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Differential reactivities of the *Arachis hypogaea* (peanut) and *Vicia villosa* B4 lectins with human ovarian carcinoma cells, grown either in vitro or in vivo xenograft model

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Abstract PNA and VVA B4 recognize the tumor-associated T antigen and its immediate precursor Tn, respectively. We found that both lectins are highly reactive in vitro, with human ovarian carcinoma cell lines, but only VVA B4 bound significantly to breast and oral cancer cells. This binding is inhibited by specific monosaccharides. The lectin binding receptors were purified, revealing a glycoprotein of 32 kDa for PNA, and two glycoproteins of 35 and 38 kDa for VVA B4. In vivo localization of PNA was almost exclusive (except for the kidneys) to the ovarian tumor xenografts. VVA B4 showed wider tissue biodistribution being preferentially accumulated in the tumors and ovaries.

Key words: Glycoprotein receptor; Intraperitoneal therapy; Lectin biodistribution; Ovarian cancer; Peanut agglutinin; *Vicia villosa* B4 agglutinin

1. Introduction

Many of the tumor-associated antigens have been identified as carbohydrate-containing structures, expressed in the form of either glycoproteins or glycolipids on the tumor cell surface [1]. These structures may be the result of: (a) the accumulation of incomplete glycosylated precursor chains (because of a decreased activity of synthesizing enzymes); (b) the production of new oligosaccharides (due to increased aberrant glycosylation of carbohydrate chains); (c) changes in the density of the carbohydrate units on the cell surface; and/or (d) exposure of cryptic sugar chains usually covered by other structures. In many cases, the changes in the carbohydrate expression was found to play a key role in determining the metastatic behavior of the tumor cells [2]. Among those specific carbohydrate structures are the Thomsen-Friedenreich (T) and Tn antigens, whose epitopes are Galβ1,3,GalNAcαO-Ser/Thr and GalNAcαO-Ser/Thr respectively, which have been identified in more than 90% of the cancers of the adenocarcinoma types, including those of the breast and the ovaries [3]. These carbohydrate-associated tumor antigens are recognized by the lectins of *Arachis hypogaea* (peanut; PNA) and *Vicia villosa* B4 (VVA B4), which react with the T and Tn antigens, respectively [4]. Both lectins, however, may interact with other closely related structures, as described by Chen et al. [4]. Recently, immunoreactive T and Tn epitope clusters have been identified in the cancer-associated mucin (MUC-1 gene product) antigen, which is a unique high molecular weight surface glycoprotein, overexpressed in more than 90% of the human ovarian cancers and in the large majority of breast carcinomas types [5].

We have previously shown that monoclonal (rodent) and polyclonal (human) anti-T and -Tn antibodies are reactive with the human ovarian IGROV-1, OVCAR-3 and SKOV-3 carcinoma cells and accumulate in vivo, in ovarian carcinomas xenografted in nude mice [6]. In vitro, the monoclonal anti-T/Tn antibodies are cytotoxic to the ovarian cancer cells in a complement-independent manner [6,7].

The present study summarizes our recent findings on the reactivities, both in vitro and in vivo, of the PNA and VVA B4 lectins with human ovarian carcinoma cell lines. We have identified the PNA and VVA B4 binding receptors by affinity chromatography on lectin agarose-linked columns, and demonstrated that both lectins accumulated in ovarian tumors xenografted in nude mice. The lectin tumor localization in vivo is associated with a rapid blood clearance of both lectins, yet being associated with a tumor suppressive effect, in the case of PNA.

2. Materials and methods

2.1. Media

RPMI 1640 and Dulbecco's modified Eagle's media (DMEM) were purchased from Gibco (Grand Island, NY). All additional tissue culture products were from BioLab (Jerusalem). Culture media were supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, non-essential amino acids (as recommended by the supplier), 50 units/ml penicillin, 50 μg/ml streptomycin and 125 μg/ml Fungizone.

2.2. Animals

Athymic CD1 female nude mice (6–8 weeks old) were obtained from the Animal Breeding Center of the Weizmann Institute of Science.

