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Flow Cytometric Analysis of the Binding of Eleven Lectins to Human T- and B-Cells and to Human T- and B-Cell Lines

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The relative surface binding of 11 lectins to human peripheral blood T- and B-lymphocytes, to Molt-4 and JM T-cell lines, and to 6410 and NC37 B-cell lines was determined by flow cytometry. The lectins from Lens culinaris (LCA), Ricinus communis (RCA), Arachis hypogaea (PNA), Abrus precatorius (APA), Ulex europaeus (UEA-F), Sarothamnus scoparius (SAS-F), Helix pomatia (HPA), Phaseolus coccineus (L-PHA), Glycine max (SBA), and Triticum vulgare (WGA) were fluoresceinated and incubated with living, formaldehyde-fixed, or neuraminidase-treated cells. Except LCA, which preferentially bound to the two B-cell lines tested in this study, none of the other lectins exhibited selective binding to the undifferentiated cells of the cell lines. The T-cell lines and, in part, the peripheral blood T-cells bound less WGA, APA, LCA, and L-PHA than the B-cell lines and the peripheral blood B-cells. Binding of PNA was found only after neuraminidase treatment of the cells; the binding of PNA, HPA, and UEA-F after neuraminidase treatment was higher for the T-cells than the B-cells from peripheral blood. No significant differences were detected between both cell types for RCA, ConA, SBA, and SAS-F.

Key terms: Flow cytometry, lectins, T-cells, B-cells, T-cell lines

Antibodies are largely used to characterize T- and B-cells at different stages of differentiation (18,19) and to preparatively separate these cells (25). The use of lectins that specifically bind to sugars is another principle for analyzing cell surfaces, and gives information on the carbohydrate pattern of the cell membrane (2,5,6). Lectins can be labeled with fluorescein-isothiocyanate (FITC). The relative amount of cell-bound lectins can then be determined by a flow cytometric measurement. It is possible to calculate the relative cell surface density of lectin-binding sites from these measurements when the volume of each cell is simultaneously determined in the flow cytometer by electrical sizing. The binding of lectins to cells of different size can be compared thereby in a standardized way (30).

The aim of the present study was to determine the surface binding of 11 lectins on human T- and B-lymphocytes and to examine whether these lectins could distinguish between mature peripheral blood T- and B-lymphocytes and the more immature blastoid cells of four human T- and B-cell lines.

MATERIALS AND METHODS

Lectins and Fluoresceinated Polycations

We isolated Lens culinaris agglutinin (LCA, D-mannose, D-glucose) (12), Canavalia ensiformis agglutinin (ConA, D-mannose, D-glucose) (1), Ricinus communis agglutinin (RCA, MW 120,000, D-galactose) (22), Arachis hypogaea agglutinin (PNA, D-galactose) (17), Abrus precatorius agglutinin (APA, MW 135,000, D-galactose) (28), Ulex europaeus agglutinin (UEA-F, L-fucose) (8), Sarothamnus scoparius agglutinin (SAS-F, L-fucose) (8), Helix pomatia agglutinin (HPA, N-acetylgalactosamine) (8), Leuko-phytohemagglutinin from Phaseolus coccineus (L-PHA, N-acetylgalactosamine) (10), Glycine max agglutinin (SBA, N-acetylgalactosamine) (7), and Triticum vulgare agglutinin (WGA, N-acetylglucosamine, N-acetyl neuraminic acid) (16) to >95% homogeneity and checked by polyacrylamide-gel electrophoresis according to the methods indicated by the authors. The procedures are in general based upon affinity chromatography, ion-exchange chromatography, and gel-filtration.

The lectins and poly-L-ornithine (mean molecular weight 220,000 D [Sigma, St. Louis, MO]) were fluoresceinated using FITC (Sigma) (20). The molar fluorescein/protein ratio was between 1.5 and 2.3 for the different

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lectins and 2.5 for fluoresceinated poly-L-ornithine (FPO). The influence of FITC-coupling to lectins was routinely evaluated by incubating cells at constant total lectin concentration with varying proportions of FITC-labeled and unlabeled lectin. Plots of FITC-lectin concentration versus cell fluorescence were linear and passed through the origin, demonstrating that the native and FITC-conjugated forms behaved functionally as a single molecular species (23).

