

## Expression of galectin-3 modulates T-cell growth and apoptosis

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**ABSTRACT** Galectin-3 is a member of a large family of  $\beta$ -galactoside-binding animal lectins. It has been shown that the expression of galectin-3 is upregulated in proliferating cells, suggesting a possible role for this lectin in regulation of cell growth. Previously, we have shown that T cells infected with human T-cell leukemia virus type I express high levels of galectin-3, in contrast to uninfected cells, which do not express detectable amounts of this protein. In this study, we examined growth properties of human leukemia T cells transfected with galectin-3 cDNA, and thus constitutively overexpressing this lectin. Transfectants expressing galectin-3 displayed higher growth rates than control transfectants, which do not express this lectin. Furthermore, galectin-3 expression in these cells confers resistance to apoptosis induced by anti-Fas antibody and staurosporine. Galectin-3 was found to have significant sequence similarity with *Bcl-2*, a well-characterized suppressor of apoptosis. In particular, the lectin contains the NWGR motif that is highly conserved among members of the *Bcl-2* family and shown to be critical for the apoptosis-suppressing activity. We further demonstrated that galectin-3 interacts with *Bcl-2* in a lactose-inhibitable manner. We conclude that galectin-3 is a regulator of cell growth and apoptosis and it may function through a cell death inhibition pathway that involves *Bcl-2*.

Normal control of cell growth hinges on the delicate balance between cell proliferation and cell death (1). Dysregulation of either event can result in pathological consequences exemplified by diseases such as cancer, where unchecked proliferation results in continuous accumulation of cells, and AIDS, where selective death of T lymphocytes results in immunodeficiency. Some proteins known to govern growth and proliferation of cells are oncoproteins, tumor suppressors, and cyclins. We report here experimental evidence that an endogenous animal lectin, galectin-3, is another factor that controls cell growth.

Galectin-3 is a member of a growing family of  $\beta$ -galactoside-binding animal lectins (2, 3). This protein is composed of a small N-terminal domain, a domain consisting of proline- and glycine-rich repeats and a C-terminal carbohydrate-recognition domain (4). Galectin-3 is expressed in a variety of tissues and cell types (5–7) and is localized mainly in the cytoplasm, although, depending on cell types and proliferative states, a significant amount of this lectin can also be detected in the nucleus (8), on the cell surface (9, 10), or in the extracellular environment (10–12). Studies from various laboratories have suggested the possible involvement of galectin-3 in diverse physiological and pathological processes, including pre-mRNA splicing (13), neoplastic transformation (14), and immune responses (15). Experimental data also suggest a relationship between galectin-3 and cell growth. For example, when 3T3 cells were stimulated to proliferate, expression of galectin-3 increased shortly after the mitogenic stimulation and before the onset of the first S-phase of the cell cycle (8, 16). In addition, in quiescent mouse 3T3 cells, galectin-3 is phosphorylated and localized mainly in the cytoplasm (8, 17).

However, in proliferating cells, this lectin is predominantly in the nucleus in both phosphorylated and unphosphorylated forms (8, 17). The definitive establishment of the role for galectin-3 in regulation of cell growth requires further investigation. We recently demonstrated that transformed T-cell lines infected with human T-cell leukemia virus type I express high levels of galectin-3, in significant contrast to uninfected cells, which do not express detectable amounts of this protein (18). This appears to result from activation of the galectin-3 promoter by the viral transactivating protein Tax (18). We therefore examined the consequences of constitutively expressing human galectin-3 in the leukemic T-cell line Jurkat as a model system for evaluating the role of galectin-3 in cell growth.

### MATERIALS AND METHODS

**Cell Line and Reagents.** The human leukemia cell line Jurkat E6–1 was from the American Type Culture Collection. Cells were maintained in RPMI 1640 medium containing 2 mM glutamine and 10% fetal bovine serum (FBS). The mouse monoclonal anti-human Fas antibody (clone CH-11) and the mouse monoclonal anti-human *Bcl-2* antibody (clone 124) were purchased from Kamiya Biomedical (Thousand Oaks, CA) and Dako, respectively. The rabbit anti-*Bcl-x* antiserum was kindly provided by John Reed (La Jolla Cancer Research Foundation, La Jolla, CA) (19). CNBr-activated Sepharose 4B and plasmid pKK-233–2 were from Pharmacia. Plasmid pREP9 was purchased from Invitrogen and pMH-Neo (20) was a gift from Barbara E. Bierer (Dana–Farber Cancer Institute, Boston). Enhanced chemiluminescence (ECL) reagents were purchased from Amersham.