2.3. Cell lines and lectins

All cell lines used in this study originated from the American Type Culture Collection (Rockville, MD, USA), except for IGROV-1 which was kindly provided by Prof. Z. Eshhar from our Department, and FS-11− which was kindly provided by Prof. M. Revel from the Department of Molecular Genetics and Virology.

All the lectins, in either native or immobilized (agarose-linked) form, were purchased from Sigma (St. Louis, MO, USA).

2.4. Tumor cell growth in vitro

All the cancer cell lines were grown in RPMI 1640 culture medium and maintained at 37°C in a 5% CO_2 humidified atmosphere, until cell confluence. Cell suspensions were prepared by a brief incubation in the presence of trypsin/EDTA (0.25%/0.02%). The cells were washed thrice in RPMI medium, harvested (800×*g*, 5 min) and resuspended

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Abbreviations: Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine; Glc, D-glucose; I.D., injection dose; PNA, peanut (*Arachis hypogaea*) agglutinin; T/B, tissue to blood ratio; VVA B4, *Vicia villosa* B4 agglutinin

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to a concentration of 2×10^5 cells per ml of the culture medium. FS-11 cells were maintained in DMEM plain medium, as described above.

2.5. Tumor cell transfer in athymic nude mice

The IGROV-1 tumor cells were cultured and harvested as above, and then injected (3×10^6 cells per mouse) subcutaneously (s.c.) into the animals. The OVCAR-3 cells were maintained in vivo, by serial transfer i.p. of the tumor cells (6×10^6 cells per mouse) into the animals. The developed ascitic OVCAR-3 tumor cells grow in large clusters making the cell count difficult, therefore the mean cell number in one cluster was estimated to be 15.

2.6. Direct [^{125}I]lectin binding and competitive radioimmunoassay (RIA)

Cell monolayers were prepared in 24-well plates (Nunc, Denmark) by plating 1×10^5 trypsinized cells in 0.5 ml of the appropriate culture medium, followed by overnight incubation at 37°C in a 5% CO_2 humidified atmosphere, to allow cell adherence. The obtained adherent cell monolayers were washed twice with PBS and incubated for 2 h at room temperature (~25°C) with various concentrations of the iodine ^{125}I-radiolabeled (using the chloramine T method, [8]) lectins suspended in 300 μl of PBS containing 1% BSA. The cells were then washed thrice with PBS, solubilized in 1 ml of 0.5 N NaOH, and the overall radioactivity was monitored in a Gamma counter (Riastar, Packard Inc., IL).

Competitive radioimmunoassay experiments with the free monosaccharides (D-galactose, Gal; *N*-acetyl-D-galactosamine, GalNAc; and D-glucose, Glc; all from Sigma), were performed by preincubation of the radiolabeled lectins with or without sugars for one hour at room temperature, before their addition to the tumor cells for an additional 1 h at room temperature. The cells were washed twice in PBS and the radioactivity levels were monitored in a Gamma counter.

2.7. Isolation of human ovarian IGROV-1 cancer cell membranes

The cell membrane preparations were prepared from IGROV-1 tumor cells grown (as described above) at logarithmic phase in plastic tissue culture flasks (Nunc, Denmark). The obtained cell monolayers (about 5×10^7 cells) were washed twice in ice-cold PBS and once in Buffer A solution (containing potassium phosphate buffer 0.01 M, pH 7.4; phenylmethylsulfonyl fluoride 0.5 mM and tosyllysinechloromethyl ketone 1 mM; all from Sigma). After cell dispersion with the aid of a sterile rubber policeman, the cells were disrupted by freeze-thawing (twice) at −80°C (for 24 h) and subsequent homogenization (10–15 strokes on ice) in a loose-fitting Dounce glass homogenizer. After centrifugation at $1500\times g$ for 15 min, the pellet (containing nuclei and cell debris) was discarded, and the supernatant centrifuged at $200\,000\times g$ (Beckman rotor SW28) for 16 h. The obtained pellet was washed in 10 ml of buffer A, centrifuged again at $200\,000\times g$ for 4 h and dissolved (in PBS containing 25 mM *n*-octyl-β-D-glucopyranoside, 5 mM EDTA and 0.01% sodium azide) to a concentration of 2.5 mg protein per ml.