Isolation of Lymphocyte Populations, Cell Lines

Human peripheral blood mononuclear cells were isolated by Ficoll-hypaque density gradient centrifugation (Pharmacia, Freiburg, FRG). T-cells were prepared by E rosetting with 1% sheep erythrocytes treated with S-(2-aminoethyl)-isothiouronium-bromide HCl (15). The rosetted mixture was layered over Ficoll-Hypaque, the E^+ pellet was recovered and treated with 0.155 M NH_4Cl. The nonrosetting population was harvested from the Ficoll interface and it contained >60% B-lymphocytes, as shown by positive immunofluorescence for surface Ig, and between 30 and 40% monocytes. The rosetting fraction contained >95% T-lymphocytes as shown by rerosetting assays and complement-mediated cell lysis with anti-T cell sera. The nonrosetting fraction contained less than 5% T-cells.

The human T-cell lines Jurkat (JM) and Molt-4 and the B-cell lines 6410 and NC37 were kindly donated by Dr. U. Schneider (Kinderklinik d. Universität Erlangen). The T-cell lines were positive for human T-lymphocyte antigen and negative for Ia-like antigen and common type leukemic antigen (18). The B-cell lines were characterized by the presence of surface immunoglobulin and the Epstein-Barr virus genomes (33). The B-cell lines were also negative for T-lymphocyte antigen.

Cell Preparation for Incubation With Lectins

All cells were washed twice (200 g, 4°C) in 5 mM tris buffered saline pH 7.4 (TBS) and fixed by 1+1 (v/v) dilution of the cell suspension with freshly prepared TBS solution containing 3.5% formaldehyde (Merck, Darmstadt, FRG) adjusted to pH 7.4 with 1.0 N NaOH. The cells were stored in the fixative for at least 4 h. Neuraminidase-treated cells were obtained by incubating 5×10^6 fixed or unfixed cells with 0.02 international units neuraminidase (Behringwerke, Marburg, FRG) in 1.0 ml isotonic saline containing 0.02 M acetate buffer (pH 5.6) and 1 mM $Ca2^+$ for 30 min at 37°C. After treatment the cells were washed twice and resuspended in TBS. The cell surface charge before and after neuraminidase digestion was determined by staining with fluoresceinated poly-L-ornithine (FPO) to monitor the amount of sialic acid removed (32).

The cell suspensions (10^6 cells/ml) were incubated with fluoresceinated lectins or FPO (20 μg/ml) for 30 min at 22°C. Unfixed cells were incubated for 1 h at 4°C. The displacement experiments were performed by using 50-mM solutions of the respective inhibitory monosaccharide. The cells were measured in the flow cytometer without prior washing in binding equilibrium.

Centrifugal Elutriation

Blood mononuclear cells were separated into lymphocytes and monocytes with an elutriator centrifuge (Beckman, Munich, FRG) at 3000 rpm by stepwise increase of the buffer flow (TBS). The volume distribution curves of the cells in the eluted fractions were determined in a Metricell flow cytometer (13). An aliquot of each cell fraction was pelleted on a microscope slide with a cytocentrifuge (Shandon Elliott, Runcorn, England) and stained with the May Grünwald-Giemsa method. Cell morphology was evaluated microscopically.

Flow Cytometric Analysis

The volume and the membrane-bound fluorescence of the stained cells were determined simultaneously with a Fluvo-Metricell flow cytometer (14), which combines electrical cell sizing and epi-illumination fluorescence measurement. The orifice for volume sizing was cylindrical, with 85 μm diameter and 100 μm length. The system was filled with TBS (65 Ohm cm at 22°C). The cells were measured with an electrical current of 0.060 mA at a suction of 0.18 kg/cm^2. Fluorescence was excited with an HBO 100 mercury lamp using a 450-nm high-pass and a 490-nm low-pass interference filter (Schott, Mainz, FRG). The fluorescent light was separated from the exciting light by a 500-nm dichroic mirror (Zeiss, Oberkochen, FRG) and an additional 500-nm high-pass filter in front of the photomultiplier. The cells were measured at 22°C at a rate of between 800 and 1,000 cells per second. The stability of the electric sizing was monitored by electronic calibration (13) and varied within 2% on a day-to-day basis. The stability of the fluorescence measurements was controlled by a reference sample prepared by daily staining of the erythrocytes from the same individual with 10 μg/ml FITC-WGA and by checking the position of the sample beam before and after each measurement. The coefficient of variation for ten repetitive measurements of the same sample was 3.7%.

