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ORIGINAL ARTICLE

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Relationships between degree of binding, cytotoxicity and cytoagglutinating activity of plant-derived agglutinins in normal lymphocytes and cultured leukemic cell lines

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Abstract *Purpose*: To clarify the relationships between the degree of lectin–cell binding, cytotoxicity and cytoagglutinating activity of plant-derived lectins in normal lymphocytes and cultured leukemic cell lines. *Methods*: Plant lectins with different quaternary structures and saccharide specificity were used: *Dolichos biflorus agglutinin* (DBA), *Soybean agglutinin* (SBA) and *Wheat germ agglutinin* (WGA). The leukemic cell lines used were: Jurkat, MOLT-4, RPMI-8402, HPB-ALL, CCR-HSB-2 and BALL-1 (derived from acute lymphoblastic leukemia); Raji and Daudi (derived from Burkitt's lymphoma); K-562 (derived from myelogenous leukemia). The lectin–cell binding was detected microscopically and fluorimetrically using FITC-conjugated lectins. Cytotoxicity was estimated by the CellTiter-Glo luminescent cell viability assay, and cytoagglutinating activity by a spectrophotometric method. *Results*: The binding of DBA and SBA to normal lymphocytes was negligible, while their binding to leukemic cells increased markedly with increasing lectin concentration. Analogous results were obtained for WGA. However, it was found that WGA also interacted to a significant degree with normal lymphocytes. The degree of lectin–cell binding increased in the order: DBA < SBA < WGA. The cytoagglutinating activity and cytotoxicity of lectins increased in the same order. DBA did not exhibit a cytotoxic effect against normal or leukemic cells, and showed a poor cytoagglutinating activity only in MOLT-4, CCR-HSB-2 and BALL-1 cells. SBA exhibited poor cytotoxicity against Jurkat, RPMI-8402, HPB-ALL and CCR-HSB-2 cells, but a well-defined cytotoxicity against Raji and Daudi cells. SBA showed poor cytoagglutinating activity in leukemic cells. In contrast, WGA at concentrations higher than 0.05 μM showed high cytotoxicity against all leukemic cell lines tested as well as against normal lymphocytes. WGA also showed a well-expressed cytoagglutinating effect in all cell lines except normal lymphocytes. There was a moderate inverse correlation between cell viability and the velocity of cytoagglutination ($r = -0.56$, $P < 0.001$), and a good correlation between cell viability and the degree of lectin–cell binding ($r = -0.75$, $P < 0.001$). There was a low positive correlation between the velocity of cytoagglutination and the degree of lectin–cell binding ($r = 0.43$, $P < 0.001$). *Conclusion*: The results suggest that the lectins that bound most strongly to leukemic cells expressed higher cytotoxic and cytoagglutinating activities.

Keywords Cultured leukemic cells · Plant-derived lectins · Cytotoxicity · Cytoagglutination · Lectin–cell binding

Abbreviations *ALL* Acute lymphoblastic leukemia · *DBA Dolichos biflorus agglutinin* · *FITC* Fluorescein isothiocyanate · *PBS(−)* Phosphate-buffered saline (Ca^{2+}- and Mg^{2+}-free) · *SBA Soybean agglutinin* · *WGA Wheat germ agglutinin*

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Introduction

Although the lectins have been known for several decades, interest in them has recently revived because of the potential for lectin-based diagnosis and therapy in cancer. Within the past few years, the lectins have become a

well-established means for elucidating various aspects of tumorigenesis and metastasis [6, 9, 11, 12, 13, 15, 23, 25, 32, 33]. They are dynamic contributors to tumor cell recognition (surface markers), cell adhesion, membrane signal transduction, augmentation of host immune defense, cytotoxicity and apoptosis [6, 9, 11, 13, 23, 25, 32, 33]. The initial step for these activities seems to be initiated by binding lectins to the cell surface carbohydrate chains, a component part of lectin-specific receptors. So, together with antibodies, the plant lectins constitute the family of carbohydrate-binding proteins and are considered a model system for protein–carbohydrate interactions [5], which are involved in many and various physiological processes.

Current research into tumor lectinology includes the design of custom-made carrier-immobilized carbohydrate ligands (neoglycoconjugates), their application for detection of specific binding sites and evaluation of potential therapeutic approaches by blocking access or by directing drug conjugates to cell surface lectin receptors, correlation of their expression with clinical parameters such as prognosis and their biochemical characterization [14]. There is also much effort directed towards the use of lectins (lectin affinity adsorbents) for the separation of cell populations and for in vitro removal and utilization of tumor cells from autologous grafts in transplantation [1, 9, 12, 30]. To advance our understanding of these lectin-dependent processes, it is necessary to clarify the relationship between lectin–cell binding and expression of lectin biological activity, and to select lectin-based (or glycoconjugate-based) tools that could be used to home into tumor cells without influencing normal ones. However, despite considerable research over many decades, structural aspects of lectin–cell binding and the relationship of structure to the biological activities of plant-derived lectins are still disputed.