2.8. Purification of the lectin binding glycoproteins from the human ovarian (IGROV-1) cancer cell membranes

The PNA-reactive glycoproteins were isolated from the tumor cell membranes, by a single step affinity chromatography on PNA-agarose-linked column. The specific adsorbed glycoproteins were eluted with Gal 0.3 M, as described in the text. VVA B4-reactive glycoproteins were similarly isolated by affinity chromatography on a VVA B4-agarose linked column, using GalNAc 0.15 M as the eluting sugar.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a 12.5% linear gradient separating gel and a 5% stacking gel, using the silver staining method, for protein detection [9].

2.9. Cell viability assay

Cell survival was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, from Sigma) staining assay, which is based on MTT reduction into formazan by viable cells [10]. Briefly, tumor cells were plated (1×10^4 cells in 0.1 ml RPMI culture medium per well) in a 96-well microtiter plate. After overnight incubation at 37°C in 5% CO_2 humidified atmosphere, the cells were treated with the lectins, at the concentrations and incubation period indicated in the text. At the end of incubation, the culture medium was removed from the wells and replaced with 100 μl of MTT solution (0.5 mg/ml of the culture medium). After 3 h incubation at 37°C, 150 μl of 0.04 N HCl in isopropanol were added to each well, to dissolve the insoluble formazan-containing crystals formed within the viable cells. Optical density was determined at 540 nm wavelength, using an automatic ELISA reader (Titertek, Multiskan MCC/340 MKII, Labsystems, Finland).

2.10. In vivo distribution of the radiolabeled lectins in ovarian tumor-bearing mice

The biodistribution of the ^{125}I-radiolabeled lectins was determined in nude mice bearing either solid subcutaneous (IGROV-1) or intraperitoneal ascitic (OVCAR-3) tumor xenografts. The mice were injected with the lectins (for IGROV-1, when the tumors reached 0.5–1.2 cm^3 volume size; and for OVCAR-3, 20 days after the tumor inoculation), and 16 h post-injection, tissue samples were removed, weighed and their radioactivity measured in a Gamma counter. The biodistribution data are expressed as percentage of the injected dose (% I.D.) per gram wet tissue.

2.11. Effects of PNA on ovarian OVCAR-3 cancer cells xenografted in nude mice

The ovarian OVCAR-3 tumor cells were prepared as described above, suspended to a concentration of 2.5×10^6 cells in 0.5 ml

Table 1
Biodistribution of [^{125}I]PNA and [^{125}I]VVA B4 in CD1 athymic female nude mice, xenografted with either solid (subcutaneous) IGROV-1 or ascitic (intra-abdominal) OVCAR-3 human ovarian tumors

Tissue	PNA[a]		VVA B4[a]	
	% I.D. ± S.D.[b]	T/B[c]	% I.D. ± S.D.[b]	T/B[c]
Lung	0.35 ± 0.09	1.17	2.76 ± 1.68	15.33
Heart	0.22 ± 0.08	0.73	0.71 ± 0.42	3.94
Liver	0.48 ± 0.31	1.60	2.84 ± 1.37	15.78
Spleen	0.35 ± 0.17	1.17	3.52 ± 2.10	19.55
Kidney	3.04 ± 2.59	10.13	5.27 ± 1.38	29.28
Skin	0.36 ± 0.03	1.20	0.78 ± 1.25	4.33
Intestine	0.38 ± 0.12	1.27	3.86 ± 1.92	21.44
Ovaries	0.50 ± 0.43	1.67	13.89 ± 5.11	77.17
Muscle	0.19 ± 0.07	0.63	0.34 ± 0.22	1.89
Tumor:				
solid (IGROV-1)	1.85 ± 1.48	6.17	0.66 ± 0.45	3.67
ascitic (OVCAR-3)	6.99 ± 0.09	23.30	10.81 ± 0.51	60.05
Blood	0.30 ± 0.06	1.00	0.18 ± 0.09	1.00

[a]The lectins were administered (i.p.) at a dose of 2.6×10^6 cpm/0.5 μg protein for PNA, and 2.1×10^6 cpm/3 μg protein for VVA B4, to the tumor-bearing mice. At 16 h post-injection, tissue samples were removed and their radioactivity measured.
[b]The results express the percentage of the injected lectin dose (% I.D.) per gram of tissue and represent mean ± S.D. values from 4–5 animals.
[c]Tissue to blood ratio.

