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FRACTIONATION OF MOUSE CYTOTOXIC T CELLS BY USE OF LECTINS*,†

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ABSTRACT

Mouse cytotoxic T lymphocytes (CTLs), induced *in vivo* and *in vitro* in mixed-lymphocyte cultures, were fractionated into agglutinated and unagglutinated cells by use of various lectins. Cytolytic activity was enriched in the cell fraction agglutinated by various 2-acetamido-2-deoxy-α-D-galactopyranose-specific lectins, namely, *Dolichos biflorus* agglutinin (DBA), *Helix pomatia* agglutinin (HPA), and *Phaseolus limensis* agglutinin (LBA). Only a little cytolytic activity remained in the unagglutinated cell fraction. Furthermore, the relationship between the binding of lectins and the cytolytic activity in various CTL cell lines was investigated with a fluorescence-activated cell sorter, and high-rank correlation was found between the cytolytic activity of these CTL cell-lines and the binding of DBA or HPA to them, but weaker rank correlation in the cases of LBA and peanut agglutinin (PNA). DBA was found to bind to almost all cells of interleukin-2-dependent CTL cell-lines tested. Although the DBA-positive CTL cell-lines have strong cytolytic activity, the DBA-negative cells, which consist of a small proportion of the CTLs, also exhibited cytolytic activity. Thus, the major part of CTLs has binding sites for α-D-GalNAc-specific lectins, particularly for DBA, and this lectin is useful for the enrichment of CTLs. However, the binding sites for DBA cannot be regarded as an exclusive differential marker for CTLs.

INTRODUCTION

The application of lectins for the separation of biologically distinct cell-subpopulations has been widely investigated by many workers. Peanut agglutinin (PNA), for example, has been used for the characterization of immature thymo-

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cytes[1], and for the concentration of concanavalin A (Con A)-induced mouse suppressor T cells[2,3]. Surface markers specific for cytotoxic T lymphocytes (CTLs) have also been investigated with lectins by Kimura *et al.*[4,5]. They found that *Vicia villosa* lectin (VVL) exhibited selective reactivity for the T145 glycoprotein that is expressed specifically on the cell surface of CTLs[4]. Furthermore, they separated CTLs in mixed-lymphocyte culture (MLC) blasts with a VVL-Sepharose 4B column[5]. However, other workers have recently reported that the receptor for VVL cannot be regarded as an exclusive marker for CTLs[6-8].

We have also attempted the separation of CTLs with various lectins. In this paper, we found that CTLs could be enriched in the cell fraction agglutinated with 2-acetamido-2-deoxy-α-D-galactopyranose-specific lectins. Furthermore, we demonstrated the correlation between lectin binding and cytolytic activity of various CTL cell-lines.

EXPERIMENTAL

Mice. — Eight to 12-week-old female C57BL/6, DBA/2, and BALB/C mice were purchased from Charles River Japan, Inc. (Kanagawa, Japan), and the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan).

Reagents. — *Dolichos biflorus* agglutinin (DBA), *Phaseolus limensis* agglutinin (LBA), *Helix pomatia* agglutinin (HPA), and their fluorescence isothiocyanate (FITC) derivatives or biotinyl derivatives, *Bandeiraea simplicifolia* agglutinin I (BSA-I), biotinyl *Arachis hypogaea* agglutinin (PNA), biotinyl *Limulus polyphenus* agglutinin (LPA), and FITC-avidin were purchased from E. Y. Laboratory (San Mateo, CA 94400) or Vector Laboratories Inc. (Burlingame, CA 94010). Soybean agglutinin (SBA), *Wistaria floribunda* agglutinin (WFA), *Bauhinia purpurea* agglutinin (BPA), and concanavalin A (Con A) were prepared by the methods of Gordon *et al.*[9], Toyoshima and Osawa[10], Osawa *et al.* [11], and Agrawal and Goldstein[12], respectively. BPA, BSA-I, WFA, and SBA were conjugated with biotin by the method of Anderson *et al.*[13]. Anti-Thy1.2 antiserum was purchased from Cedarlane Laboratories (Ontario, Canada). FITC-labeled, rabbit-antimouse μ chain antiserum was kindly provided by Dr. Sakai-Natsuume, Cancer Research Institute, Kanazawa University, Kanazawa, Japan. Anti-human, blood-group A antiserum was kindly provided by Tokyo Standard Serum Co., Ltd., Tokyo, Japan.