The maximum amplitude of the volume and fluorescence signals of each cell were stored in a 64 × 64 array of a multichannel analyzer (AEG Telefunken, Ulm, FRG). The data were transferred to a magnetic tape for permanent storage and further analysis by Fortran IV computer programs (31,32). The cell surface (S) was calculated from the cell volume (V) according to $S = 4.84 \times (V)^{0.67}$. The relative surface density of the lectin was determined as the ratio of total cellular lectin fluorescence to cell surface. The relative lectin surface density is a standardizing parameter and as so is similar to, for example, enzyme activities per gram of tissue or per milligram of protein. The relative lectin receptor density, like a number of other biochemical standardizing parameters, does not contain information on the discreteness of packing (eg, caps) or on the mean distance of labeled receptors on the cell surface. Nevertheless the relative lectin receptor density on the cell surface is useful for comparing lectin binding to cells of different size. The usefulness and experimental validation of the calculation of cell surface densities has been shown ear-

lier, when a flow cytometric method for the measurement of electrical charge density was developed (32).

RESULTS

The experiments were performed with formaldehyde-fixed cells in order to have stable conditions during a large series of measurements and to exclude the possibility of endocytosis, which would falsely increase the cellular fluorescence from ingested fluorescent lectin. Formaldehyde fixation did not change the amount of bound lectin within 5% of the binding to native cells. Neuraminidase removed sialic acid similarly from vital and fixed cells. The apparent cell volumes were decreased by fixation to approximately 25–30% of the unfixed values. Calculation of the mean lectin receptor density before and after fixation showed, however, that the characteristic differences between the cell types remained after fixation (Table 1).

Table 1
Relative Surface-Binding Density per Unit Cell Membrane of FITC-WGA (20 μg/ml) to Human B- and T-Cells Before and After Formaldehyde Fixation[a]

	Native	Formaldehyde Fixed
B-cells	0.090 ± 0.0007	0.253 ± 0.0011
T-cells	0.051 ± 0.0002	0.142 ± 0.0006
Ratio of B-cells to T-cells	1.76	1.78

[a] 1.7% v/v, 3h 4°C; ± SE, between 11, 472 and 29, 293 cells per measurement.

APA, Abrus precatorius lectin (agglutinin); CON A, concanavalin A lectin; FITC, fluorescein isothiocyanate; FPO, fluoresceinated poly-l-ornithine; HPA, Helix pomatia lectin; L-PHA, leuko-phytohemagglutinin; LCA, Lens culinaris lectin; PNA, peanut lectin; RCA, Ricinus communis lectin; SAS-F, Sarothamnus scoparius lectin; SBA, soybean lectin; TBS, tris buffered saline; UEA-F, Ulex europaeus lectin; WGA, wheat germ lectin

The JM T-cell line (Fig. 1a), the 6410 B-cell line (Fig. 1b), and the T-cell fraction of human peripheral blood (Fig. 1c) appeared as one broad cell cluster in the cell volume versus fluorescence plots. Two cell clusters were observed for the B-cell fraction (Fig. 1d). They corresponded to lymphocytes and monocytes as determined by a separation experiment with the centrifugal elutriator and microscopic examination of the May Grünwald stained cell fractions.

The T-cell lines, and in part the neuraminidase-treated T-cells from peripheral blood, showed a lower surface binding for WGA, APA, LCA, and L-PHA than the B-cell lines and the peripheral B-cells (Table 2). The binding of PNA, HPA, and UEA-F to T-cells after removal of