RPMI medium, and then injected (i.p.) to the nude mice (each experimental group consisted of at least 5 animals). The mice were treated (i.p.) with the lectin, at the doses and time schedules indicated in the text, and the tumor growth was monitored by recording the weight changes of mice.

3. Results

3.1. Binding of the lectins to human tumor cells, in vitro

The binding of PNA and VVA B4 lectins to the tumor cells in vitro, was tested by a direct radioimmunoassay, using iodine-labeled (^{125}I) radioactive lectins (Fig. 1). Both PNA and VVA B4 (2 and 5 μg/ml respectively) were found to be highly reactive with the ovarian carcinoma cells tested, revealing about 4.7, 2.7 and 2.4×10^6 PNA-binding sites (taking PNA M_r as 120 kDa) per cell for IGROV-1, OVCAR-3 and SKOV-3 cancer cell lines, respectively. The approximate number of binding sites per cell for VVA B4 (taking its M_r as 143 kDa) was even higher: 7.0, 5.5 and 2.5×10^6, respectively. Normal FS-11 fibroblastic cells used as controls, were non-reactive.

VVA B4 was also highly reactive with the KB epidermoid (about 7.4×10^6 binding sites per cell) and breast SKBr-3 (about 4×10^6 binding sites per cell) carcinoma cells. PNA

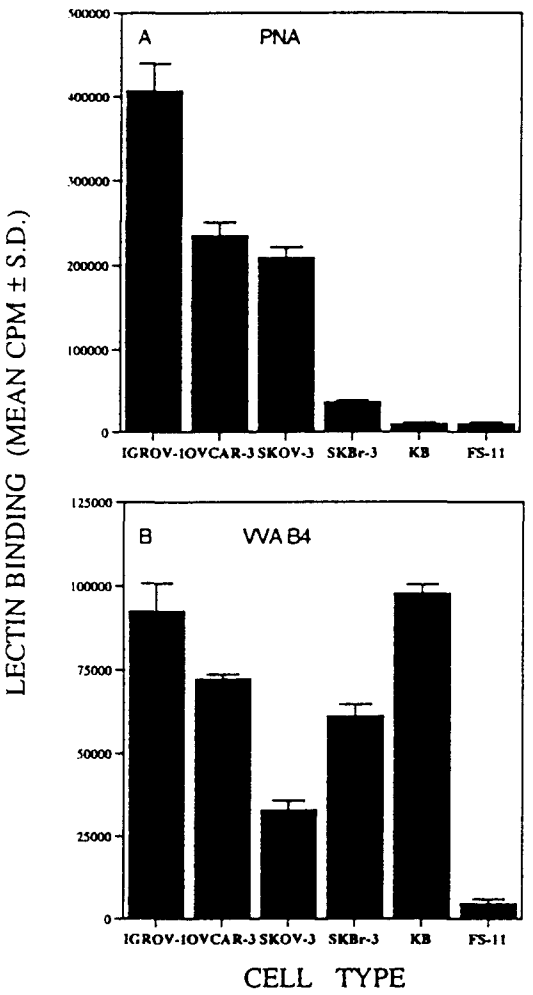

Fig. 1. Binding of [^{125}I]PNA (A) and VVA B4 (B) (added at 2 and 5 μg/ml, respectively) to human ovarian (IGROV-1, OVCAR-3 and SKOV-3), breast (SKBr-3) and oral epidermoid (KB) cancer cells, cultured (1×10^5 per well) in vitro.