Con A activation of spleen cells. — Spleen cells from C57BL/6 mice were treated with Gey's solution to lyse red blood cells. The cells were suspended at 5×10^6/mL in RPMI 1640 supplemented with 5% heat-inactivated, fetal calf serum (FCS), 25mM *N*-2-hydroxypiperazine-*N'*-2-ethanesulfonic acid (HEPES) (pH 7.2), 4mM glutamine, mM pyruvate, 50μM 2-mercaptoethanol (2-ME), 60 mg/L kanamycin [complete medium (+2-ME)], and 2.5 mg/mL of Con A. Cultures were incubated for 3 days at 37° in a humidified, 5% CO_2 atmosphere. After culturing, col-

lected cells were washed with 0.1M methyl α-D-mannopyranoside in RPMI 1640.

Induction of CTLs. — (*a*) In vivo *activation.* C57BL/6 (H-2^b) mice were immunized by i.p. injection of P815 cells (H-2^d; 2×10^7). Spleen cells were obtained after 10–11 days and treated with Gey's solution.

(b) Primary mixed-lymphocyte culture (1°MLC). C57BL/6 spleen cells (responder cells) and DBA/2 spleen cells (H-2^d; stimulator cells) were treated with Gey's solution. The cells to be used as stimulator cells in MLC were treated with mitomycin C (50 μg/mL, MMC) for 1 h at 37°. Viable responder cells (1×10^8) were mixed with MMC-treated stimulator cells in the ratio of 2:1 in MLC medium (20 mL). The MLC medium consisted of RPMI 1640, 10% heat-inactivated FCS, 25mM HEPES (pH 7.2), 4mM glutamine, mM pyruvate, 50μM 2-ME, nonessential amino acids (Gibco Laboratories Grand Island Biological Co., Grand Island, NY 14072), and kanamycin (60 mg/mL). The cells were incubated for 5 days at 37° in a 5% carbon dioxide atmosphere.

(c) Secondary mixed-lymphocyte culture (2°MLC). (i) Primary MLC was conducted by mixing responder cells (4×10^7) with an equal number of MMC-treated stimulator cells in MLC medium (20 mL). After a 14-day culture, the cells were harvested. Viable cells (4×10^7) from 1°MLC were mixed again with MMC-treated stimulator cells (8×10^7) from the same mouse strain as used for 1°MLC in MLC medium (20 mL), and recultured for 5 days. (ii) The secondary *in vitro* sensitization of *in vivo* immunized cells was carried out by the following method. Spleen T cells from C57BL/6 mice immunized 2 months earlier with P815 were purified by passage through a Nylon-wool column. The cells were restimulated with MMC-treated DBA/2 spleen cells as described earlier. These activated cells are referred to as T-2°MLC.

(d) Collection of viable cells. CTL-containing populations prepared as described earlier were purified, by the method of Kawaguchi *et al.*[14], by density-gradient centrifugation over a mixture containing 5% of Ficoll 400 (Pharmacia Fine Chemicals, Uppsala, Sweden) and 15% of Urografin (Schering, Berlin, West Germany) in RPMI 1640. The purified, viable lymphocytes were used for the cytolytic assays.

Preparation of IL-2. — BALB/C spleen cells were treated with Gey's solution. Viable cells were counted and adjusted to 1×10^7/mL in FCS-free medium with 2 μg/mL Con A. FCS-free medium was the same as MLC medium, except that FCS was not added. After incubation for 24 h at 37° in a 5% carbon dioxide atmosphere, the culture was centrifuged at 2000 r.p.m. for 10 min, and then at 20000*g* for 15 min. The supernatant was filtered through 0.45-μm Millipore filters and stored at −20° until use. This supernatant (Con A Sup) was used as crude interleukin-2 (IL-2).

IL-2-dependent CTL lines (LC cells). — C57BL/6 anti-DBA/2 MLC was performed by mixing responder cells (1×10^6/mL) with MMC-treated stimulator cells in the ratio of 2:1 for 1°MLC and of 1:2 for 2°- or 3°-MLC. The cells for successive cultures in IL-2-supplemented medium were harvested from 2°MLC and 3°MLC.