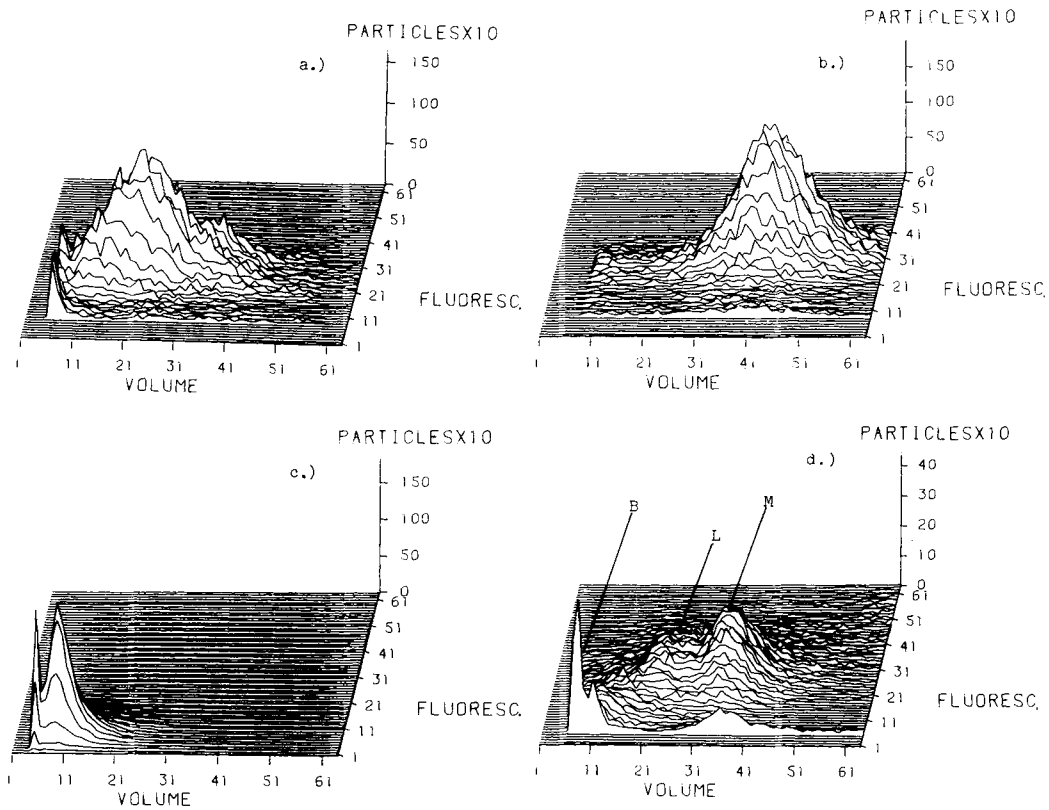

FIG. 1. Cell volume versus membrane-bound FITC-WGA (20 μg/ml) fluorescence of formaldehyde-fixed cells measured after 30 min incubation at 22°C. a) JM T-cell line (total cell count [TC] = 105,339). b) 6410 B-cell line (TC = 75,075). c) T-cell fraction from human peripheral blood (TC = 136,580). d) B-cell enriched fraction from human peripheral blood (TC = 107,953; B = background, L = lymphocytes, M = monocytes). The volume and fluorescence signals were measured using 2,5 logarithmic decade pulse amplifiers. A difference of approximately six volume or fluorescence classes in the 64 × 64 channel array of the multichannel analyzer corresponds to a twofold increase of signal amplitude.

Table 2
Relative Surface Binding of Fluoresceinated Lectins and Poly-Ornithine to Neuraminidase-Treated, Formaldehyde-Fixed Human Cells in Percent of the Calculated Mean Fluorescence Density per Unit Cell Surface of JM T-Cell Line and T-Cells (±SE, between 31,784 and 597,727 cells per measurement)[a]

	WGA	APA	LCA	L-PHA	HPA	PNA	UEA-F	PO
T-cell lines								
JM	100 ± 0.15	100 ± 0.30	100 ± 0.25	100 ± 0.45	100 ± 0.17	100 ± 0.39	100 ± 0.75	100 ± 0.22
Molt-4	63 ± 0.14	84 ± 0.22	49 ± 0.09	93 ± 0.19	57 ± 0.25	65 ± 0.57	105 ± 0.52	74 ± 0.16
B-cell lines								
6410	165 ± 0.34	129 ± 0.54	285 ± 1.27	153 ± 0.67	111 ± 0.45	103 ± 0.58	148 ± 0.67	95 ± 0.26
NC37	137 ± 0.41	118 ± 0.40	188 ± 0.34	122 ± 0.42	100 ± 0.28	103 ± 0.10	129 ± 0.41	72 ± 0.26
Peripheral								
T-cells	100 ± 0.15	100 ± 0.15	100 ± 0.07	100 ± 0.08	100 ± 0.13	100 ± 0.09	100 ± 0.13	100 ± 0.25
B-cells	177 ± 0.33	234 ± 0.34	76 ± 0.17	122 ± 0.41	48 ± 0.20	47 ± 0.15	45 ± 0.16	16 ± 0.20

[a]For abbreviations see Table 1.