Plant lectins are a structurally diverse class of proteins of non-immune origin that bind carbohydrates in a reversible fashion and do not exhibit enzymatic activity towards their ligands [18]. The monomers of lectins of the same family usually exhibit high levels of sequential and structural identity, varying only in a few amino acid residues of the oligopeptide chains. However, the fine differences between the monomers result in a surprising variety of fine differences in quaternary structure, which in turn directly result in differences in carbohydrate specificity and binding of the lectins with tumor cell surface receptors [3, 4, 17]. This phenomenon may play a role in the regulation of receptor crosslinking and subsequent signal transduction [7, 10].

The legume lectins *Dolichos biflorus agglutinin* (DBA) and *Soybean agglutinin* (SBA) are examples. DBA and SBA are tetrameric proteins consisting of similar oligopeptide chains and quaternary structures: DBA-2α2β chains (Mr approximately 110,000) and SBA-4α chains (Mr approximately 120,000). Both lectins have analogous carbohydrate affinity to galactosyl-containing residues of cell-surface receptors. However, it has been established that SBA interacts more strongly with pure carbohydrate ligands than DBA [5, 20]. It is not known whether these fine differences in the structure of the two lectins and in their binding with carbohydrate ligands influence the expression of their biological activity against native cells.

In our previous work [29] we established that even a few sequence differences between the *Wheat germ agglutinin* isolectins (WGA-1, WGA-2 and WGA-3, coded by WGA-A, WGA-D and WGA-B genome, respectively; Mr approximately 43,200) result in different degrees of interaction with leukemic cells and different cytoagglutinating and cytotoxic activities. The degree of lectin–cell binding seems to be representative and predictive of the biological activity of a lectin against native and leukemic cells. Moreover, we found that preliminary establishment of the relationship between lectin–cell binding and the biological activity of a lectin is necessary when using lectin-affinity adsorbents for separation of normal cells from leukemic cells [1, 30]. The lectins most well-bound to the cells are often not the best choice.

The present study was designed to clarify the relationships between the degree of lectin–cell binding, cytotoxicity and cytoagglutinating activity in normal lymphocytes and leukemic cells, using lectins with different structures and different potentials to bind to the cell surface.

Materials and methods

Preparation of cells

The human leukemic cell lines Jurkat, MOLT-4, RPMI-8402, HPB-ALL, CCR-HSB-2 and BALL-1 (derived from acute lymphoblastic leukemia, ALL), Raji and Daudi (derived from Burkitt's lymphoma), and K-562 (derived from myelogenous leukemia) (Hayashibara Biochemical Laboratories, Okayama, Japan) were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 µg/ml streptomycin, and 100 U/ml penicillin at 37°C in a humidified atmosphere containing 5% CO_2. The cell lines were a generous gift from Dr. J. Minowada (Hayashibara Biochemical Laboratories). Normal lymphocytes were purified from heparinized peripheral blood obtained from normal adults (aged 38–40 years) by Lymphosepar I. The cells used for the assays were in the logarithmic growth phase. They were sedimented by centrifugation (1000 rpm, 10 min) and washed three times with PBS(−) before use in experiments.

Measurement of cytotoxicity

The cytotoxicity of the lectins against normal and leukemic cells was measured using the CellTiter-Glo luminescent cell viability assay [8]. The bioluminescence of ATP was used as a marker of cell proliferation and viability. Briefly, the lectins were dissolved in PBS(−) and were dispensed in 10-µl aliquots into 96-well microplates. Eight independent experiments were done for each lectin concentration and for each cell line. The cells were suspended in the culture medium to a concentration 5×10^5 cells/ml and then were added in 90-µl aliquots to each patch. After a 24-h incubation at 36.9°C in an incubator (under an atmosphere containing 5.1% CO_2), CellTiter 96 kit (Promega) was added in aliquots of 100 µl to each patch and incubated with the cell suspensions for 1 h, following the procedure recommended by the manufacturer. The luminescence, produced by the luciferase-catalyzed reaction of

luciferin and ATP was measured using an Inter Med Immunomini NJ-2300 multiwell scanning spectrophotometer. The data were normalized to the control group. The lectin cytotoxicity was determined as the percentage decrease in luminescence in comparison with the control.

Cytoagglutination assay

Cytoagglutination was evaluated by the method of Ohba et al. [28] using a Jasco V-550 spectrophotometer (Tokyo, Japan) equipped with a magnetic stirrer. Briefly, the cells were washed three times with PBS(−) and resuspended in the same buffer to a concentration 2×10^6 cells/ml. The cell suspension and PBS(−) were placed into the sample and reference cuvettes, respectively, and were incubated until the baseline at 600 nm became constant. A solution of lectin (DBA, SBA or WGA) was added to the cell suspension at different concentrations and the decrease in turbidity at 600 nm (OD_{600}) was recorded under continuous stirring. The measurement was stopped when the OD_{600} reached a plateau. The velocity of cytoagglutination (ΔOD_{600}/min) was determined from the kinetic curves as described by Ohba et al. [28].