**Recombinant Human Galectin-3 and the Carboxyl-Terminal Fragment of Human Galectin-3 (Galectin-3C).** Recombinant full-length human galectin-3 was purified as described (21). To generate DNA encoding the C-terminal domain (lectin domain) of galectin-3, primers 5'-CCCCAGC-CATGGCACACATTG-3' and 5'-CCCCAAGCTTCTTTCAC-TGTAATATTT-3' were used in a PCR using the plasmid pDH- $\epsilon$ BP (21) as the template. A 427-bp product was purified and inserted into *Nco*I/*Hind*III-digested vector pKK-233–2. Recombinant galectin-3C, which contains amino acid residues 112–250 of the galectin-3 molecule, was purified in the same way as galectin-3 (21).

**Generation of Stable Jurkat Transfectants Expressing Human Galectin-3.** The cDNA encoding human galectin-3 was excised from clone 2.2 (22) with *Eco*RI and inserted into plasmid pREP9 at the *Hind*III site or into pMH-Neo at the *Eco*RI site, generating the expression plasmid pREP9-galec3 or pMH-galec3 with the galectin-3 cDNA in the sense direction to the Rous sarcoma virus long terminal repeats or the Friend spleen focus-forming virus long terminal repeat, respectively.

Abbreviations: FBS, fetal bovine serum; galectin-3C, carboxyl-terminal fragment of galectin-3.

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Purified plasmid DNAs were used to transfect recipient cell lines by electroporation (23). Two days after electroporation, cells were selected by culturing in the presence of 1 mg/ml of G418 for at least 2 weeks before being used for functional studies. G418-resistant cells transfected with pMH-galec3 were cloned by limiting dilution and individual clones were used for further studies. Because pREP9 is an episomal vector containing the Epstein-Barr virus replicon and is capable of autonomous replication independent of the genome of recipient cells (24), all G418-resistant cells transfected with pREP9-galec3 should contain the galectin-3 gene under control of the independent viral promoter and express galectin-3. Therefore, these G418-resistant transfectants were used directly without further cloning.

**Immunoblotting.** Expression of galectin-3 was determined by immunoblotting. Briefly, cells were lysed with a Triton X-100-containing lysing buffer (25) and the lysates were mixed with lactosyl-Sepharose 4B (22). The bound protein was eluted and separated by SDS/12.5% PAGE and subjected to immunoblot analysis (22) using a rabbit anti-galectin-3 antiserum (26) and the ECL kit. For cells transfected with pMH-Neo-based constructs, cell lysates were directly mixed with SDS sample buffer and subjected to SDS/PAGE and immunoblotting.

**Assays for Cell Growth and Viability.** To assess the growth of Jurkat transfectants in low serum medium, cells at a density of  $10^5$  cells/ml were cultured in RPMI 1640 medium containing 1% FBS. Viable cell numbers were determined by the MTS assay (27), using a CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay kit (Promega). For apoptosis assay, transfected Jurkat cells ( $1 \times 10^6$  cells/ml) were treated with either 50 ng/ml of mouse monoclonal anti-human Fas IgM or 0.5  $\mu$ M of staurosporine in RPMI 1640 medium containing 10% FBS. Viable cell numbers were determined by the MTT assay (28), using the reagent from Sigma. The MTS and MTT assays are similar in principle; both reagents are tetrazolium salts that are reduced to the respective formazans by mitochondrial dehydrogenase from the cells.

**Flow Cytometry.** Expression of Fas on cell surface of Jurkat transfectants was determined by flow cytometry. Cells ( $1 \times 10^6$ ) were washed with HBSS staining buffer (containing 0.1% NaN<sub>3</sub> and 1% BSA) and incubated for 20 min at 4°C in 50  $\mu$ l of staining buffer containing 50 ng/ml or 10  $\mu$ g/ml of mouse anti-human Fas IgM. After two washes with staining buffer, cells were incubated for 20 min at 4°C with fluorescein isothiocyanate-labeled goat anti-mouse IgM (Sigma; 1:200 dilution) in 50  $\mu$ l of staining buffer. Cells were then washed twice with staining buffer and analyzed on a FACScan (Becton Dickinson) and  $10^4$  events were recorded.