The last MLC was performed for 2 days and the preceding 1°- or 2°-MLC was performed for 10–12 days. The cells from the last MLC were resuspended in MLC medium supplemented with 20% of Con A Sup at densities of 4–10 × 10^4 viable cells/mL with or without 1 × 10^6/mL MMC-treated stimulator cells, and placed in 24-well, multi-well, culture plates. The cells were harvested from the cultures at days 5 to 7 and recultured in fresh culture medium. After several passages, the cells were diluted to 2 × 10^4 viable cells/mL in fresh culture medium with or without stimulator cells, and successively cultured.

Fractionation of lymphocytes with lectins. — Cells were separated by the agglutination–sedimentation method with lectins[15]. Briefly, cells (1 × 10^8) in RPMI 1640 (0.5 mL) were incubated with a lectin (500 μg) for 10 min at room temperature, and then layered over 50% of heat-inactivated FCS. In the case of LC cells, cells (1.5 × 10^7) in RPMI 1640 (0.5 mL) were incubated with 50 μg of a lectin. After 30–60 min at room temperature, the cells were divided into 3 fractions, namely, top, middle, and bottom fractions. The fractionated cells were washed with a solution of a suitable haptenic sugar for the lectin. 50mM *N*-Acetyl-D-galactosamine was used for DBA, LBA, HPA, and SBA; 0.1M D-galactose for BSA-I; and 0.3M lactose for WFA, SBA, and PNA.

Determination of the proportions of T and B cells, and macrophages in cell preparations. — Thy1.2-positive cells were determined by a modification of the microcytotoxic test described by Amos *et al.*[16]. Surface IgM-positive cells were detected by the fluorescence-staining assay with FITC-anti-μ serum. Nonspecific, esterase-positive cells were detected by the method of Li *et al.*[17].

Treatment of lectin-fractionated cells with antisera. — Lectin-fractionated cells (1 × 10^7/mL) were incubated with anti-Thy1.2 (final dilution, 1:5) or anti-human, blood-group A antiserum (final dilution, 1:5) on ice for 60 min. These cells were then treated with selected rabbit serum (as complement, final dilution, 1:15) for 30 min at 37°.

Cytolytic assay. — P815 (H-2^d) or EL4 (H-2^b) cells (5–10 × 10^6) as target cells in RPMI 1640 (1 mL) supplemented with 5% of heat-inactivated FCS, 25mM HEPES (pH 7.2), 4mM glutamine, mM pyruvate, and 60 mg/L kanamycin [complete medium (2-ME)] were incubated with $Na_2^{51}CrO_4$ (100 μCi) (New England Nuclear, Boston, MA 02118) for 1 h at 37° with occasional gentle shaking. The ^{51}Cr-labeled target cells thus prepared were thoroughly washed and suspended at a concentration of 2 × 10^5/mL in complete medium (2-ME). The ^{51}Cr-labeled target-cell suspension (100 μL) and the effector-cell suspension (100 μL), adjusted to an appropriate cell-density, were mixed in wells of round-bottom microtiter plates, centrifuged at 1000 r.p.m. for 1 min, and then incubated for 4 h at 37° in an atmosphere of 5% of carbon dioxide. After incubation, the plates were centrifuged at 1000 r.p.m. for 15 min. The supernatant (100 μL) in each well was collected and the radioactivity counted in a gamma counter. All assay results represent means of triplicate experiments.

The percent of specific ^{51}Cr release was calculated as follows: specific ^{51}Cr release (%) = [experimental release (c.p.m.) − spontaneous release

(c.p.m.)] · [maximum release (c.p.m.) − spontaneous release (c.p.m.)]$^{-1}$ · 100. One lytic unit (LU) was defined as the number of lymphocytes required to lyse 50% of 2×10^4 target cells within a 4-h, ^{51}Cr-release assay, unless otherwise stated.

Analysis, with a fluorescence-activated cell sorter, of cells labeled with fluorescent lectins. — Viable cells (6×10^6) were incubated for 30 min on ice with an FITC-lectin or a biotinyl lectin at the following concentrations: biotinyl DBA, 5 μg/mL; FITC-HPA, 30 μg/mL; FITC-LBA, 100 μg/mL; biotinyl PNA, 10 μg/mL; biotinyl BPA, 25 μg/mL; biotinyl BSA-I, 3 μg/mL; biotinyl SBA, 5 μg/mL; and biotinyl WGA, 5 μg/mL. The cells were washed with 0.1M sodium phosphate buffer–0.15M sodium chloride (PBS) containing sodium azide. The cells treated with a biotinyl lectin were then incubated with PBS containing sodium azide. The labeled cells were analyzed with a FACS II (Becton–Dickinson Electronics Laboratory, Mountain View, CA 94040). Viable cells were separated on the basis of their light-scattering characteristics. The results are exressed as fluorescence histograms (cell number *vs.* logarithmic fluorescence-intensity).

