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A Novel β-Galactoside-Binding Lectin in Cultured Murine Lymphocytic Leukemia Cells

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Using affinity chromatography on lactosyl-Sepharose, a β-galactoside-binding protein of 38 kDa was detected in mouse L1210 lymphocytic leukemia cells. Immunoblotting analysis revealed that it is distinct from any known larger molecular weight galectin. The partial amino acid sequences of the 38 kDa protein indicated that it is a novel member of the galectin family. This 38 kDa lectin is expressed in lymphocytic cell lines but not macrophage-like cell lines.

Key words: β-galactoside-binding lectin, galectin, L1210, leukemic cell.

Galectins, previously known as animal S-type or S-Lac lectins, are a growing family of β-galactoside-binding lectins, which exhibit specificity for β-galactosides and share characteristic amino acid sequences (*1*). Though their function is not yet fully understood, they could participate in cell growth regulation (*2*), cell adhesion (*3, 4*), and immune responses (*5, 6*). Three groups of galectins have been studied extensively. They are classified according to their molecular architecture into the following types: proto- (galectin-1, -2, -5, and -7) (*7, 8*), chimera- (galectin-3) (*9*), and tandem-repeat (galectin-4 and -8) (*10, 11*) types. The chimera- and tandem-repeat type galectins have large molecular weights of 29,000-36,000, whereas the molecular weights of the proto-type ones are 14,000-18,000 (*12*). There have been many reports concerning the synthesis and secretion of galectins by leukocytes (*13-15*), but few of those by lymphoid cells. In previous studies (*16*), we identified a β-galactoside-binding lectin in extracts of adult rat kidney, and named it rkCBP17.5. In this study, using an antibody against rkCBP17.5, we detected a 38 kDa lectin in lymphoid cells.

L1210 cells, a murine lymphocytic leukemia cell line, were collected into PBS buffer, pH 7.4 (8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl) by centrifugation and washed three times with the same buffer, and then extracted with 1% Triton X-100/TBS using the isolation procedure for rkCBP17.5 (*16*). The extracts of L1210 cells were applied to a column of lactosyl-Sepharose, and proteins retained specifically by the column were eluted with 500 mM lactose. Several polypeptide bands were found on SDS-13% PAGE (Fig. 1A). When immunoblotted with the monoclonal antibody against rkCBP17.5, a single band was observed at a position corresponding to a polypeptide of $M_r = 38,000$ (Fig. 1B, lane 1). This 38 kDa protein band was excised, subjected to SDS-PAGE again with a different acrylamide concentration, and then visualized by silver staining, and a single band corresponding to a molecular weight of 38,000 was obtained (data not shown). The antibody against rkCBP17.5 is a highly specific antibody which was screened and cloned for immunoreaction to rkCBP17.5 as described previously (*16*), and it does not react with galectin-4 or galectin-8, as indicated below. The observation that the anti-rkCBP17.5 antibody recognized the 38 kDa lectin raises the possibility that rkCBP17.5 is a proteolytic fragment of the 38 kDa lectin, and an alternative possibility that the cross reaction of anti-rkCBP17.5 with the 38 kDa lectin might be due to the high homology in the antibody recognition region between rkCBP17.5 and the 38 kDa lectin.

An attempt to determine the N-terminal sequence by direct application of the 38 kDa lectin failed. The N-terminal amino group seems to be blocked, as in the case of known galectins. Thus, peptides derived from the 38 kDa lectin were generated with *Staphylococcus aureus* V8 protease (endoproteinase Glu-C) digestion by the Cleveland method (*17*). Briefly, the β-galactoside-binding proteins of L1210 cells were resolved by 1st SDS-13% PAGE, and then the proteins were visualized by Coomassie blue staining. The 38 kDa protein band was excised and digested with *S. aureus* V8 protease (endoproteinase Glu-C; Boehringer Mannheim Biochemica), and the resulting peptides were separated by 2nd SDS-15% PAGE, transferred to a Glassybond membrane (Biometra, Germany), visualized by Coomassie Blue staining, and sequenced with an Applied Biosystems model 476A gas-phase sequencer. The obtained sequences of several peptides were compared with the sequences of known proteins stored in the NCBI GenBank database. Two peptide sequences (GYVVDNTK-QNGQXG and FKVMVNKKFQVQYQH) were highly

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Abbreviations: rkCBP17.5, a rat kidney carbohydrate-binding protein with a subunit molecular weight of 17,500; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; TBS, 50 mM Tris, pH 7.5, 150 mM NaCl, 1.0 μg/ml pepstatin and leupeptin, 1 mM phenylmethylsulfonyl fluoride; Mac-2, a cell surface marker of thioglycolate-elicited macrophages; IgEBP, IgE-binding protein.