**Detection of *Bcl-2* Binding to Galectin-3-Sepharose 4B.** Recombinant human galectin-3 or galectin-3C were immobilized on CNBr-activated Sepharose 4B according to the manufacturer's procedures. For detection of galectin-3/*Bcl-2* interaction,  $10^7$  Jurkat cells were lysed with lysing buffer (20 mM HEPES-KOH/50 mM NaF/25 mM disodium glycerophosphate/1 mM sodium vanadate/5 mM L-phenylalanine/0.2% Nonidet P-40, pH 7.5) containing protease inhibitors (1  $\mu$ g/ml leupeptin/0.5  $\mu$ g/ml pepstatin/10  $\mu$ g/ml aprotinin/0.5 mM phenylmethylsulfonyl fluoride) for 30 min on ice and cellular debris was removed by centrifugation at  $12,000 \times g$  for 10 min at 4°C. The supernatant was incubated with constant mixing at 4°C for 1 h with 10  $\mu$ l of galectin-3-Sepharose 4B or galectin-3C-Sepharose 4B, in the absence or presence of 25 mM of lactose or sucrose. After the beads were washed three times with lysing buffer, the bound proteins were eluted with SDS sample buffer, separated by SDS/PAGE, transferred to Immobilon-P membrane (Millipore) and *Bcl-2* was detected with a mouse anti-human *Bcl-2* monoclonal antibody. The immunoblot was developed with the ECL system.

## RESULTS

**Expression of Galectin-3 in Transfectants.** Galectin-3 expression in Jurkat transfectants was analyzed by immunoblotting. As shown in Fig. 1A, cells transfected with the parental vector pREP9 did not express detectable amounts of galectin-3, whereas those transfected with pREP9-galec3 expressed the lectin. The identity of the expressed protein was established by its binding to lactosyl-Sepharose 4B, its molecular weight identical to the authentic galectin-3, and its immunoreactivity with anti-galectin-3 antibodies. Fig. 1B shows the expression of galectin-3 in four clones obtained from the G418-resistant cells transfected with pMH-galec3. Like Jurkat cells transfected with the vector pMH-Neo (lane 2), clones 1 and 2 did not express detectable amounts of galectin-3 (lanes 3 and 4). In contrast, clones 3 and 4 expressed high levels of the lectin (lanes 5 and 6). Because all four clones were obtained from parental cells transfected with the same construct, clones 1 and 2 served as ideal controls for clones 3 and 4 in experiments described below.

**Cells Expressing Galectin-3 Grow Faster than Cells That Do Not Express the Lectin in Low Serum Medium.** Effects of galectin-3 expression on cell growth in culture were then tested. Cells were grown in RPMI 1640 medium containing 1% FBS and viable cell numbers were determined by the formazan colorimetric method using MTS. As shown in Fig. 2A, Jurkat transfectants expressing galectin-3 grew significantly faster than control transfectants. In cultures of pREP9-galec3 transfectants, the number of viable cells doubled after 2 days in culture and the cells continued to proliferate after day 4 when the cell numbers had increased to approximately 270%. The control transfectants, however, had ceased proliferating by day 3, after the cell numbers had increased to 170% (Fig. 2A). Similar results were obtained with pMH-galec3-transfected cells (Fig. 2B). Thus, after a 4-day culture in 1% FBS, viable cell numbers in clones that did not express galectin-3 (clones 1 and 2) increased less than 2-fold, whereas greater than 3-fold accumulation of viable cells occurred in clones expressing galectin-3 (clones 3 and 4, Fig. 2B). Cell viability was also measured by trypan blue exclusion in separate experiments. The cell densities at days 2, 3, and 4 were 1.89, 2.57, and 3.80

