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Human T Lymphotropic Virus-I Infection of Human T Lymphocytes Induces Expression of the β-Galactoside-Binding Lectin, Galectin-3

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Animal lectins play important roles in a variety of biological processes via their recognition of glycoconjugates. Galectin-3 is a β-galactoside-binding lectin previously designated as εBP (IgE-binding protein), CBP35, Mac-2, L-29, and L-34, and its expression has been associated with various physiological and pathological processes, including cell growth, tumor transformation, and metastasis. Galectin-3 is widely distributed in various tissues and cell types and is expressed in many leukocytes, with the notable exception of B and T lymphocytes. We now report that galectin-3 is abundantly expressed in a number of human T lymphotropic virus (HTLV)-I-infected human T cell lines, including F6T, HUT 102, K3T, MT-2, and SLB-I, but is not expressed in non-HTLV-I-infected T cell lines such as Jurkat, CEM, and MOLT-4. In addition, the galectin-3 level was markedly increased in human thymocytes after infection with HTLV-I as compared with uninfected thymocytes. The up-regulation of galectin-3 expression appeared to correlate well with HTLV-I gene expression, as undetectable or very low levels of galectin-3 were found in the S1T and ATL-1K cell lines, which are nonproductively infected with HTLV-I. In co-transfection experiments, the galectin-3 promoter was significantly up-regulated by expression vectors encoding the 40-kd Tax protein, a potent transactivator in HTLV-I. Analysis of various Tax mutants suggested that galectin-3 promoter induction is dependent on activation of the cyclic-AMP-responsive element binding protein/activation transcription factor family of transcription factors and, to a lesser extent, nuclear factor-κB/Rel induction. Transfection of human promonocytic U-937 cells with an HTLV-I Tax expression vector induced galectin-3 expression in this cell line. Functionally, galectin-3 was shown to activate interleukin-2 production in Jurkat T cells. Together, these findings raise the possibility that HTLV-I Tax production induces the transcription and subsequent synthesis and secretion of galectin-3, which in turn may further activate these T cells and contribute to the altered properties of cell growth found in adult T cell leukemia induced by HTLV-I. (Am J Pathol 1996, 148:1661–1670)

Human T leukemia/lymphoma virus (HTLV)-I is a type C retrovirus that produces disease within the $CD4^+$ subset of T lymphocytes and is clinically manifested as adult T-cell leukemia/lymphoma (for reviews see Refs. 1–4). *In vitro* infection of T lymphocytes by HTLV-I results in up-regulation of a number of cellular proteins and immortalization of these cells. Many of the biological effects of HTLV-I infection on T cells are believed to be the result of the viral Tax protein, which augments expression of all HTLV-I gene products through effects on the HTLV-I long terminal repeat but also activates many cellular genes includ-

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ing interleukin (IL)-2 and the α-subunit of the high affinity IL-2 receptor complex.[2] In this communication, we report that HTLV-I infection of T cells results in a marked increase in expression of the β-galactoside-binding lectin, galectin-3, and furthermore, that these effects involve the action of the viral transactivator Tax.

Galectin-3 is a member of a newly defined and growing family of animal lectins[5,6] previously designated as IgE-binding protein (εBP) for its IgE-binding activity[7–9] and as CBP35,[10,11] CBP30,[12] Mac-2,[13,14] L-29,[15] and L-34.[16,17] This M_r 31,000 protein is composed of two apparent domains; the amino-terminal domain consists primarily of tandem repeats of nine amino acids and the carboxyl-terminal domain contains the carbohydrate-binding region.[18]

Studies from a number of laboratories suggest that galectin-3 may have a wide range of functions. This lectin was found to be up-regulated in proliferating fibroblasts as compared with quiescent cells, and the protein was found to be located in the nucleus of the proliferating cells, suggesting an association of its expression with cell growth.[19] More recent preliminary data have suggested a positive role for this protein in pre-mRNA splicing.[20] Expression of galectin-3 has also been correlated with the neoplastic transformation and metastatic potential of tumor cells.[21] Galectin-3 is also present on the cell surface, and the laminin-binding activity of this lectin has been identified.[22,23] Thus, this lectin may play a role in cell adhesion to basement membranes.[23] Finally, galectin-3 is secreted by various cells[13,24,25] and may function extracellularly to modulate various properties of different cell types.[26]

The distribution of galectin-3 is quite diverse, and its expression in various leukocytes has been observed, including neutrophils,[27] macrophages,[13] and eosinophils.[28] In view of the wide tissue distribution of galectin-3, it is remarkable that this lectin is not present or only sparingly expressed in normal lymphocytes and several lymphoid cell lines.[7,29] However, recent studies involving the HTLV-I-infected T cell line HUT 102 revealed that galectin-3 consistently co-purified with the viral envelope protein gp46 (S. R. Hammes and W. C. Greene, unpublished results). This finding prompted us to perform more extensive analyses of the relationship between galectin-3 expression and HTLV-I infection and the mechanism underlying its unexpected expression in these immortalized human T cells.