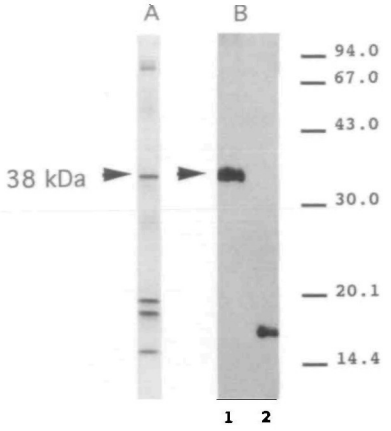

Fig. 1. **SDS-PAGE and immunoblot of the β-galactoside-binding proteins from L1210 cells.** β-Galactoside-binding proteins eluted from lactosyl-Sepharose were subjected to SDS-13% PAGE under reducing conditions, followed by silver staining (A), or electrotransferred onto a nitrocellulose membrane and immunoblotted with the anti-rkCBP17.5 monoclonal antibody (B, lane 1). The sample in lane 2 of B was rkCBP17.5. The numbers on the right denote molecular weight markers.

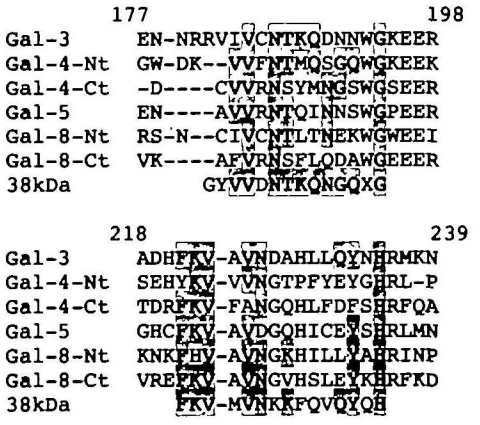

Fig. 2. **Amino acid sequence of the peptides obtained on endoproteinase Glu-C digestion of the 38 kDa lectin and sequence alignment with known galectins.** Gal-3, mouse galectin-3 (*9*); Gal-4-Nt and Gal-4-Ct, N terminal and C-terminal halves of rat galectin-4, respectively (*10*); Gal-5, rat galectin-5 (*7*); Gal-8-Nt and Gal-8-Ct, N terminal and C-terminal halves of rat galectin-8, respectively (*11*). The amino acid positions correspond to those of galectin-3. Identical amino acid residues are shaded.

homologous with the known galectins (Fig. 2). Based on these data, we conclude that this 38 kDa β-galactoside-binding protein is a member of the galectin family.

The known larger molecular weight galectins are galectin-3, galectin-4, and galectin-8. Galectin-3 has been cloned in mouse, rat, man, and other species. The 38 kDa lectin was found to be distinct from galectin-3, on comparison with mouse galectin-3, in sequences. Galectin-4 and galectin-8 have been sequenced in rat but not in mouse. In order to determine whether the 38 kDa protein is a novel lectin or the mouse homologue of galectin-4 or galectin-8, immunoblotting analysis was performed. β-Galactoside binding proteins obtained from rat liver and 4 M guanidine-HCl extracts of fresh rat large intestine were used to detect galectin-8 and galectin-4, respectively, as described previ-

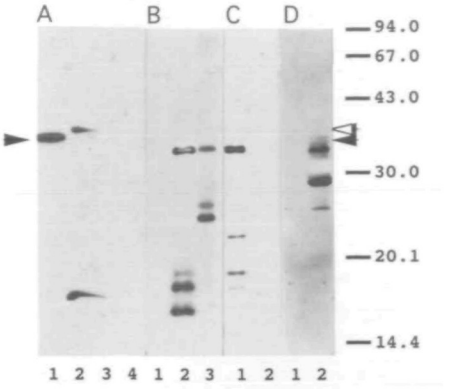

Fig. 3. **Western blot analysis of the 38 kDa lectin.** Samples were prepared, subjected to SDS-13% PAGE, electrotransferred to nitrocellulose membranes, and then immunoblotted. Panel A (Western blot using anti-rkCBP17.5): lane 1, L1210 β-galactoside-binding proteins; lane 2, rat liver β-galactoside-binding proteins; lane 3, guanidine-HCl extracts of rat large intestine; lane 4, recombinant galectin-4. Panel B (Western blot using anti-galectin-4 domain I): lane 1, L1210 β-galactoside-binding proteins; lane 2, guanidine-HCl extracts of rat large intestine; lane 3, recombinant galectin-4. Panel C (Western blot using anti-galectin-4 domain II): lane 1, recombinant galectin-4; lane 2, L1210 β-galactoside-binding proteins. Panel D (Western blot using anti-galectin-8): lane 1, L1210 β-galactoside-binding proteins; lane 2, rat liver β-galactoside-binding proteins, the upper stained band is thought to be galectin-8 (*11*), the lower bands may be proteolytic fragments of it. The numbers on the right denote molecular weight markers. The black arrows indicate the position of migration of the L1210 38 kDa lectin. The blank arrow indicates the position of migration of the rat liver 38.5 kDa protein.