Lectin–cell interaction: microscopic determination

FITC-marked lectins (FITC-DBA, FITC-SBA, FITC-WGA) were added to the respective cell suspension (2×10^6 cells/ml) and the mixtures were incubated for 15 min at room temperature. The cells were sedimented by centrifugation at 1800 rpm for 10 min, washed twice with PBS(−) and resuspended in PBS(−), and the lectin–cell conjugates were analyzed by fluorescent microscopy using an Olympus IX70 microscope.

Lectin–cell interaction: spectrofluorimetric determination

In parallel, the degree of interaction of FITC-marked lectins with normal and leukemic cells was estimated spectrofluorimetrically at an excitation wavelength of 485 nm and an emission wavelength of 538 nm using a BioRad Fluoromark instrument (BioRad, Japan).

Statistical analysis

For multiple group comparisons one-way analysis of variance (ANOVA) was employed followed by Bonferroni's test for truly significant differences. Statistical significance was defined as $P < 0.05$. The statistical procedures were performed with GraphPad InStat software. Data are expressed as means ± SD.

Reagents

All reagents were of analytical grade and were obtained from Amersham Pharmacia Biotech, Becton Dickinson, Gibco BRL, Seikagaku, and Wako Pure Chemical Industries.

Results

Binding of DBA, SBA and WGA with normal lymphocytes and cultured leukemic cells

The degree of binding of lectins with normal and leukemic cells as a function of lectin concentration was determined spectrofluorimetrically, using FITC-lectins as fluorescent markers. The results are shown in Fig. 1. The binding of DBA (Fig. 1A) and SBA (Fig. 1B) with normal lymphocytes was negligible, while their binding with leukemic cells increased markedly with increasing lectin concentration. The degree of DBA binding with leukemic cells increased in the order: K-562 (BALL-1) < HPB-ALL < RPMI-8402 < Daudi < Raji ≤ CCRF-HSB-2 (MOLT-4) < Jurkat. The degree of SBA binding with leukemic cells increased in a similar order: K-562 (BALL-1) < HPB-ALL < RPMI-8402 < MOLT-4 (Daudi) < Raji < Jurkat ≤ CCRF-HSB-2. SBA interacted with leukemic cells to a higher degree than DBA. The differences in the

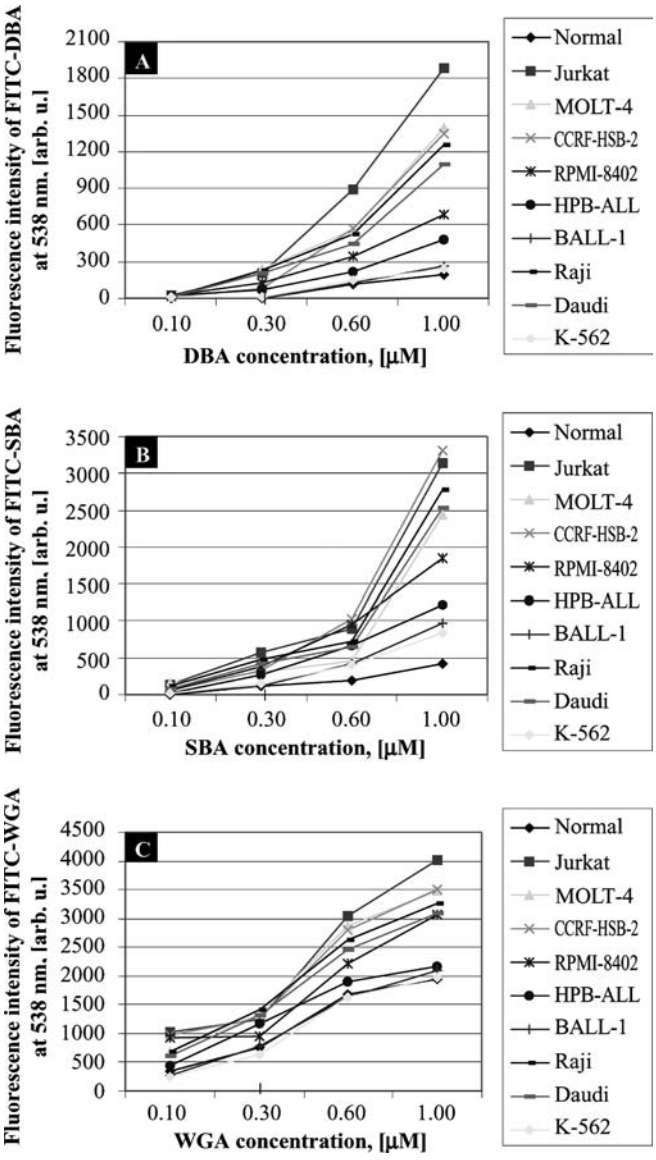

Fig. 1A–C Binding of DBA (**A**), SBA (**B**) and WGA (**C**) with normal lymphocytes and leukemic cells. The degree of binding was evaluated using a spectrofluorimetric method and FITC-lectins as fluorescent markers. The fluorescence intensity of lectin–cell conjugates detected ($\lambda_{ex} = 485$ nm, $\lambda_{em} = 538$ nm) was a function of the FITC-lectin concentration

degree of binding were statistically significant at all lectin concentrations in the same leukemic cell line, with *P*-values in the range 0.05–0.001.