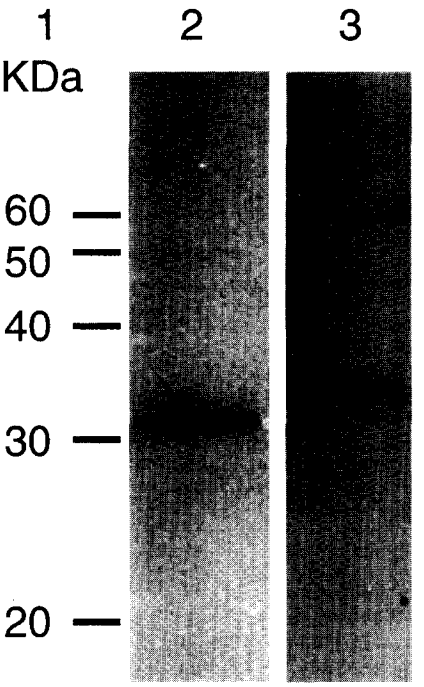

Fig. 2. SDS-polyacrylamide gel electrophoresis of the affinity chromatography purified PNA (lane 2) and VVA B4 (lane 3) reactive glycoproteins, isolated from the ovarian IGROV-1 cancer cell membranes. Lane 1 represents the 10 kDa protein ladder molecular weight standard markers from Gibco.

in contrast, exhibited only a weak reactivity (about 3.83-fold higher as compared to that obtained with the FS-11 cells) with the human SKBr-3 cancer cells (Fig. 1), and did not bind at all to the KB epidermoid cancer cells.

3.2. Inhibition of the lectin binding to the tumor cells by free monosaccharides

The binding of both PNA (610 000 ± 35 000 cpm at 2 μg/ml) and VVA B4 (100 568 ± 19 344 cpm at 4 μg/ml) lectins to the IGROV-1 cancer cells was significantly ($P<0.005$; Student's t-test) inhibited by the addition of Gal (25 mM). This sugar led to decrease of the PNA binding by 62% (231 800 ± 18 544 cpm) and that of VVA B4 by 49% (50 853 ± 3156 cpm). GalNAc, which is regarded as the haptenic immunodominant carbohydrate of Tn antigen, indeed inhibited the VVA B4 binding to IGROV-1 cells by 59% (41 452 ± 10 902 cpm), but caused only 16% (512 400 ± 37 405 cpm) reduction of the PNA binding. The irrelevant non-haptenic Glc sugar led to only a slight inhibition (16–18%) of either lectins.

3.3. Isolation of the lectin-reactive glycoproteins from human ovarian tumor cells

The PNA-reactive glycoproteins were isolated from the tumor cell membranes by affinity chromatography on a PNA-agarose linked column. Essentially, 2 ml (5 mg protein) of the crude cell membrane material was applied to the column (0.5×3.5 cm) equilibrated with PBS 0.01 M; pH 8. After washing with the same buffer, the specific adsorbed glycoproteins were eluted with Gal 0.3 M, and submitted to extensive dialysis against PBS 0.01 M; pH 7.2. The protein content was estimated by absorbance at 280 nm, and the samples were lyophilized to one tenth of the original volume.

The VVA B4-reactive glycoproteins were similarly isolated

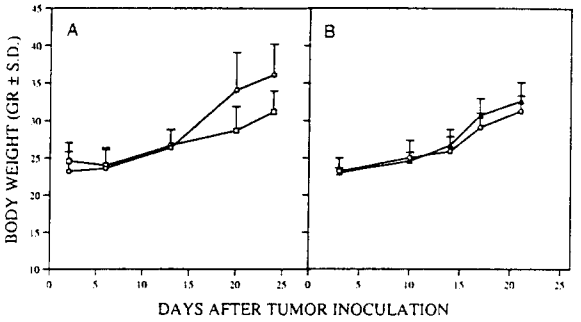

Fig. 3. Effects of PNA (panel A; □) and VVA B4 (panel B; △) treatment (2 i.p. injections of 200 μg each, administered 3 days apart) on the development of human ovarian (OVCAR-3) cancer cells intraperitoneally xenografted (2.5×10^6 cells per mouse) in female CD1 nude mice. The ascites tumor cell development was monitored by recording the changes in the total weight (g ± S.D.) in the animals. The open circles depict the cell growth in the untreated group of mice.

by application of 1.2 ml (3 mg protein) of the crude cell membrane material to a VVA B4-agarose linked column equilibrated with PBS 0.01 M; pH 7.2. After washing of the column with the same buffer, the specific adsorbed glycoproteins were eluted with GalNAc 0.15 M, pooled, dialyzed against PBS and lyophilized to one fifth of the original volume.