Table 3
Surface Binding of Fluoresceinated Lectins and Poly-Ornithine to Neuraminidase-Treated, Formaldehyde-Fixed Human Cells in Percent of the Calculated Mean Fluorescence Density per Unit Cell Surface Before Neuraminidase Treatment (±SE, between 81,014 and 612,936 cells per measurement)[a]

	WGA	APA	LCA	L-PHA	HPA	PNA	UEA-F	PO
T-cell lines								
JM	113 ± 0.02	92 ± 0.28	124 ± 0.31	119 ± 0.54	133 ± 0.23	563 ± 0.98	99 ± 0.74	93 ± 0.20
Molt-4	76 ± 0.17	155 ± 0.41	106 ± 0.19	116 ± 0.24	111 ± 0.49	429 ± 0.76	127 ± 0.63	63 ± 0.13
B-cell lines								
6410	99 ± 0.20	131 ± 0.55	215 ± 1.12	169 ± 0.74	156 ± 0.63	670 ± 1.19	87 ± 0.39	73 ± 0.33
NC37	108 ± 0.32	141 ± 0.48	129 ± 0.23	156 ± 0.54	157 ± 0.44	693 ± 1.07	80 ± 0.25	78 ± 0.25
Peripheral								
T-cells	91 ± 0.14	139 ± 0.21	101 ± 0.07	109 ± 0.09	177 ± 0.23	187 ± 0.17	99 ± 0.13	35 ± 0.09
B-cells	84 ± 0.16	164 ± 0.23	102 ± 0.23	179 ± 0.60	125 ± 0.52	182 ± 0.58	74 ± 0.26	35 ± 0.44

[a]For abbreviations see Table 1.

sialic acid by neuraminidase was higher than to B-cells (Table 3). The removal of sialic acid from the cells resulted in a lower surface charge density, as demonstrated by lower binding of FPO. Peanut lectin did not bind to the four cell lines prior to neuraminidase treatment. No significant differences of lectin binding between B- and T-cells were observed for RCA, Con A, SBA, and SAS-F. Ricinus communis lectin and Con A had a high and similar binding to all cell lines that was increased by neuraminidase incubation. Soybean lectin bound only weakly to the cell lines and binding was not influenced by neuraminidase. Binding of SAS-F was in all cases close to the noise background of the assays. The results shown in Table 2 are from measurements made on a single day. The measurements were repeated several times with a smaller series of lectins and with new cells, and similar results were obtained. The cells appeared as broad distributions in the cell volume versus fluorescence plots (Fig. 1a–d) with coefficients of variation for cell volume and cell fluorescence between 18 and 59%. The coefficient of variation of the means for cell volume and fluorescence in ten repetitive assays of the same cell suspension was 3.7%. Differences of the mean values of various histograms that are greater than two standard deviations, ie, 7.4%, were therefore estimated to be significant.

All experiments were performed at a lectin concentration of 20 μg/ml. This concentration was below the level of lectin receptor saturation and cell agglutination that would prevent the flow cytometric measurements. The dose-response curve gives a nearly linear increase of fluorescence intensity with the logarithm of lectin concentration. This is shown with WGA for the different cell types tested (Fig. 2). Similar curves which roughly parallel the WGA curve were obtained for other lectins; SAS-F and UEA-F exhibited very weak fluorescence in this range of concentrations. The specificity of lectin binding was examined by displacement studies with the inhibitory monosaccharides. The fluorescent lectin could be displaced by the corresponding sugars, with the exception of WGA and APA where 50 mM N-acetyl-glucosamine and D-galactose respectively were sufficient to remove only approximately 80% of the bound lectin from non-neuraminidase-treated cells.

DISCUSSION

There was no selective binding of any one of the eleven lectins to a particular cell type before or after neuraminidase treatment. A possible exception may be LCA, which bound well to the B-cell lines but not to the T-cell lines. A similar observation was reported by others (4,29). The PNA binding to non-neuraminidase-treated