ously (*11, 10*). Figure 3A shows the results for blots probed with the anti-rkCBP17.5 monoclonal antibody. The anti-rkCBP17.5 reacts with the 38 kDa lectin from L1210 cells (lane 1), and rat liver β-galactoside-binding proteins of molecular weights of 38.5 and 17.5 kDa (lane 2). The stained band at about 38.5 kDa in lane 2 is not galectin-8 as judged, when its mobility was compared with galectin-8 (Fig. 3D, lane 2), and it may be a rat homologue of the L1210 cell protein. No binding of the anti-rkCBP17.5 to intact galectin-4 from guanidine-HCl extracts of fresh rat large intestine (Fig. 3A, lane 3), or recombinant galectin-4 (Fig. 3A, lane 4) was ever observed. Furthermore, when immunoblotted with anti-galectin-4 polyclonal or anti-galectin-8 polyclonal antibodies, the 38 kDa lectin did not react with anti-galectin-4 domain I (Fig. 3B, lane 1), anti-galectin-4 domain II (Fig. 3C, lane 2), or anti-galectin-8 (Fig. 3D, lane 1). These results suggest that the 38 kDa lectin is distinct from galectin-4 or galectin-8, and that it is a novel member of the galectin family. We are now conducting further structural studies in order to determine the complete primary structure of the 38 kDa lectin.

The L1210 lymphoid cells were found to express the 38 kDa lectin but did not express other known larger galectins, particularly galectin-3 expressed in monocytic cells (*15, 18*). Therefore, we investigated whether the 38 kDa lectin was expressed in other cultured lymphoid cells or macrophage-like cells. β-Galactoside-binding proteins were obtained from various cells, and immunoblotted with the anti-rkCBP17.5 antibody or anti-galectin-3 antibody. The 38 kDa lectin was detected in all of the lymphoid cell lines, L1210, L5178Y, and BW5147 (Fig. 4A). However, no

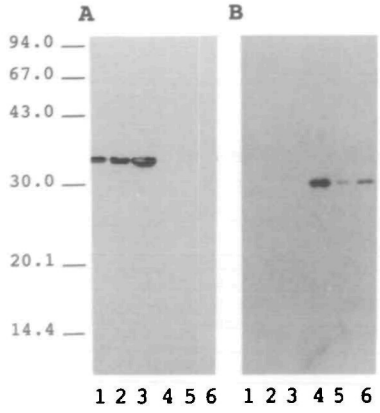

Fig. 4. **Expression of the 38 kDa lectin and galectin-3 in various cell lines.** Western blot using anti-rkCBP17.5 (A) or anti-galectin-3 (B). Lane 1, L1210 (lymphocytic leukemia); lane 2, BW5147 (lymphocyte-like T cell lymphoma); lane 3, L5178Y (lymphocyte-like leukemia); lane 4, WEHI-3 (macrophage cells); lane 5, J774A.1 (monocyte-macrophages); lane 6, P388-D1 (macrophage-like leukemia). The numbers on the left denote molecular weight markers. The cell lines used here were obtained from the Japanese Cancer Research Resources Bank (Japan).

immunoreactive material was ever found in any of the macrophage-like cell lines examined (WEHI-3, J774A.1, and P388-D1). In contrast, when immunoblotted with the anti-galectin-3 antibody, a single band corresponding to a molecular weight of about 35,000 was observed for macrophage-like cells only (Fig. 4B). This band co-migrated with galectin-3 (data not shown). This result suggests that these two lectins were not co-expressed in leukocytic cells. As far as examined, no expression of the 38 kDa lectin was detected in the other cell types, including macrophage-like cells (in this study), fibroblasts, epithelials, transformed cells, and many tumor cells (data not shown). On the other hand, in contrast to the 38 kDa lectin, galectin-3, also known as Mac-2 or IgEBP (*18, 19*), was found in all of the above examined cell lines but not lymphoid cell lines. There is an intriguing possibility that this variation of galectin expression in leukocytic cells may be correlated with the differentiation from hemopoietic stem cells. Several studies have indicated the importance of endogenous lectins like galectin-1 or galectin-3 in cell-cell communication, adhesion, immunosuppression, and apoptosis (*20, 21, 5*). Since the 38 kDa lectin seems to be specifically expressed in lymphocytic cells, it might also play a role in modulating immune systems.

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