SDS-PAGE of the purified lectin-reactive glycoproteins revealed the presence of one major band migrating to a relative molecular weight (M_r) of 32 kDa for PNA, and at least two bands with M_r of 35 and 58 kDa respectively, for VVA B4 (Fig. 2).

3.4. *Viability of human ovarian carcinoma cells cultured in the presence of the lectins*

The effects of PNA and VVA B4 (native forms) on the cell viability of human ovarian IGROV-1 cancer cells cultured in vitro was performed by the method described by Mosmann [10]. Essentially, the cells were seeded at 10 000 cells per well, and treated 24 h later with various doses (2–100 μg/ml) of the lectins, for an additional incubation period of 72 h. The cell viability was evaluated by the MTT ('tetrazolium') staining assay, which is based on MTT reduction into formazan by the viable cells with functional mitochondrial dehydrogenases. Neither PNA nor VVA B4 (up to 100 μg/ml) did exert any significant inhibitory/stimulatory effect on the growth of the tumor (IGROV-1) cells, cultured for up to 72 h in vitro (data not shown). In contrast, the same IGROV-1 cancer cells were highly sensitive to the toxic effect of another Gal/GalNAc-binding lectin from *Ricinus communis* (RCA 60, also referred to as ricin). This lectin (which belongs to the ribosome-inactivating protein [RIP] family) induced a 80% (± 2) suppression ($P<0.0005$) of the ovarian tumor cell growth (observed after a 16 h incubation period) at lectin concentration as low as 0.1 μM, which corresponds to 0.6 μg/ml lectin concentration, taking the ricin molecular weight as 60 kDa.

3.5. *Biodistribution of the radiolabeled PNA and VVA B4 lectins in athymic nude mice bearing human ovarian tumor xenografts*

The radiolabeled [^{125}I]PNA (2.6×10^6 cpm/0.5 μg protein) and VVA B4 (2.1×10^6 cpm/3 μg protein) lectins were administered (i.p.) to the nude mice bearing either solid IGROV-1 (subcutaneous) or ascitic OVCAR-3 (intra-abdominal) tumor xenografts (Table 1). The tissue distribution was measured 16 h post-injection and calculated as % I.D. per gram tissue. The tissue to blood (T/B) ratio, which is an important factor in tumor radioimaging and historadiographic studies, was also calculated. As shown in Table 1, when [^{125}I]PNA was injected i.p. to the tumor-bearing mice, it did accumulate at relatively high levels in the ascitic OVCAR-3 (T/B = 23.30) and solid IGROV-1 (T/B = 6.17) tumor xenografts. It did not show accumulation in other organs, except for the kidneys (T/B = 10.13). This lectin accumulation in the kidneys is due to natural clearance of the lectin from the plasma, as previously shown by others [11]. The biodistribution of [^{125}I]VVA B4 lectin (injected i.p.) revealed a remarkable lectin uptake in the ovaries (T/B = 77.17) and in the ascitic OVCAR-3 tumors (T/B = 60.05). This lectin, however, showed significant accumulation in almost all the organs tested, except for the muscle tissue.

Both [^{125}I]PNA and VVA B4 were effectively cleared from the blood, giving average % I.D. g^{-1} of 0.30 (± 0.06) for PNA, and 0.18 (± 0.09) for VVA B4 (Table 1).

3.6. *Effect of the lectin injection on the ovarian cancer cells xenografted in nude mice*

The effect of the PNA injection on human ovarian OVCAR-3 cells, grown i.p. in female nude mice is depicted in Fig. 3. Injection (i.p.) of this lectin (2 doses of 200 μg each, given 3 days apart, starting at 4 days after the tumor inoculation) to the tumor-bearing mice, was associated with a slight, but statistically significant ($P<0.05$; Student's t-test) suppression of the tumor development in the mice. This tumor growth inhibitory effect (which was not observed with VVA B4) was accompanied by an increase in the mean survival time (MST) of the mice, from 28.25 ± 6.04 days for the untreated group to 36.70 ± 8.10 days in the lectin-treated one ($P<0.05$).