Analogous results were obtained with WGA (Fig. 1C). The degree of WGA binding with leukemic cells increased in the order: K-562 (BALL-1) < HPB-ALL < RPMI-8402 ≤ Daudi ≤ Raji < CCRF-HSB-2 (MOLT-4) < Jurkat. WGA interacted with leukemic cells to a higher degree than DBA and SBA. The differences in the degree of binding between all lectins were statistically significant, with *P*-values in the range 0.05–0.001. In contrast to the other lectins, WGA also bound to a significant degree to normal lymphocytes.

The binding of normal lymphocytes and leukemic cells with FITC-lectins was visualized by fluorescent microscopy (Fig. 2). Fluorescent DBA and SBA cell conjugates were observed only with leukemic cells (Fig. 2A, C), while fluorescent WGA cell conjugates were observed with both leukemic and normal cells (Fig. 2B).

Cytoagglutinating activity of DBA, SBA and WGA in normal lymphocytes and cultured leukemic cells

The cytoagglutinating activity of DBA, SBA and WGA in normal lymphocytes and leukemic cells is shown on Table 1. DBA and SBA at concentrations up to 1 μ*M* did not show cytoagglutinating activity in normal lymphocytes, while WGA showed slight activity. In leukemic cells, the cytoagglutinating activity of lectins depended on the lectin and on the cell line tested. DBA induced cytoagglutination only in MOLT-4, CCRF-HSB-2 and BALL-1 cells, and the effect was slight. SBA at 1 μ*M* induced cytoagglutination in all leukemic cell lines except Raji and K-562. The effect was slight, but greater than with DBA. WGA showed a well-defined cytoagglutinating activity in all leukemic cell lines. The velocity of cytoagglutination in the presence of 1 μ*M* WGA was highest in CCRF-HSB-2 and K-562 cells.

Cytotoxicity of DBA, SBA and WGA against normal lymphocytes and cultured leukemic cells

As shown in Fig. 3A, DBA did not affect the viability of normal lymphocytes or leukemic cell lines. SBA showed a well-expressed cytotoxicity against Raji and Daudi cells, and slight cytotoxicity against Jurkat, RPMI-8402, CCRF-HSB-2 and HPB-ALL cells, but showed no cytotoxicity against normal lymphocytes, MOLT-4, BALL-1 or K-562 cells (Fig. 3B). In contrast, WGA at concentrations of 0.5 μ*M* and higher exhibited strong cytotoxicity against all cell lines, and its cytotoxicity increased in the order: normal (Raji, K-562, Daudi, BALL-1) < Jurkat < HPB-ALL < MOLT-4 < CCRF-HSB-2 ≤ RPMI-8402 (Fig. 3C).

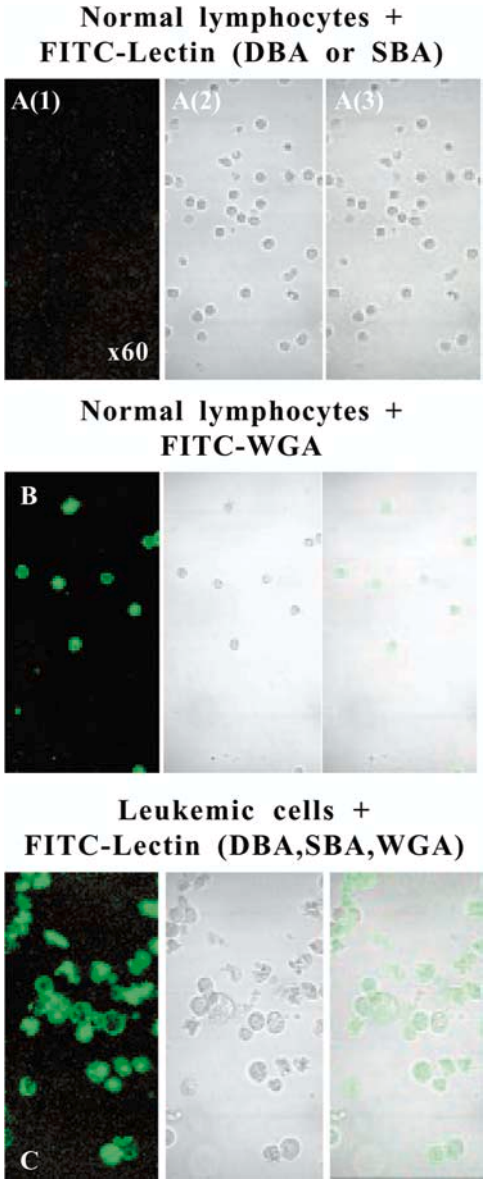

Fig. 2A–C Microscopic detection of lectin–cell interactions. **A** The results are representative of two experiments using either FITC-DBA or FITC-SBA. Note that normal lymphocytes did not interact with DBA or SBA: no fluorescent conjugates are apparent. **B** Normal lymphocytes interacting with WGA: many fluorescent WGA–cell conjugates are apparent. **C** Interaction between FITC-DBA and Jurkat cells representative of several experiments in which different FITC-lectin and leukemic cell lines were used. Note that all leukemic cells interacted with DBA, SBA and WGA. Panels *(1)* fluorescence; panels *(2)* transmission; panels *(3)* fluorescence and transmission