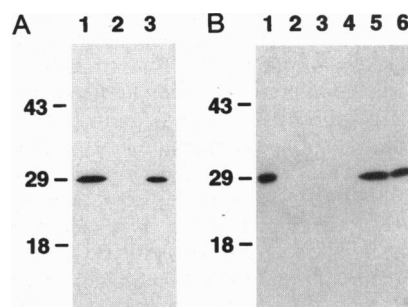


Fig. 1. Expression of galectin-3 protein in Jurkat transfectants. (A) Cells transfected with pREP9 constructs. Bulk G418-resistant lines ( $1 \times 10^7$  cells) were lysed, absorbed with lactosyl-Sepharose 4B, and the bound protein was eluted and subjected to immunoblotting with a rabbit antiserum to human galectin-3. Lane 1, purified recombinant human galectin-3; lane 2, lactose-binding protein from Jurkat cells transfected with pREP9; lane 3, lactose-binding protein from Jurkat cells transfected with pREP9-galec3. (B) Cells transfected with pMH-Neo-based constructs. Transfected cells were lysed and the cell lysates were mixed with SDS sample buffer and subjected to SDS/PAGE and immunoblotting with a rabbit anti-human galectin-3 antiserum. Each lane represents lysate from  $1.2 \times 10^6$  cells. Lane 1, purified human galectin-3; lane 2, Jurkat cells transfected with pMH-Neo; lanes 3-6 represent individual clones 1, 2, 3, and 4, respectively, from the G418-resistant cell population transfected with pMH-galec3. Molecular weight markers ( $\times 10^{-3}$ ) are shown in the left margin.

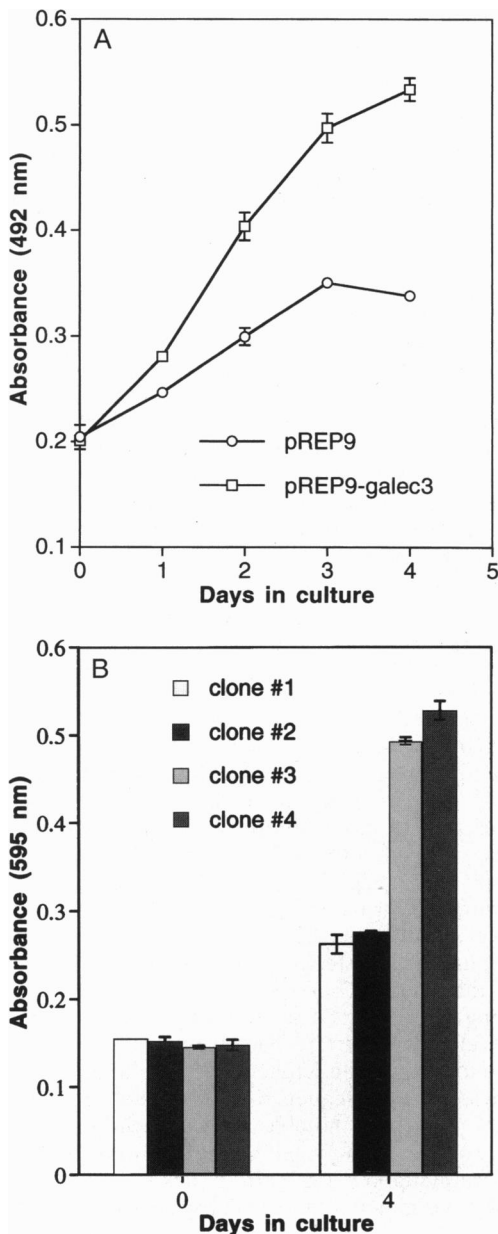


FIG. 2. Growth of Jurkat transfectants in low serum medium. Jurkat cells ( $10^5$  cells/ml) transfected with pREP9- (A) or pMH-Neo-based (B) constructs were cultured in RPMI 1640 medium containing 1% FBS. Viable cell number were determined by the MTS assay (A) or MTT assay (B). Absorbance due to formazan formation that reflects viable cell numbers is expressed as mean  $\pm$  SD from three cultures. Similar results were obtained in three independent experiments. Clones 1 and 2 (galectin-3 negative) and clones 3 and 4 (galectin-3 positive) are described in Fig. 1B. Differences between control and galectin-3-expressing transfectants were tested for statistical significance by analysis of variance (ANOVA), using the software package STATVIEW (version 4.01, Abacus Concepts, Berkeley, CA). Expression of galectin-3 has a significant effect on the growth of transfectants ( $P < 0.0001$ ).

$\times 10^5$ , respectively, for clone 2, and  $2.47$ ,  $4.07$ , and  $6.20 \times 10^5$ , respectively, for clone 4.

