect was seen on the other two cell lines (data not shown).

Discussion

In tumour pathology, aberrant glycosylation is a common attribute of neoplastic growth and is considered to be one of the major determinants of cancer-related phenomena such as invasive growth or metastasis (Feizi 1985; Hakomori 1991; Dennis 1992; Dabelsteen 1996).

Lectins, which selectively and reversibly bind to particular carbohydrate glycoconjugate structures (Barondès 1988), have been demonstrated to be able to

induce and/or control a number of metabolic and proliferative cell processes. Furthermore, over the last few years there has been an increasing interest in investigating the presence and function of endogenous lectins. In this respect carrier-immobilised glycans (neoglycoconjugates) have been used to monitor the presence of specific carbohydrate-binding sites in tissues and cells (Gabius and Gabius 1993; Lee and Lee 1994; Danguy et al. 1995; Gabius and Bardosi 1991). A logical extension of this work is to test these substances as elicitors of cellular responses, as was done in the present study.

In this study we investigated the roles of five plant lectins, three animal lectins and six neoglycoconjugates (see Materials and methods) in the growth of three sarcoma cell lines.

Numerous publications have suggested that lectins act as stimulators or inhibitors of cell growth, depending on the tissue targeted and the lectin tested. Indeed, PNA lectin, which binds to galactose-β1,3-*N*-acetylgalactosamine, inhibits breast cancer cell proliferation (Marth and Daxenbichler 1988) while stimulating smooth muscle and pulmonary arterial cell proliferation (Sanford and Harris-Hooker 1990) as well as benign and malignant colorectal cells (Ryder et al. 1994a, b). In our laboratory, previous studies undertaken on prostatic cancer (Camby et al. 1996) and astrocytic cell lines (Camby et al. 1997) demonstrated that the same plant lectins had a globally significant dose-dependent toxic effect. Nevertheless, low doses of GSA-IA4 and PHA-L significantly increased cell proliferation in one of the prostatic cancer cell lines (Camby et al. 1996).

Our present data, obtained in vitro on the three above-mentioned sarcoma cell models, showed that, among the lectins under study, ConA, GSA-IA4, CL-14, CL-16 and, to a lesser extent, PHA-L had the most inhibitory effect on cell proliferation at a 50-µl/ml dose. The lectin-induced modifications to the three cell lines investigated can be prevented by the addition of 10–50 nM each exolectin-specific glycan to the culture medium (data not shown); this has been already reported in a previous study of a similar type devoted to melanoma cell lines (Lorea et al. 1997). However some transient, but nevertheless statistically significant, lectin-induced stimulation of cell proliferation was also observed. These transient stimulatory effects at low doses have also been reported in other cancer cell lines studies (Camby et al. 1996, 1997; Lorea et al. 1997). Recently the mechanism involved in the cytotoxic effect of some lectins (WGA, GSI-A4) was partly elucidated. Kim et al. (1993) found that, in various murine tumour cell lines, lectins induced apoptosis with DNA fragmentation. These authors also demonstrated that the binding of a lectin to cell-surface carbohydrates is not sufficient to trigger tumour cell lysis. This indicates that lectin internalisation is probably required for cell lysis by means of programmed cell death. On the other hand, in a study of the effect of ConA on in vivo fibroblast models, Kulkarni and Mc Cullogh (1995) showed that lectin binding on cell-surface carbohydrates can induce cell death by inhibiting protein synthesis (the inhibition of ribosomal elongation). Preliminary data related to apoptosis measurements (performed by means of a photometric enzyme immunoassay) agree with the hypothesis that apoptosis could be a mechanism of the inhibitory effect of lectins in cell growth. More extensive experiments are being undertaken in our laboratory.

These data on plant-lectin-induced changes in cell proliferation raise the question of whether this is solely an in vitro phenomenon, or whether it has some biological relevance in vivo. Lectins are plentiful in fruit, vegetables and cereal products, and the importance of such dietary constituents in human metabolism is now readily accepted (Englyst et al. 1988, 1989). It has also been stressed that, with respect to PHA-L, 10% of initially intragastrically administered lectin can be found in blood and most peripheral organs of the body within 3 h (de Oliveira et al. 1988). Other lectins, WGA for example, are also transported through epithelial cells into capillaries and the lacteal subepithelial tissues of the intestinal wall (Putszai et al. 1991). It thus appears that one of the major characteristics of lectins is that they can cross the intestinal barrier in appreciable amounts in certain of the cases studied hitherto. It therefore seems reasonable to suggest that lectins can reach any part of the body via the systemic circulation and bind glycan-rich structures.

Although the three animal lectins influence the cell proliferation in sarcoma cell lines, their effect is not totally identical. These three lectins are members of the galectin family, which is ubiquitous in the animal kingdom, and located intra- and extracellularly. The relative binding discrepancies of these three galectins emphasise the fact that subtle differences in the binding-site architecture, as recently inferred for a tailor-made sugar derivative (Solis et al. 1996), may be crucial for this phenomenon. Galectin-1 is known to bind to a variety of naturally occurring galactosides; the nature of true in vivo ligands such as poly-*N*-acetyllactosamines requires further studies (Barondès et al. 1994a, b; Kasai and Hirabayashi 1996; Gabius 1997). Interestingly enough, it has been reported that this endogenous mammalian lectin induces the apoptosis of T lymphocytes, suggesting its involvement in regulating the immune response (Perillo et al. 1995).

To the best of our knowledge this is the first report emphasising the influence of sugar- and blood-group-antigen-exposing neoglycoconjugates on cell growth. Globally PL-Lac and PL-Mel, the receptor specificities of which are β- and α-galactoside respectively, have a similar effect on cell growth. However, at doses of 50 µl/ml, PL-Lac has a strong inhibitory effect on Hs 729 cell growth. As observed with the lectins tested, the lowest doses have transient stimulatory effects on cell proliferation. Biotinylated polyacrylamide-attached trisaccharides that represent the ABH histo-blood-group antigens have been applied to sections and cells to determine the importance of these substances in human pathology.

Remarkably enough, the expression of the binding sites of the histo-blood-group antigens A and H trisaccharides correlates with patient survival in cases of lung cancer (Kayser et al. 1994); transformed leukaemic and lymphoid cells interact more intensely than normal ones (Korchagina and Bovin 1992); differences in the histochemical expression of such probes have also been reported in various human lesions (Hassid et al. 1996). In the light of these findings we hypothesise that blood-group-related oligosaccharides might have an effect on cell proliferation. The response depends on both the cell line and the polyacrylamide-attached trisaccharides under study. An overall inhibitory effect on cell proliferation is the rule, but a stimulatory effect on SK-UT-1 growth was observed with PAA-A (0.5 μg/ml and 50 μg/ml) at the end of the culture. Interestingly enough, a tetrasaccharide related to histo-blood-groups A and Le^d has been demonstrated as reducing proliferation in astrocytes, gliomas and neuroblastomas at micromolar concentrations (Santos-Benito et al. 1992). Although blood-group oligosaccharides have been known for a long time, their biological role has not been fully established. Both our data and those reported by Santos-Benito and colleagues (1992) on transformed neural cells strongly suggest that these glycans may be involved in controlling cell proliferation.

In conclusion, the findings reported in the present study show that plant lectins, galectins and neoglycoconjugates, including histo-blood-group trisaccharide structures attached to a carrier, can modulate growth in human sarcoma cell lines. The data thus raise the possibility of controlling pathological cell division and may have practical importance. Further histopathological and biological cell studies are thus to be encouraged in this new area.

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