Correlations between the degree of binding, cytotoxicity and cytoagglutinating activity of DBA, SBA and WGA in normal lymphocytes and cultured leukemic cells

The results shown in Table 2 show the correlations between all analyzed parameters. A moderate inverse correlation was observed between cell viability and velocity of cytoagglutination in the presence of lectin

Table 1 Velocity of cytoagglutination of normal lymphocytes and leukemic cell lines following the addition of 1 μ*M* DBA, SBA or WGA

Cell line[a]	Velocity of cytoagglutination (ΔOD_{600}/min)		
	DBA	SBA	WGA
Normal	0	0	0.004 ± 0.001
Acute T-lymphoblastic leukemia			
RPMI-8402 (II)	0	0.006 ± 0.002	0.038 ± 0.007
HBP-ALL (II)	0	0.002 ± 0.001	0.258 ± 0.015
Jurkat (III)	0	0.010 ± 0.002	0.084 ± 0.010
MOLT-4 (III)	0.005 ± 0.001	0.007 ± 0.002	0.172 ± 0.021
CCRF-HSB-2 (IV)	0.004 ± 0.001	0.050 ± 0.007	0.424 ± 0.025
Acute B-lymphoblastic leukemia			
BALL-1 (III)	0.004 ± 0.001	0.008 ± 0.002	0.063 ± 0.007
Burkitt's lymphoma			
Raji	0	0	0.185 ± 0.016
Daudi	0	0.013 ± 0.004	0.166 ± 0.009
Myeloblastic leukemia			
K-562	0	0	0.323 ± 0.021

[a]Parentheses indicate the differentiation stage of the cell line

($r = -0.56$, $P < 0.001$), and a good correlation was observed between cell viability and degree of cell–lectin binding ($r = -0.75$, $P < 0.001$). A low but significant positive correlation was found between the degree of cell–lectin binding and the velocity of cytoagglutination ($r = 0.43$, $P < 0.001$).

Discussion

The present study provides direct evidence of the relationship between the degree of lectin-cell binding and expression of cytoagglutination and cytotoxicity of some plant-derived lectins (DBA, SBA and WGA) in normal lymphocytes and cultured leukemic cell lines, derived from ALL, myelogenous leukemia and Burkitt's lymphoma. The choice of lectins was based on the assumption that variations in their quaternary structure have significant functional implications for the binding of multivalent sugar ligands on tumor cell surfaces, which often initiates signal transduction cascades [3, 18, 19].

DBA and SBA consist of similar polypeptide chains ($2\alpha 2\beta$ for DBA, and 4α for SBA) but have different quaternary structures [5, 19]. Both lectins have a similar carbohydrate specificity: DBA is a GalNAc-α1-3GalNAc-binding protein, and SBA is a GalNAc-α1-3Gal-binding protein. However, the fine differences in their quaternary structure seems to be very important for the strength of cell binding and expression of their biological activities. We established that SBA bound to a significantly greater extent with leukemic cells than DBA (Fig. 1). Probably, this was a result of some structural differences between DBA and SBA. First, the substitution of an aromatic residue at positions 127 by a shorter aliphatic leucine or valine residue in the DBA molecule has been shown to abolish its carbohydrate binding [16]. Second, the α-helices have been found to increase the stability of the quaternary structure of SBA in comparison with that of DBA [20, 31], and to increase the ability of SBA to bridge large distances and to crosslink multivalent carbohydrate ligands on the cell surface [5]. The freedom of movement of the epitopes on the carbohydrate ligands would also affect lectin–cell interactions [5, 20].

The third lectin, WGA, is outstanding among plant-derived lectins in its potent cytotoxicity against tumor cells such as hepatoma, choriocarcinoma and osteosarcoma [9, 10, 15, 23, 25, 34]. However, less is known about its biological effects against leukemic cells. WGA is a dimeric molecule with a molecular weight about three times lower than that of DBA and SBA [25]. WGA also has a different carbohydrate specificity. It is an *N*-acetyl-D-glucosamine/*N*-acetyl-D-neuraminic acid binding substance [27]. The degree of WGA–cell interaction was higher than that of DBA and SBA, especially at low concentrations (0.1–0.6 μ*M*, $P < 0.001$). It seems that leukemic cells contain more glucosyl/neuraminic acid residues than galactosyl residues on their surface membrane. However, the concentration curve of WGA–cell binding showed a tendency to plateau at 1 μ*M*, while those of DBA and SBA strongly increased in the region 0.1–1 μ*M*. Therefore, the higher degree of WGA–cell binding was probably the result of the higher affinity of WGA to the respective cell surface receptors and the higher stability of the WGA–cell conjugates compared with those of DBA and SBA.