RESULTS

Cytolytic activity of in vivo*-activated spleen cells fractionated with lectin.* — Spleen lymphocytes were obtained from C57BL/6 mice immunized 10–11 days earlier with P815 and fractionated by the agglutination–sedimentation method with various lectins. The cells were divided into the bottom fraction (agglutinated cells), the middle fraction, and the top fraction (unagglutinated cells). The middle fraction contained smaller aggregates than the bottom fraction. The agglutinated cells were dissociated into single cells by washing with haptenic, sugar-containing medium and the cytolytic activity of the separated cells against P815 was tested. The results are shown in Fig. 1. On the basis of lytic unit/10^7 cells, the bottom fraction obtained by use of LBA, DBA, or HPA had a 2-3-fold higher cytolytic activity than the top fraction. In the case of fractionation with BSA-I, SBA, or WFA, no significant difference in lytic activity was found among the three fractions.

Cytolytic activity of MLC cells fractionated with lectins. — LBA, DBA, and HPA were used to fractionate 1°MLC cells. As shown in Fig. 2, the cytolytic activity was enriched in the bottom fraction. The bottom fraction generally had a 6–7-fold higher cytolytic activity against ^{51}Cr-labeled stimulator cells than the top fraction. Furthermore, when T-2°MLC cells were separated with DBA, the bottom fraction showed a 12-fold higher cytolytic activity than the top fraction (data not shown).

Characterization of MLC cells fractionated with lectins. — When 1°MLC cells or the cells in the LBA-bottom fraction were treated with anti-Thy1.2 antiserum and complement, the cytolytic activity of the treated cells was found to be completely abolished. These results indicate that the cytolytic cells in these cell preparations are apparently T cells. However, treatment with anti-blood-group A antiserum and complement did not affect the cytolytic activity. Subsequently, the cell-surface markers of the cell fractions were analyzed, after separation of 1°MLC cells

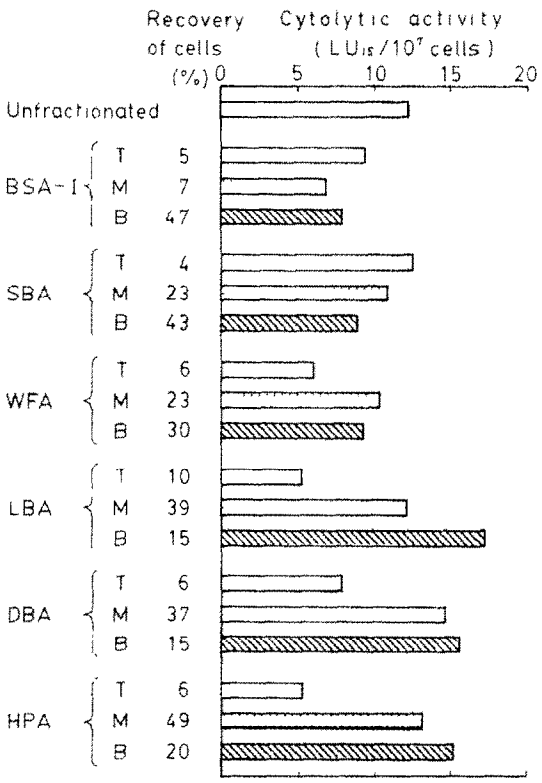

Fig. 1. Cytolytic activity of lectin-fractionated lymphocytes primed with P815 *in vivo*. The lymphocytes were fractionated into the top (T), middle (M), and bottom (B) fractions by the agglutination–sedimentation method with various lectins as described in the Experimental section. One unit (LU_{15}) is defined as the number of lymphocytes required to lyse 15% of 2×10^4 P815 cells as target cells within a 4 h, ^{51}Cr-release assay

with LBA, DBA, or HPA (Table I). However, no significant difference was observed in the proportions of Thy1.2-positive cells (T cells) and surface IgM-positive cells (B cells) among these cell fractions, indicating that the three lectins cannot separate the cells into T and B cells. The aforementioned results demonstrate that CTLs can be enriched in the cell fractions agglutinated by LBA, DBA, and HPA.