4. Discussion

T antigen and its immediate precursor Tn, are oncofetal antigens overexpressed in many epithelial malignancies, including ovarian, breast and other adenocarcinomas [3]. A wide variety of lectins have been identified that recognize these epitopes with high binding affinities and minimal binding to normal tissues. In the present study we investigated both the in vitro and in vivo reactivities of PNA (which recognizes the Galβ1,3 GalNAc-O-Ser/Thr motif of T antigen), and VVA B4 (which is reactive with the GalNAc-O-Ser/Thr structure of Tn antigen) on human ovarian carcinoma cell lines. Both lectins, however, are not strictly specific for T and Tn and may recognize other closely related structures [4].

Our results demonstrate that both PNA and VVA B4 lectins are highly reactive with the human ovarian IGROV-1, OVCAR-3 and SKOV-3 cancer cell lines, detecting about $2.4\text{-}7.0\times10^6$ lectin binding sites per cell in these carcinoma cells. These values are very close to those previously reported by others for the binding of PNA to poorly differentiated hepatoma cells [12]. Similarly high levels of lectin binding sites were also observed for VVA B4 in human breast SKBr-3 and oral KB carcinoma cells (Fig. 1B). In contrast however, the PNA binding to SKBr-3 carcinoma was relatively low, and no PNA binding could be detected in KB carcinoma cells (Fig. 1A). The weak or absence of responsiveness of these cell lines

with PNA does not correlate our previous results using immunochemical methods, which demonstrated that both polyclonal and monoclonal anti-T antibodies are highly reactive with these cancer cells [6,7]. It is possible therefore, that the lectins and antibodies recognize different molecular structures anchored on the tumor cell surface. Another possibility is that the type (e.g. α/β anomeric forms), number ('clustering') and/or location of the Galβ1,3 GalNAc structural units along the T antigen backbone, are critical for the PNA lectin binding, but do not play an important role in binding to antibodies.

The specificities of the interactions of the PNA and VVA B4 lectins with the tumor cells, was demonstrated by inhibition tests using free monosaccharides. In the case of PNA, Gal was the most potent inhibitor while GalNAc and Glc induced only a feeble inhibitory effect. In contrast, VVA B4 binding to the same tumor cells was inhibited by both GalNAc and Gal, but not by Glc, used as an irrelevant sugar.

Isolation and characterization of the PNA and VVA B4 binding receptors from the IGROV-1 carcinoma cells was performed by affinity chromatography on appropriate immobilized (agarose-linked) lectins, revealing a band of 32 kDa as the major PNA-specific glycoprotein receptor, and at least two bands of 35 and 58 kDa respectively, as specific glycoprotein receptors for VVA B4 (Fig. 2). None of these glycoproteins reacted (in Western immunoblotting) with the polyclonal anti-T and anti-Tn antibodies (unpublished results), although both T and Tn antigenic receptors were previously detected in this human cancer cell line by both direct (e.g. radioimmunoassay) and indirect (e.g. immunoperoxidase staining and FACS analysis) immunochemical methods [6,7]. These results suggest therefore, that in addition to the immunoreactive T and Tn antigens, PNA and VVA B4 react with other glycoprotein or glycolipid structures anchored on the cell surface of the ovarian IGROV-1 cells, although the precise nature of these binding receptors is unknown. Such PNA-binding receptors with closely similar molecular weight (around 30 kDa) were described in human urinary bladder carcinomas [13] and in the rat tumor BSp73 cell lines [14]. PNA-binding receptors with relatively high molecular weights (more than 110 kDa) were also reported, for example in human gastric carcinoma cells and in the plasma membranes of rat hepatoma cells [12,15,16].

Since some of the 'anti-T/Tn'-like lectins are toxic to certain cell lines that bear the appropriate recognition epitopes [17,18], and others have growth-stimulatory effects on certain gastrointestinal cell lines and tissue explants [19], we have performed both in vivo and in vitro experiments to determine whether the binding of PNA and VVA B4 to the human ovarian cancer cells, is associated with such inhibitory/stimulatory effects. Neither PNA nor VVA B4 lectins (examined at doses of 2–100 μg/ml) exhibited any stimulatory or inhibitory effect on the growth of the ovarian (IGROV-1) cancer cells, grown at up to 3 days in cultures. The injection of PNA (2 doses of 200 μg each, administered i.p., 3 days apart) to the tumor-bearing mice, was even associated with a limited suppressive rather than stimulatory effect (Fig. 3) on the tumor cell growth.