A strong interaction of WGA with normal lymphocytes was also shown by microscopic analysis (Fig. 2). This indicates that glucosyl/neuraminic acid-containing receptors are not specific for leukemic cells and WGA cannot be used as a qualitative marker for their characterization. However, quantitative analysis of WGA–cell binding may be able to distinguish between leukemic cells (except BALL-1) and normal cells, because this parameter was about two times lower in the case of normal lymphocytes. On the other hand, the structures of DBA-binding and SBA-binding galactosyl-containing receptors appear to be characteristic of leukemic cells and they are not expressed in normal lymphocytes.

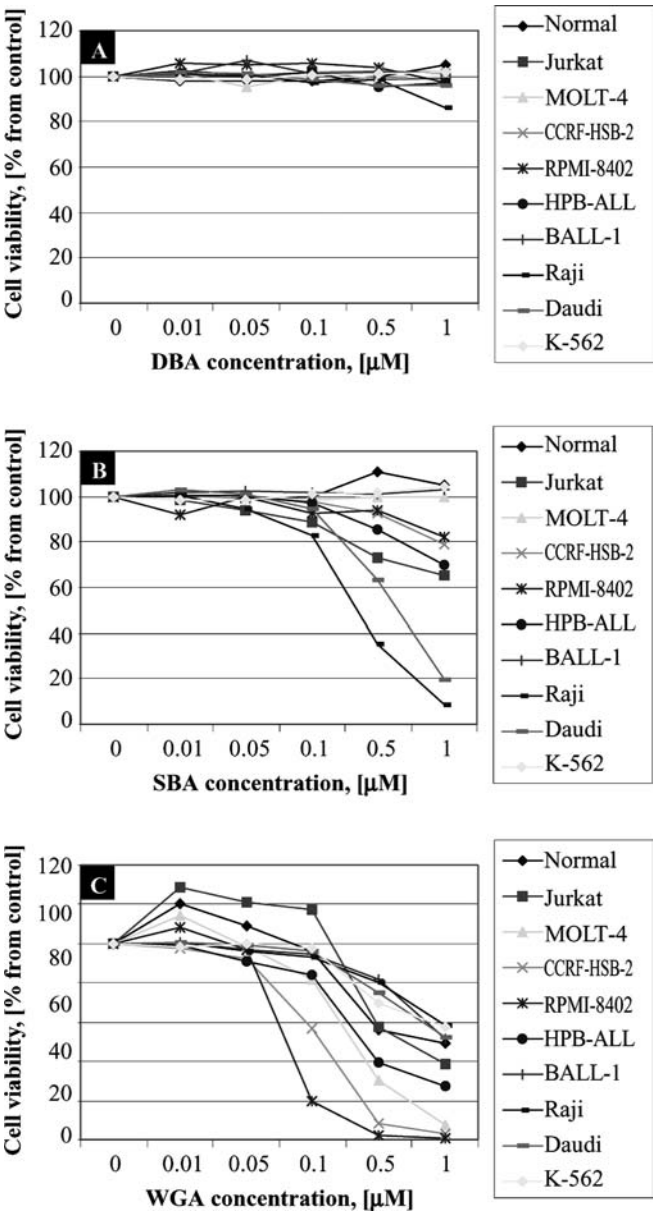

Fig. 3A–C Cytotoxicity of DBA (**A**), SBA (**B**) and WGA (**C**) against normal lymphocytes and leukemic cells. Lectin-induced cytotoxicity was evaluated using the CellTiter-Glo luminescent cell viability assay, as described in Materials and methods. Lectin cytotoxicity was calculated as the percentage decrease in luminescence in comparison with the control group

These observations are in agreement with previously reported results indicating that the binding of DBA and SBA to normal lymphocytes is generally low, and these lectins can be used as tumor diagnostic markers [2, 6, 11, 12]. It has also reported that SBA binding gradual increases with the stage of differentiation of leukemic cells [2].

The degree of lectin binding was different for each leukemic cell line (Fig. 1). These differences could be considered to be the result of differences in the cell surface glycoproteins. Several reports emphasize changes in the structure and distribution of the cell surface

Table 2 Correlation between degree of binding, cytotoxicity and cytoagglutinating activity of DBA, SBA and WGA in normal lymphocytes and leukemic cells

Parameters	Correlation coefficient (r)	P value
Cell viability (%)/velocity of cytoagglutination (ΔOD_{600}/min)	−0.564893	<0.001
Cell viability (%)/degree of lectin–cell binding (fluorescence intensity 538 nm)	−0.7522742	<0.001
Velocity of cytoagglutination (ΔOD_{600}/min)/degree of lectin–cell binding (fluorescence intensity 538 nm)	0.4261964	<0.001