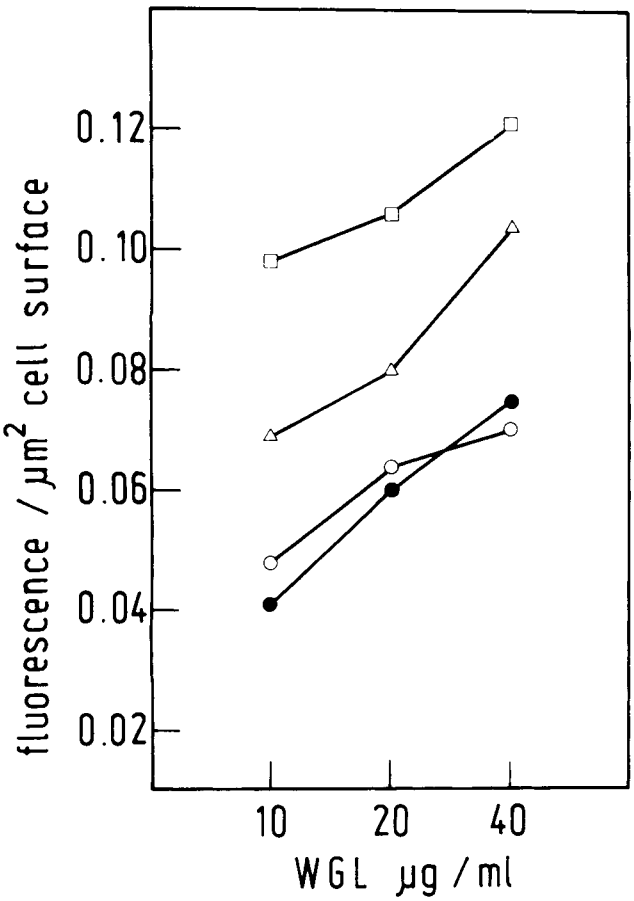

FIG. 2. Concentration dependence of FITC-WGA binding to formaldehyde-fixed cells of JM T-cell line (●—●), 6410 B-cell line (△—△), human peripheral blood T-cells (○—○), and human peripheral blood B-cells (□—□).

cells is suggestive of less differentiated cell membrane with an incomplete glycosylation of membrane glycoproteins (26). Peanut lectin was found in man to bind to non-neuraminidase-treated cells in germinal centers, to some cortical thymocytes, and to follicular lymphoma cells (27). The four cell lines tested in this study, however, did not bind PNA before neuraminidase treatment.

The seven lectins listed in Table 1 showed significant differences of binding between peripheral T- and B-cells. A similar quantitative difference between the T- and the B-cell lines was only detected for WGA. This was unexpected, since WGA as well as RCA and Con A are known to interact strongly with a great variety of cells from different tissues (5).

Although several lectins tested in the present study have an identical monosaccharide specificity (such as ABA, PNA, and RCA for D-galactose; HPA, L-PHA, and SBA for N-acetyl-D-galactosamine; or LCA and Con A for D-mannose/D-glucose), they exhibit different binding patterns to various cell types. This is probably due to the fact that lectin-binding affinity is influenced by the presence of particular membrane oligosaccharides (6). The flow cytometric results are in general agreement with observations gained by other methods (2,3,4,8, 11,29). Conflicting results possibly reflect the influence of the cell volume on the evaluation of the cell-bound fluorescence (21), or may be due to the use of much higher lectin concentrations (8) and different reaction conditions which make further binding sites accessible (9).

It seems particularly important that the cells can be measured by flow cytometry in biochemical equilibrium with the free lectin in solution. Washing, which is often used in biochemical procedures, may result in uncontrolled desorption of lectin. A restriction for the use of flow cytometry is that one has, in general, to measure lectin binding below saturation. This is, however, not an important drawback, since the primary goal of flow cytometric studies is to establish relative binding patterns for different lectins. These patterns are not much influenced by the lectin concentration, since the binding curves of the lectins used in this study essentially parallel each other in the dose range 10 to 100 µg/ml.

The results show that the relative packing density of fluoresceinated lectins to cells of the human T- and B-cell lineages may be quantitatively determined by flow cytometry. Different cell types can be compared readily in a standardized way, which is more difficult in biochemical studies. The lectin-binding patterns are characteristic for the cell lines and the isolated cell types.

In heterogeneous cell populations the situation is more difficult because cell clusters largely overlap. It is not possible to identify the T- and B-cells in the unseparated peripheral human blood by the lectins tested in this study. The application of lectins for cell surface characterization seems, therefore, mainly useful for cell line characterization.

Minor modifications in the carbohydrate cell surface structure that occur on malignant transformation of cultured cells may be monitored by flow cytometry, as demonstrated for the Con A binding of human lymphoma lines before and after infection with Epstein-Barr virus in vitro (30). There is also evidence that the metastatic potential of tumor cell lines is influenced by surface determinants that bind lectins: Murine lymphosarcoma cell lines that were sequentially selected for decreased adherence to immobilized Con A yielded variant lines with increased in vivo malignancy, whereas in vitro selection on immobilized WGA led to variant lines with decreased malignancies (24). In this sense the characterization of cell lines by lectins seems a useful supplement to the available antibody methodology.

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