**Galectin-3 Suppresses Apoptosis in Jurkat Cells.** Components of the signaling pathways for proliferation may also be components of the pathways leading to apoptosis (29). Thus, it was of interest to determine whether galectin-3 also participates in the regulation of apoptosis. We first used a monoclonal antibody against human Fas to induce apoptosis in Jurkat transfectants. Fas is a cell surface antigen of the tumor

necrosis factor receptor family (30) and its crosslinkage by Fas ligand or anti-Fas antibodies triggers apoptotic signals in susceptible cell types such as Jurkat (31). As shown in Fig. 3A, greater than 50% of the cell population perished in Jurkat subclones that do not express galectin-3 (clones 1 and 2) within 2 h after the treatment with 50 ng/ml of the anti-Fas antibody. In contrast, about 80% of the cells expressing galectin-3 (clones 3 and 4) remained viable 2 h after the treatment. After 4 h, survival of galectin-3-negative clones dropped to approximately 20%, whereas approximately 70% of cells from galectin-3-positive clones were still alive. In a separate experiment cell viability was also determined by trypan blue exclusion after induction of apoptosis with 100 ng/ml anti-Fas antibody in clone 2 (galectin-3 negative) and clone 4 (galectin-3 positive) cells. Five hours after induction, cell viability for clone 2

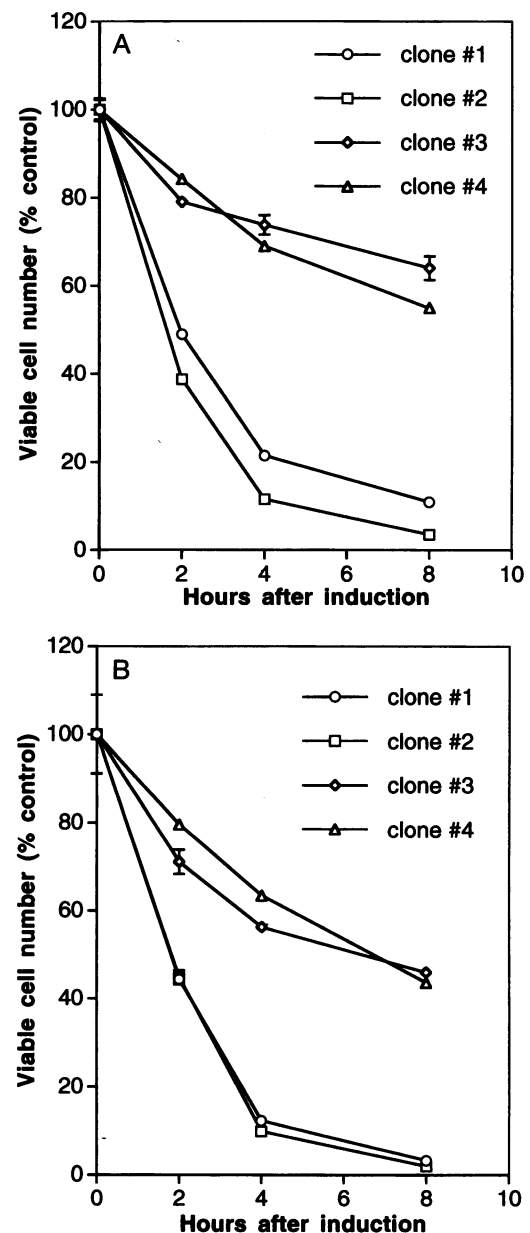


FIG. 3. Suppression of induced apoptosis by expression of galectin-3 in Jurkat cells. Jurkat transfectants were treated with either 50 ng/ml anti-Fas antibody (A) or with  $0.5 \mu\text{M}$  staurosporine (B), and viable cell numbers were determined with the MTT assay. Results are expressed as mean  $\pm$  SD from three cultures. Similar results were obtained in three independent experiments. Clones 1-4 are as described in Fig. 1B.

dropped to 7.8%, whereas 43.7% of the cells from clone 4 were still alive. Using flow cytometry, we quantified the expression of Fas on the cell surface of Jurkat transfectants and observed slight reduction in the level of Fas on the surface of galectin-3-expressing transfectants as compared with controls, when 10  $\mu$ g/ml of the anti-Fas antibody was used (data not shown). However, all clones tested showed virtually identical fluorescent intensities when 50 ng/ml of the antibody was employed, the concentration used to induce apoptosis (data not shown).

We next tested the resistance of Jurkat transfectants to apoptosis-induced with a protein kinase inhibitor, staurosporine, which is believed to be a universal inducer of apoptosis that activates a common apoptotic pathway (32). Again we found that galectin-3-expressing transfectants were remarkably more resistant to staurosporine-induced apoptosis than the galectin-3-negative controls (Fig. 3B).