glycoproteins during differentiation of tumor cells, reflecting lectin–cell binding [21, 22, 24, 26]. In T-ALL cell lines, all lectins showed strong binding with the relatively differentiated Jurkat, CCRF-HSB-2 and MOLT-4 cells in comparison with relatively non-differentiated RPMI-8402 and HBP-ALL cells (Fig. 1). In contrast, in the case of B-ALL cell lines the relatively differentiated BALL-1 cells showed weak binding with all the plant-derived lectins studied. The carbohydrate specificity together with the degree of cell binding of DBA, SBA and WGA could provide information about the carbohydrates on the surface of the leukemic cells. In the case of cell lines derived from ALL, it may be supposed that glycoconjugates having α-linked or β-linked galactopyranosides and/or glucopyranosides at the non-reducing end may appear during differentiation of T-ALL cells, and they may disappear during more pronounced differentiation of B-ALL cell lines. A decrease in high molecular weight oligosaccharides on human lymphocytic cell membranes during differentiation has been reported [24, 26], and this may also reflect the degree of lectin–cell binding. It appears that the binding of lectins with low molecular weight oligosaccharides on the cell surface of T-ALL cells is stronger than that with high molecular weight oligosaccharides. This is not surprising in view of the structure and stability of lectin–carbohydrate complexes [5].

The most important observation in our study was that the strength of lectin–cell binding reflected the biological activities of the lectins (cytoagglutination and cytotoxicity) in leukemic cells.

DBA is known to agglutinate red blood cells of group A and to precipitate the derived glycoconjugates [6, 20, 31]. However, we have shown that, despite binding to the leukemic cell surface, DBA manifested poor cytoagglutinating activity only in MOLT-4, CCRF-HSB-2 and BALL-1 cells (Table 1). It did not exhibit cytotoxicity. In contrast, SBA exhibited a well-defined cytotoxic effect against Burkitt's lymphoma cells (Raji and Daudi), and a slight but significant cytotoxicity against ALL-derived RPMI-8402, CCRF-HSB-2, HPB-ALL and Jurkat cells, but did not show a cytotoxic effect against MOLT-4, BALL-1 or K-562 cells (Fig. 3B). SBA also showed a higher cytoagglutinating activity than DBA (Table 1). WGA showed high cytotoxicity and cytoagglutinating

activity in leukemic cells and marked cytotoxicity against normal lymphocytes (Fig. 3C). Both parameters were several times higher than for SBA and DBA.

There are two possible explanations for these findings. First, the stronger interaction of WGA with the cell surface may lead to a higher cytoagglutinating and cytotoxic activity. A good inverse correlation was found between cell viability and degree of lectin–cell binding ($r = -0.75$, $P < 0.001$), and a poor but significant positive correlation between the velocity of cytoagglutination and the degree of lectin–cell binding ($r = 0.43$, $P < 0.001$). Second, cell surface receptors containing glucosyl and neuraminic acid could be considered better triggers of cytoagglutinating and cytotoxic action than galactosyl-containing receptors. However, both possibilities are speculative and need verification.

In conclusion, the results suggest that plant-derived lectins that show a higher degree of interaction with leukemic cells and normal lymphocytes have better expressed cytoagglutinating and cytotoxic activities. However, the binding of a lectin to the cell surface is not enough to trigger the mechanisms leading to expression of its biological activity. A comparison of our results with previously published data shows that the dimeric lectins (WGA, abrin-a, CEL-III, etc.) usually interact with leukemic cells to a higher degree than tetrameric lectins (DBA, SBA), but they are not highly selective for leukemic cells. Moreover, some of dimeric lectins show high cytoagglutinating activity and cytotoxicity not only against leukemic cells but also against normal lymphocytes, and it is impossible to use them diagnostically or for separation of cell populations. Our observations indicate that the tetrameric lectins probably have the greatest clinical potential, despite the fact that they show lower cytotoxicity than the dimeric lectins. The optimal variants appear to be tetrameric lectins consisting of identical monomers. They interact to a high degree only with leukemic cells and possess low cytotoxic and cytoagglutinating activities in leukemic cells, but have no effect on the viability of normal lymphocytes.