Characterization of LC cells. — IL-2-dependent CTL cell-lines having strong cytolytic activity, LC4, LC5, LC6, and LC5(-f), were established. LC4 and LC7 were long-term culture, blast-cell lines originating from 2°MLC between C57BL/6 (responder) and DBA/2 (stimulator), and LC5 originated from 3°MLC in the same system. LC4 and LC5 cells were successively cultured in IL-2 supplemented medium in the continuous presence of MMC-treated stimulator cells. LC7 cells were cultured in the absence of stimulator cells. In the case of a portion of the LC5 cells, stimulator cells were removed 7 weeks after the start of the culture, and thereafter maintained only in the presence of IL-2. This cell line was designated as

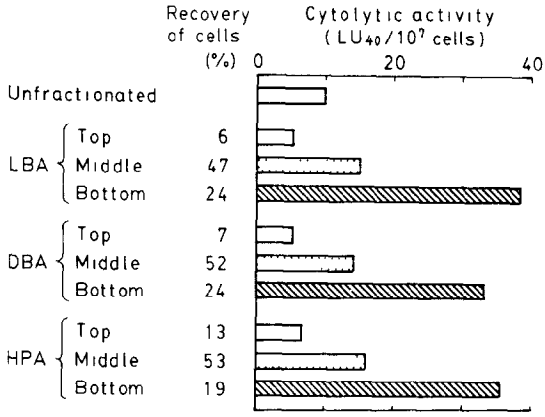

Fig. 2. Cytolytic activity of lectin-fractionated lymphocytes activated by primary MLC. One unit (LU_{40}) is defined as the number of lymphocytes required to lyse 40% of 2×10^4 P815 cells as target cells in a 4-h, ^{51}Cr-release assay.

TABLE I

CELL MARKERS OF THE LECTIN-FRACTIONATED CELLS [a]

Cells	*Unfractionated cells*	*Lectin*					
		LBA		*DBA*		*HPA*	
		Top	*Bottom*	*Top*	*Bottom*	*Top*	*Bottom*
s-IgM$^+$	46	45	38	43	36	45	39
Thy-1	48	52	45	45	51	32	50
Esterase$^+$	4.5	1.6	3.8	2.0	4.0	1.3	3.7

[a]The values in this table are percentages of the respective cells of total populations and the means of three independent experiments.

TABLE II

CYTOLYTIC ACTIVITIES OF CTL POPULATIONS

Cells	*Cytolytic activity (LU/10^6 cells)*
Con A-activated spleen lymphocytes	0.036
Spleen lymphocytes primed *in vivo*	0 21
1°MLC cells	1.8
2°MLC cells	4.8
LCS(-f) cells	6.0
LC7 cells	6.9
LC4 cells	25
LC5	192
LC5T^8	152
LC5B^5	227

LC5(-f). These four cell-lines were found to express Thy1.2 antigen, to adhere to a plastic surface, and not to respond to Con A. LC4 and LC5 cells retained strong cytolytic-activity against P815 for more than 9 months, but LC7 and LC5(-f) cells retained weaker cytolytic-activity against P815 (Table II) Furthermore, it was found that all cell lines exerted very weak cytolytic-activity against syngeneic EL4 cells. Thymocyte-costimulating and killer-helper activity of these cell lines could not be detected by the method of Narimatsu and Saito[18] (data not shown).

Relationship between lectin binding and cytolytic activity. — The relationship between cytolytic activity of various CTL cell-lines and the binding of various lectins to these cell lines was examined. The cytolytic activities of these cell lines are