**Galectin-3 Shares Sequence Similarity with *Bcl-2*.** The ability of galectin-3 to inhibit apoptosis prompted us to investigate a possible link between this protein and *Bcl-2*, a well-characterized repressor of apoptosis (33, 34). We found that galectin-3 exhibits low but significant sequence similarity with *Bcl-2* (Fig. 4): There is 28% identity and 48% similarity between these two protein sequences. In comparison, human *Bcl-2* and another member of the *Bcl-2* family, BHRF-1, show 24% identity and 41% similarity. The amino-terminal portions of both galectin-3 and *Bcl-2* contain regions rich in proline (P), glycine (G), and alanine (A): Amino acid residues 36–85 of human *Bcl-2* show a P + G + A content of 62%, whereas residues 30–113 of human galectin-3 contain 75% P + G + A. Significant sequence similarity is also found in the carboxyl-terminal regions of the two proteins. In particular, the highly conserved NWGR motif within the BH1 domain of members of the *Bcl-2* family (36) is present in galectin-3 (residues 180–183 in galectin-3 and residues 143–146 in human *Bcl-2*).

**Galectin-3 Interacts with *Bcl-2*.** Because both galectin-3 and *Bcl-2* have a tendency to self-associate (21, 37), sequence homology between these two proteins suggests the possibility

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10  ALSGSGNPNPQGWPGAWGNQAGAGGYPGASYPGAYPGQAPPAYPGQAP 59
   | . . . . . : : : : : . . . . . || . . . . . : . . . . . |
2   AHAGRTGYDNRREIVMKYIHYKLSQRGY... EWDAQGVGAAPPAAAE... 44

60  PGAYHGAAPGAYPGAPAPGVYPGPPSPGPGAYPSSGQ. PSAPGAYPATPGYPG 108
   ||| . . . . . : : : : : . . . . . : . . . . . | . . . . . | . . . . . | . . . . . |
45  . . . . . APGIFSSOPGHTPHTAASRDVFARTSPLQTPAARFGA... AAGPAL 86

109 APAGPLIVPYNLPLPGGVVPRMLITILGTVKPNANRIALDFQRGNDVAFH 158
   . . . . . : . . . . . : . . . . . : . . . . . : . . . . . : . . . . . : . . . . . :
87  SPVPP. VVHLTLRQAGDDFRRYRDFAEMSRQ.....LH 120

159 FNPRFNENRRVIVCNTKLDN... NWGRE... ERQSVFPFESGKP... 197
   . . . . . | . . . . . : . . . . . : . . . . . : . . . . . : . . . . . : . . . . . :
121 LTP. FTARGRFATVVEELFRDGVNWGRIVAFFEFGGVMCVESVNREMSPL 169
   BH1
198 . . . . . FKIQVLVEPDHFKVAVN... DAHLLQYNHRVKKLNEISKLGISG 238
   . . . . . | . . . . . : . . . . . : . . . . . : . . . . . : . . . . . : . . . . . :
170 VDNIALWMTTEYLNRHLHTWIQDNGGWDAFVVELYGPMSRPLDFDFSWLSLKT 219
   BH2
239 ..DIDLTSASYTM 249
   . . . . . : . . . . . : . . . . . :
220 LLSLALVGACITL 232
  
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FIG. 4. Comparison of *Bcl-2* and galectin-3 protein sequences. The upper sequence is for human galectin-3 (22) and the lower one is for human *Bcl-2* (35). Alignment was performed with the program BESTFIT of the GCG package (Genetics Computer Group, Madison, WI). Vertical lines indicate amino acid identity, colons denote amino acid comparison value  $\geq 0.5$ , periods indicate amino acid comparison value  $\geq 0.1$ . The BH1 and BH2 domains in *Bcl-2* are underlined. Two regions of high similarity between galectin-3 and *Bcl-2* in the amino-terminal portions are double underlined. One region in galectin-3 shares significant sequence similarity with the BH1 domain, including the presence of an NWGR motif (boldface letters). Sequences corresponding to the BH2 domain of *Bcl-2* are not present in galectin-3.