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References

1. Bakalova R, Ohba H (2003) Purification of normal lymphocytes from leukemic T-cells by lectin-affinity adsorbents—correlation with lectin–cell binding. Cancer Lett 192:59
2. Ben-Bassat H, Weksler ZS, Shlomai Z, Prokocimer M (1987) Interaction of soybean agglutinin with human leukemia-lymphoma lines at various stages of differentiation. Leuk Res 11:589
3. Brewer CF (1996) Multivalent lectin-carbohydrate cross-linking interactions. Chemtracts Biochem Mol Biol 6:165
4. Bouckaert J, Hamelryck T, Wyns L, Loris R (1999) Novel structures of plant lectins and their complexes with carbohydrates. Curr Opin Struct Biol 9:572
5. Buts L, Dao-Thi M-H, Loris R, Wyns L, Etzler M, Hamelryck T (2001) Weak protein-protein interactions in lectins: the crystal structure of a vegetative lectin from the legume *Dolichos biflorus*. J Mol Biol 309:193
6. Caselitz J (1987) Lectin and blood group substances as ''tumor markers''. Curr Top Pathol 77:245
7. Chay CH, Pienta KJ (2000) Evidence for lectin signaling to the nuclear matrix: cellular interpretation of the glycocode. J Cell Biochem 35:S123
8. Crouch SPM (1993) The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. J Immunol Methods 160:81
9. Gabius HJ (1997) Concepts of tumor lectinology. Cancer Invest 15:454
10. Gabius HJ (2001) Probing the cons and pros of lectin-induced immunomodulation: case studies for the mistletoe lectin and galectin-1. Biochimie 83:659
11. Gabius HJ, Engelhard R, Cramer F (1985) Endogenous tumor lectins: a new class of tumor markers and targets for therapy? Med Hypotheses 18:47
12. Gabius HJ, Engelhard R, Cramer F (1986) Endogenous tumor lectins: overview and perspectives. Anticancer Res 6:573
13. Gabius HJ, Vehmeyer K, Gabius S, Nagel GI (1988) Clinical application of various plant and endogenous lectins to leukemia. Blut 56:147
14. Gabius HJ, Kayser K, Gabius S (1995) Protein-carbohydrate recognition. Foundation and medical application with illustrations of tumor lectin studies. Naturwissenschaften 82:533
15. Gorelik F, Galili U, Raz A (2001) On the role of cell surface carbohydrates and their binding proteins (lectins) in tumor metastasis. Cancer Metastasis Rev 20:245
16. Hamelryck TW, Loris R, Bouckaert J, Dao Thi M-H, Strecker G, Imberty A, Fernandez E, Wyns L, Etzler ME (1999) Carbohydrate binding, quaternary structure and a novel hydrophobic binding site in two legume lectin oligomers from *Dolichos biflorus*. J Mol Biol 286:1161
17. Heldin CH (1995) Dimerization of cell surface receptors in signal transduction. Cell 80:213
18. Lis H, Sharon N (1998) Lectins: carbohydrate-specific proteins that mediate cellular recognition. Chem Rev 98:637
19. Loris R, Hamelryck T, Bouckaert J, Wyns L (1998) Legume lectin structure. Biochim Biophys Acta 1383:9
20. Loris R, De Greve H, Dao Thi M-H, Messens J, Imberty A, Wyns L (2000) Structural bases of carbohydrate recognition by lectin II from *Ulex europeus*, a protein with promiscuous carbohydrate binding site. J Mol Biol 301:987
21. Mann PL, Lopez-Colberg I, Kelley RO (1987) Cell surface oligosaccharide modulation during differentiation: I. Modulation of lectin binding. Mech Ageing Dev 38:207
22. Mann PL, Swartz CM, Holmes DT (1988) Cell surface oligosaccharide modulation during differentiation: III. Lectin affinity class distribution. Mech Ageing Dev 44:1
23. Misra RR, Pinsky PF, Srivastava S (2000) Prognostic factors for hematologic cancers. Hematol Oncol Clin North Am 14:907
24. Mizoguchi A, Takasaki S, Maeda S, Kobata A (1984) Changes in asparagine-linked sugar chains of human promyelocytic leukemic cells (HL-60) during monocytoid differentiation and myeloid differentiation. Decrease of high-molecular-weight oligosaccharides in acidic fraction. J Biol Chem 259:11949
25. Mody R, Joshi S, Chaney W (1995) Use of lectins as diagnostic and therapeutic tools for cancer. J Pharmacol Toxicol Methods 33:1
26. Motoyoshi F, Kondo N, Orii T (1990) Appearance of bisected N-acetylglucosamine residue of biantennary sugar chains and decrease of high molecular weight oligosaccharides of human lymphocytic cell membranes during differentiation. Clin Immunol Immunopathol 54:495
27. Muraki M, Ishimura M, Harata K (2002) Interaction of wheat germ agglutinin with GlcNAc-b1,6-Gal sequence. Biochim Biophys Acta 1569:10
28. Ohba H, Toyokawa T, Yasuda S, Hoshino T, Itoh K, Yamasaki N (1997) Spectroscopic analysis of the cytoagglutination

activity of abrin-b isolated from *Abrus precatorius* seeds against leukemic cells. Biosci Biotech Biochem 61:737
29. Ohba H, Bakalova R, Muraki M (2002) Cytoagglutination and cytotoxicity of wheat germ agglutinin isolectins against normal lymphocytes and cultured leukemic cell lines—relationship between structure and biological activity. Biochim Biophys Acta 1619:144
30. Ohba H, Bakalova R, Moriwaki S, Nakamura O (2002) Fractionation of normal and leukemic T-cells by lectin-affinity column chromatography. Cancer Lett 184:207
31. Olsen LR, Dessen A, Gupta D, Sabesan S, Sacchettini JC, Brewer CF (1997) X-ray crystallographic studies on unique cross-linked lattices between four isomeric biantenary oligosaccharides and soybean agglutinin. Biochemistry 36: 15073
32. Raedler A, Schreiber S (1988) Analysis of differentiation and transformation of cells by lectins. Crit Rev Clin Lab Sci 26:153
33. Vaickus L, Ball ED, Foon KA (1991) Immune markers in hematologic malignancies. Crit Rev Oncol Hematol 11:267
34. Wang H, Ng TB, Ooi VEC, Liu WK (2000) Effects of lectins with different carbohydrate-binding specificities on hepatoma, choriocarcinoma, melanoma and osteosarcoma cell lines. Int J Biochem Cell Biol 32:365