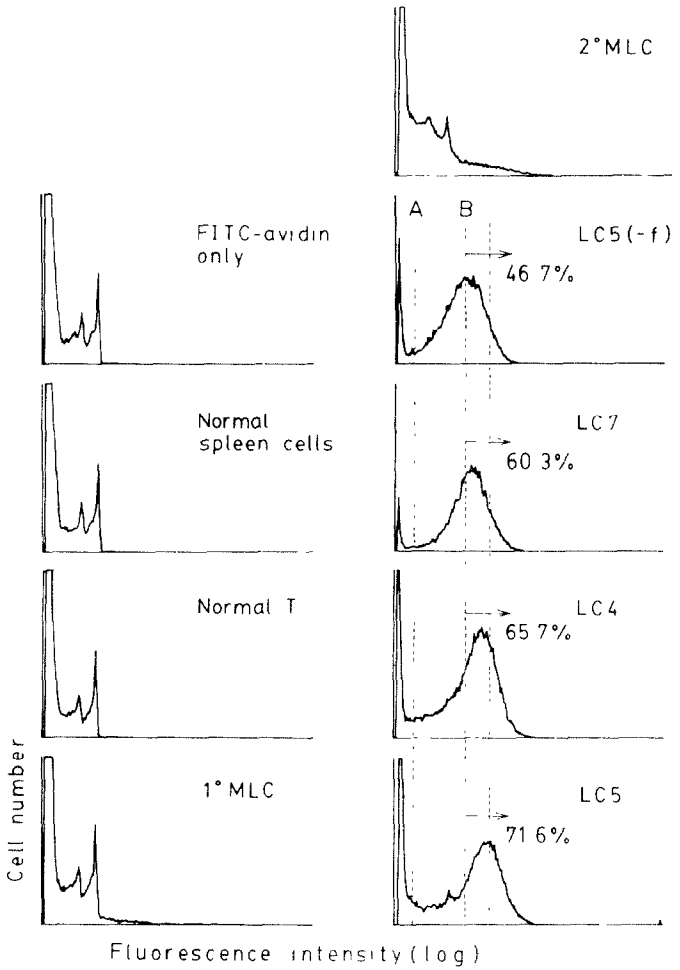

Fig 3. Fluorescence profiles of normal cells and CTL cell-lines labeled with biotinyl DBA and FITC-avidin The values shown as percentages represent the ratio of (area having a higher fluorescence intensity than B in a peak) × 100/(area having a higher fluorescence intensity than A in a peak)

shown in Table II. On the other hand, the binding of lectins was analyzed by a fluorescence, activated-cell sortor (FACS II). Fig. 3 shows the results of staining of the cell lines with biotimyl DBA and FITC-avidin. Normal spleen cells and normal T cells showed no significant fluorescence. However, 2°MLC cells and CTL cell-lines showed brighter fluorescence. The proportion of bright cells and the fluorescence intensities at the peaks in the fluorescence profiles increased almost parallel to the cytolytic activity.

Fig. 4 shows the fluorescence profiles of CTL populations that had been stained with FITC-HPA. Although more cells were stained with HPA than with DBA, the tendency of the relationship between cytolytic activity and intensity of the staining was similar to that in the staining with DBA.

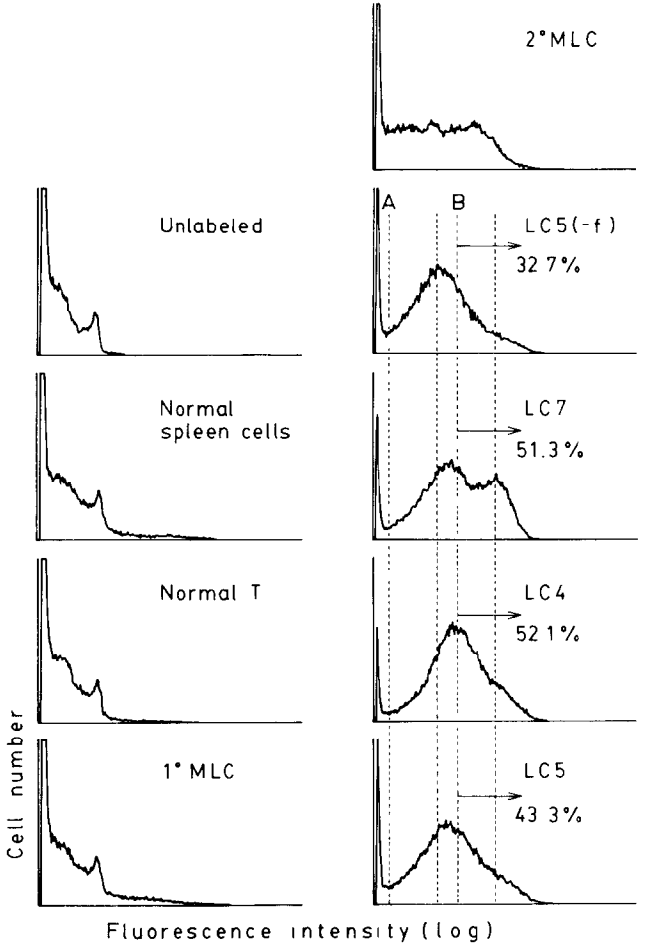

Fig. 4. Fluorescence profiles of normal cells and CTL populations labeled with FITC-HPA. The same expressions were used as in Fig. 3.