that they may interact with each other. Indeed, when the Jurkat cell lysate was mixed with galectin-3-Sepharose 4B, *Bcl-2* was found to be specifically adsorbed, as evidenced by the detection of a protein that comigrated with *Bcl-2* in SDS/PAGE ( $M_r$  of 26,000) and was recognized by a monoclonal anti-*Bcl-2* antibody (Fig. 5A, lane 3). The specificity of the interaction was demonstrated by the lack of detection of this protein when protein A-Sepharose 4B was used (Fig. 5A, lane 2), or when an isotype-matched control antibody was employed in the immunoblot analysis (data not shown). The specificity was also demonstrated when the immunoblot was probed with an antiserum against *Bcl-x*, which are members of the *Bcl-2* family (38). Fig. 5B shows that whereas *Bcl-x<sub>L</sub>* is present in Jurkat cells (lane 1), it is not bound to galectin-3 (lane 3). Lactose, an effective ligand of galectin-3, but not sucrose, inhibited the binding of *Bcl-2* to galectin-3-Sepharose 4B (Fig. 5C, lanes 2 and 3), suggesting the relevance of the carbohydrate-recognition domain in the galectin-3-*Bcl-2* association. This conclusion was further supported by the binding of *Bcl-2* to Sepharose 4B conjugated with galectin-3C, which contains the carbohydrate-binding site (Fig. 5C, lane 4).

## DISCUSSION

By using the human T-cell line Jurkat transfected with human galectin-3, we demonstrated that this protein promotes growth of cells cultured in restrictive culture conditions. When cultured in low serum medium, cells expressing galectin-3 grew significantly faster than counterparts that do not express galectin-3. Moreover, we found that galectin-3 expression in cells confers resistance to apoptosis. This was demonstrated in apoptosis induced both by Fas receptor ligation and by staurosporine, suggesting that galectin-3 could be a cell death suppressor interfering with a common pathway of apoptosis. We have shown that the level of Fas is not significantly different among transfectants expressing galectin-3 and those lacking this lectin. Therefore, the resistance of galectin-3-expressing cells to anti-Fas-induced apoptosis is not due to lower levels of cell surface Fas on these cells.

A clue to the galectin-3 function in regulation of cell growth was provided by the sequence similarity between this protein and *Bcl-2*. The most notable finding is the presence of the NWGR motif in both proteins. The importance of this motif in cell death suppression by *Bcl-2* has been previously confirmed by site-directed mutational studies (39). This motif is

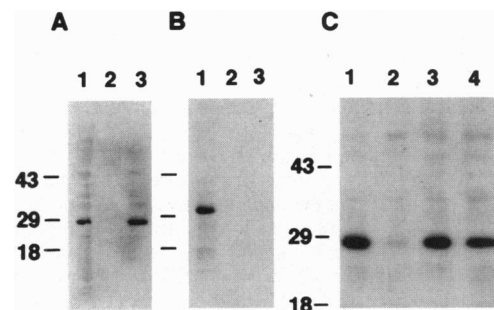


FIG. 5. Interaction of galectin-3 with *Bcl-2*. (A) Total lysate from  $2 \times 10^5$  cells was applied to lane 1. Lysate from  $10^7$  cells was adsorbed with protein A-Sepharose (lane 2) or galectin-3-Sepharose (lane 3). Bound and subsequently eluted proteins were probed with an anti-*Bcl-2* antibody in immunoblot. (B) Same as A, except that the immunoblot was probed with an anti-*Bcl-x* antiserum. (C) Lysate from  $10^7$  cells was mixed with galectin-3-Sepharose (lanes 1–3) or galectin-3C-Sepharose (lane 4) in the absence (lanes 1 and 4) or presence of 25 mM lactose (lane 2) or sucrose (lane 3). The bound and subsequently eluted protein was probed with an anti-*Bcl-2* monoclonal antibody in immunoblot. Molecular weight markers ( $\times 10^{-3}$ ) are shown in left margin.

highly conserved in galectin-3 among different species: human, hamster, and rat galectin-3 all contain this motif, whereas mouse galectin-3 contains the charge-conserved substitution of arginine (R) by lysine (K) (22). In dog galectin-3, the corresponding motif is IWGK (40), where the N-to-I substitution may reflect species divergence in this region. In particular, divergence of the leading residues of this motif is also observed among species homologues of the *Bcl-2* family (39). Among all galectins only galectin-3 contains the NWGR motif, although tryptophan (W) and glycine (G) residues are found in the corresponding positions in all other galectins (3, 41–43). An additional intriguing observation is that this tryptophan residue is likely to be indispensable for the carbohydrate-binding activity of all galectins given that it has been shown to be in direct contact with the bound carbohydrate in the x-ray crystallographic structure of galectin-2 (44).