These results are not in accordance with our recent data on the effects of specific anti-T and anti-Tn monoclonal antibodies on human tumor cells grown in vitro. According to those data, both anti-T and anti-Tn monoclonal antibodies exert cytotoxic effects (in a complement-independent manner) toward human ovarian and breast cancer cells grown in cultures, but not toward human normal breast cells ([6], Avichezer et al., in preparation). Moreover, we have recently shown that a bacterial lectin (PA-I of *Pseudomonas aeruginosa*) which like PNA and VVA B4 is a Gal/GalNAc binding lectin, but in contrast to them interact preferentially with B, P^k, P_1 and I (rather than T and Tn) blood group antigens, is highly cytotoxic to human ovarian and oral cancer cells cultured in vitro [20]. Its toxicity however, was much lower than that of ricin which is toxic to these cells in the ng dose range. Ricin is a protein composed of two different polypeptide chains A and B (the lectin subunit) linked by a single disulfide bridge. It exerts its toxic activity (towards a wide variety of both transformed and non-transformed cells), through the A chain unit which is capable of inactivating eukaryotic ribosomal RNA leading to inhibition of the tumor cell protein synthesis [21]. All these results taken together suggest, therefore, that the mechanism of tumor cell suppression, induced by either the antibodies or lectins, is unlikely to be simply due to recognition of Gal/GalNAc terminal residues, but represents a much more complex profile of ligand-tumor cell interactions through specific cancer-associated carbohydrate antigens. In some cases, these interactions may represent the first step in the triggering of the cascade events involved in the programmed cell death.

We have examined the potential of PNA and VVA B4 (^{125}I-labeled) for tumor localization studies in a mouse xenograft model. PNA was shown to have a high degree of selective reactivity for human ovarian tumors xenografted in nude mice, as compared to the other organs examined, except for the kidneys which also accumulated significant levels of the radioactive lectin (Table 1). This lectin accumulation in the kidneys is due to natural clearance of the lectin from the plasma, as previously described [11,22]. VVA B4 was also found to accumulate in the ovarian tumor xenografts, even at higher levels than PNA, as judged by the remarkable elevated tissue to blood (T/B) ratios (Table 1). This lectin however, showed a wide tissue binding distribution reflected by relatively high levels of radioactivity uptake in almost all the tissues tested. Since the expression of Tn and other related (e.g. sialyl Tn) antigens recognized by this lectin is very limited (because they are masked by additional sugar residues) in normal tissues and secretions, we presume that the observed interactions of VVA B4 with the normal tissues is due to cross-reactivity with non-specific cross-reacting antigens. Support to this assumption came from our recent observation on the in vivo accumulation in the tumor of specific polyclonal and monoclonal anti-Tn antibodies (^{125}I-labeled), which was not associated with such a pronounced accumulation in the normal tissues (except for the kidneys) as that observed here with VVA B4.

The results of the present study demonstrate therefore, the differential reactivities (both in vitro and in vivo) of PNA and VVA B4 with human carcinoma cells. Of particular importance are the results obtained with PNA. This lectin localized remarkably in human ovarian tumor cells cultured in vitro, and exhibited a selective targeting to the ovarian tumors xenografted in nude mice. The lectin injection was associated with a rapid clearance from the body, yet being associated with a certain suppressive effect on the tumor cell growth. All these properties taken together indicate therefore, that this lectin is a potentially good candidate molecule for delivery of drugs, radionuclides and toxins for the treatment of

ovarian cancer, more particularly for therapy of the residual malignant peritoneal (including ascitic) disease, which remains the major problem in post-operative patients. Yet, due to species-specific differences in tissue glycosylation, the immunogenicity and pharmacokinetic properties of both PNA and VVA B4 lectins in tumor-bearing patients need to be more extensively investigated.

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