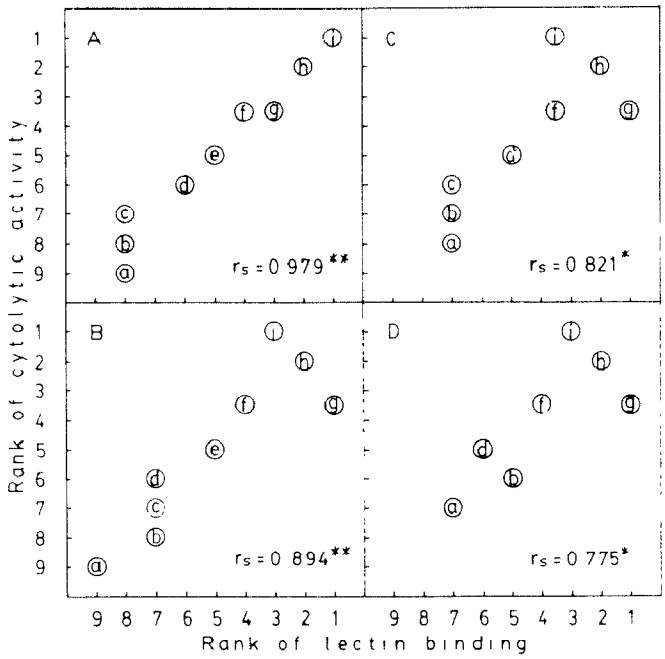

Fig 5 Correlation between cytolytic activity and lectin binding in CTL populations The binding of four kinds of lectins to various CTL cell-lines was analyzed with a FACS II: (A) DBA, (B) HPA, (C) LBA, and (D) PNA. The rank of lectin binding was determined on the basis of fluorescence profiles; * and ** indicate that the Spearman rank-correlation coefficient (rs) of the values is significant at a level of 5 and 1%, respectively. CTL populations: (a) normal T cells; (b) Con A-activated spleen lymphocytes; (c) spleen lymphocytes primed with P815 *in vivo*; (d) 1°MLC cells, (e) 2°MLC cells; (f) LC5(-t) cells; (g) LC7 cells, (h) LC4 cells; and (i) LC5 cells.

The fluorescence intensities of the four types of CTL cell-lines stained with these fluorescent lectins (DBA and HPA) were very high, and a difference in fluorescence intensity among CTL cell-lines was observed, but it was not as marked as the difference in cytolytic activity. On the other hand, when cells were stained with biotinyl PNA adn FITC-avidin, Con A-activated spleen cells and LC7 cells were found to contain more highly fluorescent cells in spite of their weak cytolytic activity (data not shown).

The relevance of lectin binding to cytolytic activity of CTL cell-lines was investigated by calculating the Spearman rank-correlation coefficients. Fig. 5 shows the relevance for DBA, HPA, LBA, and PNA. For DBA and HPA, strong rank-correlations between lectin binding and cytolytic activity were observed, but weaker ones for LBA and PNA.

Cytolytic activity of LC cells fractionated with DBA. — LC5 cells, which had the strongest cytolytic activity, were fractionated by the agglutination–sedimentation method with DBA. The major part (90%) of the cells was recovered in the agglutinated fraction, and a much smaller part (3%) was found to be not agglutinated. However, the difference in cytolytic activity was not so marked between

these two cell populations. Both cell populations obtained by DBA-fractionation of LC5 cells were recultured in fresh medium, and the selection, namely, DBA-fractionation and reculturing, was repeated several times for both cell populations to further purify the DBA-agglutinated and DBA-unagglutinated cells. The cytolytic activity of unagglutinated cells ($CL5T^8$ cells; the selection was repeated 8 times) and agglutinated cells ($LC5B^5$ cells; the selection was repeated 5 times) thus obtained was not very different from that of the original LC5 cells (Table II). These results show that a minor group of CTLs has no binding sites for DBA. This is consistent with the observation that the cells having weak cytolytic-activity remained in the top fraction, that is, were unagglutinated cells as shown in Figs. 2 and 3.

