Selective Binding of Leukemia and Lymphoma Cell Lines to Lectins

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Lectins are carbohydrate-binding substances of nonimmune origin with unique characteristic carbohydrate specificities [8, 18, 26]. By utilizing these subtle differences in their carbohydrate specificities, isolation and characterization of glycoconjugates and fractionation of lymphocyte subpopulations or malignant cells have been carried out [8, 18, 26]. Peanut (PNA) and soybean (SBA) agglutinins have been most frequently used to isolate lymphocyte subpopulations [16, 17, 23–25] and to screen lymphoma and leukemia cells [3, 5, 11, 22, 27]. Attempts were made to examine the binding abilities of plant lectins, other than PNA and SBA, to human leukemia and lymphoma cell lines in hope of finding suitable screening tools for malignant cells.

Human cell lines used were Molt 4 [14], CCRF-CEM [7] and TALL-1 [15] from T cell leukemias, Daudi [10] and Raji [21] from Burkitt lymphomas, and K562 [13] from myelogenous leukemia. They were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/ml penicillin, 100 μg/ml streptomycin and 4 mM L-glutamine. For removal of sialic acids, cells (1×10⁶/ml) were treated at 37°C for 1 hr in saline with 100 μg/ml of neuraminidase of Clostridium perfringens. Lectins were purchased from E. Y. Laboratories. Rabbit anti-lectin antibodies were isolated by affinity chromatography on lectin-coupled CNBr-activated Sepharose 4B [6]. Appropriate dilutions of antibodies which showed an absorbance reading of 2.0 at 492 nm against homologous lectin were used in the following enzyme linked immunosorbent assay (ELISA). The lectin binding on the cell line was carried out in quadruplicate with microtiter ELISA plates (Becton Dickinson). Ten microliter of cell suspension containing 1–2×10⁵ cells and 100 μl of purified lectin (1 to 10,000 ng) were separately added to each well of ELISA plates precoated with 0.15 M phosphate buffered saline (PBS, pH 7.2) containing 0.5% bovine serum albumin (BSA). The lectin-bound cells were washed with the same buffer by brief centrifugation (100×g, 1 min, 3 times). One hundred microliter of purified rabbit anti-lectin antibodies were then added to each well. After washing similarly, 100 μl of peroxidase-coupled goat anti-rabbit IgG (Cappel Laboratories, diluted to 1: 20,000) was added to each well. One hundred microliter of the substrate solution containing 0.03% H₂O₂ and 1 mg/ml o-phenylenediamine in 0.1 M citrate-phosphate buffer, pH 5.0, was finally added to each well and allowed to develop color in the dark at room temperature for 30 min. To stop the reaction, 25 μl of 10 N H₂SO₄ was added to each well. The enzyme-substrate reaction was determined spectrophotometrically with a microplate photometer (Corona Electric) at 492 nm. The lectin binding on cells was determined by the absorbance at 492 nm, using an absorbance reading of 2.0 at 492 nm as 100% binding.

The binding patterns of different lectins to human leukemia and lymphoma cell lines are shown in Fig. 1. Among concanavalin A (Con A), Pisum sativum (pea) and Vicia faba lectins specific for mannose (Man) and/or glucose (Glc), Con A showed the high binding to all human cell lines although no selective binding was found for those cell lines used (Fig. 1). Pea lectin was reactive with Raji and TALL-1 but not with other human cell lines. It was most specific for Raji (Fig. 1). Its binding to Raji was much higher than that of Con A. Vicia faba lectin exhibited much poorer binding. These findings may be consistent with the previous reports [1, 9, 12, 28] that these three lectins show their own carbohydrate specificities although they are specific for Man and/or Glc in terms of monosaccharide specificity.

Although the in vitro carbohydrate specificities of ricin and PNA are similar to certain extents [2, 20], their binding to human cell lines were different from each other as shown in Fig. 1; ricin was most reactive with Daudi and was slightly
more reactive with Raji and Molt 4 when compared to PNA. With K562, TALL-1 and CCRF-CEM, however, similar binding was found between these two lectins. The PNA binding to human cell lines other than Raji was found to be similar before and after removal of terminal sialic acids by the neuraminidase treatment. Although terminal sialic acids inhibit the PNA binding [8, 26], the above results suggest the possibility that binding sites for PNA on CCRF-CEM, TALL-1, Daudi and K562 were not fully sialylated. On the other hand, SBA with the N-acetyl-D-galactosamine (GalNAc) specificity [19] was poorly reactive with all human cell lines (Fig. 1), suggesting that O-glycosidically linked oligosaccharides are rarely present on these cell lines.

Ricinus communis agglutinin (RCA-1) and wheat germ agglutinin (WGA) did not show specific binding to any of human cell lines used (Fig. 1) although they have been used to characterize and isolate tumor cells [22], B cellspecific antigen [25], or mouse B lymphocytes [4]. The poor binding of WGA may be attributable to the poor interaction with its binding site or less binding site exposed on cell surfaces. Thus, these two lectins may not be useful in characterizing human leukemia and lymphoma cell lines used in this study.

Although peanut (PNA) and soybean (SBA) agglutinins have been most frequently used for characterization of malignant cells [3, 5, 11, 22, 27], their binding to human cell lines such as Molt 4, CCRF-CEM, TALL-1, Raji, and K562 were found to be lower than had been expected, possibly because their binding sites on these malignant cells are not fully exposed. In light of these findings, it appears that PNA and SBA are not suitable screening lectins for characterizing all of human leukemia and lymphoma cell lines. In contrast, pea lectin and ricin were found to be quite reactive with human Burkitt lymphoma cell lines Raji and Daudi, respectively. This finding suggests the possibility that the binding sites for these two lectins on these two cell lines may be properly exposed and/or orientated, making pea lectin and ricin useful reagents in screening and characterizing these two cell lines.

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要約

植物性レクチンのヒト白血病、リンパ腫由来の株化細胞に対する結合について（短報）：杉井俊二（北里大学
衛生学部血清学教室）——植物性レクチンによる腫瘍細胞の分類、細胞表面上の複合糖質の検索（解析）の可
能性を探るため、植物性レクチンのヒト白血病、リンパ腫由来の株化細胞に対する結合を酵素抗体法で調べた。
エンドウ豆レクチンはRaji細胞、リシンはDaudi細胞と特異的に結合することが判明したが、他の細胞
（TALL-1, Molt 4, CCRF-CE M, K562）に対しては特異的な結合を示さなかった。またこれらの株化細胞
は他のレクチン（コンカバリンA、ソラ豆レクチン、ビーナッツレクチン、ヒマ豆レクチン、小麦胚芽レクチ
ン、大豆レクチン）とも特異的な結合を示さなかった。以上の成績から、エンドウ豆レクチンおよびリシンは
Raji細胞、Daudi細胞の分類およびこれらの細胞の表面複合糖質の検索、解析などに有用である可能性が示唆さ
れた。