Another clue to the galectin-3 function in regulation of cell growth was provided by the demonstration that galectin-3 interacts with *Bcl-2*. The presented data are consistent with either a direct or indirect interaction between these two proteins. However, preliminary studies support direct galectin-3 binding to *Bcl-2* (data not shown). This finding is consistent with the known property of *Bcl-2* to form heterodimers with homologous proteins, such as Bax (37, 45). An intriguing finding, however, was the lactose-inhibitable *Bcl-2*-galectin-3 interaction, particularly since *Bcl-2* is not a glycoprotein. The result suggests that the carbohydrate-binding site in galectin-3 may be closely involved in this molecular interaction, or lactose binding to galectin-3 induces a conformational change in the critical region of this protein that participates in the interaction with *Bcl-2*. Interestingly, the NWGR motif in *Bcl-2* has been shown to be critical for *Bcl-2/Bcl-2* homodimerization and *Bcl-2/Bax* heterodimerization (37). The same motif in galectin-3, which, as mentioned above, is probably present within the carbohydrate-binding site, may be involved in binding to *Bcl-2*.

The exact mechanisms of action of galectin-3 in cell growth control remain to be determined. Total *Bcl-2* levels in galectin-3-expressing cells do not differ significantly from control cells (data not shown). Therefore, the effect of galectin-3 is not due to its upregulation of *Bcl-2*. However, the interaction between galectin-3 and *Bcl-2* suggests that galectin-3 may regulate apoptosis by participation within a cell death inhibition pathway involving *Bcl-2*. By immunoblotting of cellular fraction prepared by using the freeze-thaw method (7) we found that galectin-3 is mainly present in the cytosol in the transfected Jurkat cells, similar to the endogenous lectin expressed in other cell types (7, 46). Therefore, galectin-3 has potential to interact with *Bcl-2* family proteins, which are located on the outer membrane of mitochondria (47). The sequence similarity between galectin-3 and *Bcl-2* suggests that these two proteins may be evolutionarily related. It is interesting that galectin-3 appears to have acquired two identities: while belonging to a family of animal lectins capable of functioning through their carbohydrate-binding activity, it may also be a part of a family of intracellular proteins destined to regulate cell growth.

Although our finding of the galectin-3 cell growth regulation property was made with transfected T cells, it may be relevant to the role of this lectin in normal T cells, because the level of galectin-3 in these cells is likely to be upregulated by certain stimuli, especially those capable of activating the CREB and/or NF- $\kappa$ B transcriptional factor pathways (18). Our conclusion may also be generalized to various normal cell types constitutively expressing galectin-3, but may be of particular relevance to the growth properties of certain neoplastically transformed cells. It has been reported that galectin-3 expression is either induced or upregulated in a wide range of neoplasms, including spontaneously occurring ones as well as those induced by virus, ultraviolet light, or chemicals (48–50). Galectin-3 expression has been found to increase significantly

in *ras*-transformed cells with a maximum level found in those cells that have lost their anchorage-dependence in growth (50). In mouse fibroblasts, galectin-3 expression appears to be regulated in a manner comparable to other mitogen-activated genes, including the oncogenes *c-fos* and *c-myc* (16). In addition, transfection of the BALB/c-3T3-A31 fibroblast cell line with galectin-3 cDNA resulted in cells with morphological transformation (14).

Our results may be of particular relevance to the role of galectin-3 in the biological behavior of human T-cell leukemia virus type I-infected T cells, previously shown to overexpress this lectin (18). Other investigators have shown that these cells exhibit reduced susceptibility to anti-Fas-induced apoptosis, despite the high-level expression of cell surface Fas (51). These findings and the present study strongly suggest that overexpressed galectin-3 in human T-cell leukemia virus type I-infected T cells may contribute to the altered growth properties of these cells, and culminate in tumor transformation.

In summary, we conclude that, in addition to other possible functions, galectin-3 is a cell growth regulator. It appears to confer a survival advantage in cells by inhibiting apoptosis and sustaining growth under restrictive conditions. Although the mechanism of this function of galectin-3 awaits further investigation, the preliminary data suggest the involvement of this protein's interaction with intracellular regulators of cell growth or apoptosis, such as *Bcl-2*. The finding that such interaction is inhibited by the saccharide ligands of galectin-3 suggests that certain specific glycoconjugates may have a profound effect on cell growth through binding to this lectin. Interestingly, another member of the galectin family, galectin-1, has recently been shown to induce apoptosis when added to cell cultures of activated human T cells and T-cell lines (52). Thus, it appears that various members of the galectin family may play an important role in regulation of cell growth and apoptosis. Moreover, the opposite effect of galectin-1 and galectin-3 is reminiscent of that found for members of the *Bcl-2* family—despite sequence similarities some members inhibit apoptosis, whereas others promote it (53).

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