TABLE III

RELATIVE LABELING INTENSITIES OF VARIOUS CELLS WITH LECTINS[a]

Cells	*Lectin*				
	DBA	*WFA*	*SBA*	*BPA*	*BSA-I*
Peripheral-blood T				+	+
Con A-activated T	±	+	+	++	+
LC5	++	++	++	++	+

[a]The relative labeling intensity was estimated with a fluorescence-activated cell sorter after labeling with the respective fluorescence-labeled lectin.

Binding of other lectins to CTL-line LC5. — LC5 cells were stained with other fluorescent-labeled α-D-GalNAc-binding lectins, followed by analysis with a FACS II (Table III). BSA-I, WFA, SBA, and BPA, which are not α-D-GalNAc-specific lectins, bound to LC5 cells. They also bound to Con A-activated cells, whereas DBA hardly bound to Con A-activated cells. These data agree with the observation that BSA-I, WFA, and SBA are not effective lectins, but DBA is an effective lectin for the separation of CTLs, as shown in Fig. 1.

DISCUSSION

In this study, it was demonstrated that CTLs could be enriched, in the agglutinated-cell fraction, by the agglutination–sedimentation method with the lectins that have binding specificity for a terminal α-D-GalNAc group in the sugar chain on the cell surface. This is in agreement with the finding of Kimura *et al.*[5] that CTLs could be separated by adherence to an affinity adsorbent prepared by coupling VVL, which was found to be one of the α-D-GalNAc-binding lectins[19]. Moreover, we also confirmed the positive correlation between the stainability with these lectins and the cytolytic activity of the cells in various CTL cell-lines. This indicates that most CTLs have abundant terminal α-D-GalNAc groups in the sugar chains on the cell surface. However, this correlation is not absolute, because a

minor proportion of the strong CTL cells was not stained with these lectins. Therefore, our data suggest that the binding sites for α-D-GalNAc-specific lectins cannot be regarded as an exclusive differential marker for CTLs. This assumption is consistent with the results of other investigators[6–8], which indicate that VVL does not bind selectively to CTLs and all CTLs do not necessarily express the binding site for VVL (membrane glycoprotein T145), although we have not yet identified the binding sites for α-D-GalNAc-binding lectins used in this study as membrane glycoprotein T145. However, since the major part of CTLs can be agglutinated by these lectins, their use may have practical value for the enrichment of CTLs.

Among the α-D-GalNAc-specific lectins employed in this study, the highest rank-correlation between the cytolytic activity of CTLs and the lectin binding to them was observed in the case of DBA. DBA is known to be the most specific lectin toward α-D-GalNAc residues, and its most potent inhibitor is a blood-group A active pentasaccharide[20], α-D-Gal*p*NAc-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-β-D-Gal*p*NAc-(1→6)-R. The other lectins used, HPA and LBA, appear to be less specific toward α-D-GalNAc groups[21,22] and may also react, to a more limited extent, with terminal α-D-Gal groups. It may, therefore, be reasonable to assume that this wider sugar-binding specificity of the last-named two lectins is reflected in their lower selectivity for CTLs. SBA is also a specific lectin toward α-D-GalNAc residues[23,24], but an α-L-fucosyl group in the blood-group A active pentasaccharide is known to greatly diminish the inhibitory activity of the oligosaccharide toward SBA. Since SBA is ineffective in the separation of CTLs (Fig. 1), most CTLs may express a blood-group A, antigen-like structure including an L-fucosyl group, and DBA can bind most specifically to this sugar structure. In this context, it is also of interest to note that the blood-group A active pentasaccharide is much less active as an inhibitor toward VVL than is[19] α-D-Gal*p*NAc-(1→3)-D-Gal. Although VVL was not tested in this study owing to its unavailability, it may be less selective for CTLs than DBA. Membrane receptors of CTLs for DBA may not exactly be a blood-group A antigen itself, because immune, anti-human blood-group A serum used in this study could not react with CTLs. Another possibility may be that the anti-A antiserum recognizes the larger portion of the blood-group A antigen. Thus, the characterization of membrane receptors of CTLs for DBA, even if they are not exclusive, differential cell-surface markers for CTLs, is of interest.

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