Materials and Methods

Reagents

Recombinant human galectin-3 was prepared as described.[30] Rabbit polyclonal anti-human galectin-3 was generated using a standard protocol as previously described.[31] Recombinant murine IL-2[32] generously provided by Dr. H. Karasuyama, Basel Institute for Immunology, Switzerland, was obtained through Dr. H Kishimoto, Scripps Research Institute.

Cells

Cell lines included 1) HTLV-I-infected human T cell lines F6T,[33] HUT 102,[34] MT-2,[35] and SLB-1,[36,37] which express viral proteins, 2) HTLV-I-infected but nonproductive cell lines ATL-1K[38] and S1T,[33,39] 3) uninfected human T cell lines CEM, MOLT-4, and Jurkat, 4) a murine B cell line M12.4.5, which is an oubain-resistant subclone of M12.4.2[40] and was originally from Dr. R. Asofsky (National Institutes of Health, Bethesda, MD), 5) a human promonocytic cell line, U-937,[41] 6) human thymocytes and the HTLV-I-infected human thymocyte cell line TC91$^+$, kindly provided by Dr. C. Tsoukas (San Diego State University, San Diego, CA) and demonstrated to be transformed and expressing viral proteins,[42] and 7) Jurkat E6–1[43] and CTLL-2, obtained from American Type Culture Collection (Rockville, MD). ATL-1K, MT-2, and SLB-I cell pellets were kindly provided by Dr. W. Wachsman (University of California, San Diego, La Jolla, CA). The remaining cell lines were cultured in Dulbecco's minimal essential medium or RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 2 mmol/L glutamine.

Detection of Galectin-3 by Immunoblotting Analysis

Cell lysates were prepared as previously described,[44] and galectin-3 present in the lysates was purified by affinity adsorption with lactosyl-Sepharose 4B[45] and detected by immunoblotting analysis. Specifically, cell lysates containing 100 to 400 μg of total cellular proteins were adsorbed with 25 μl of lactosyl-Sepharose 4B, and bound proteins were eluted and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis.[46] Separated proteins were then transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA) by electroblotting,[47] and galectin-3 was detected by using a rabbit anti-galectin-3 antibody followed

by goat anti-rabbit IgG-alkaline phosphatase conjugate and chemiluminescent detection using a Tropix kit (Bedford, MA) or by ^{125}I-labeled protein A and autoradiography. Quantitative comparisons of galectin-3 were performed by adsorption of equivalent protein contents from each cell lysate. Densitometry was performed on an LKB Ultroscan XL.

Construction of Expression Vectors

HTLV-I Tax wild-type (pcTaxWT) and mutant expression vectors transactivating through the cyclic-AMP-responsive element binding protein/activation transcription factor (CREB/ATF) pathway, pcTaxM22 (HTLV-I Tax expression vector impaired for CREB/ATF transactivation pathway only), the nuclear factor (NF)-κB/Rel pathway, pcTaxM47 (HTLV-I Tax expression vector impaired for NF-κB/Rel pathway only), and inactive vectors pcTaxM20, pcTaxM21, and pcTaxM44 were described previously.[48] In brief, mutant vectors generated by site-directed mutagenesis resulted in replacement of wild-type amino acid residues to Ala-Ser at ^{131}Thr-Leu for pcTaxM22 (The location of pcTaxM22 has been confirmed to be different from that published earlier.[48] However, the phenotype of this mutation remains exactly as described.[49]), ^{132}Gly-Leu for pcTaxM20, ^{134}Pro-Asp for pcTaxM21, or the replacement to Arg-Ser at position ^{319}Leu-Leu for pcTaxM47. Mutants were evaluated for their abilities to induce the CREB/ATF or NF-κB/Rel transcription pathways by their influence on HTLV-I long terminal repeats or HIV-I long terminal repeat promoters, respectively.[48]

The murine galectin-3 promoter region,[50] from the *Bsr*BI site in exon I and approximately 2 kb upstream to an *Eco*RI site was obtained by gel purification of the appropriate band and inserted into the pCAT-basic vector (Promega, Madison, WI) to drive an *Escherichia-coli*-derived chloramphenicol acetyltransferase (CAT) gene. The pCAT-basic vector used did not contain enhancer elements other than those introduced within the insert.

Transfection of Cells

The murine B lymphoid cell line M12.4.5 was cultured in Dulbecco's minimal essential medium (Bio-Whittaker, Walkerville, MD) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Salt Lake City, UT) and 2 mmol/L glutamine and transfected by electroporation.[51] Briefly, actively dividing cells were cooled on ice, washed once in serum-free medium, and suspended in electroporation buffer at 2×10^7 cells/ml. DNA mixtures were precipitated in ethanol, resuspended in electroporation buffer, and added to 0.2-ml aliquots of cell suspensions before electrical discharge in a BioRad Gene Pulser apparatus at 150 V and capacitance setting of 960 μF in 0.2-cm cuvettes.

Chloramphenicol Acetyltransferase Activity Assays

Two days after transfection, cells were harvested, washed sequentially with phosphate-buffered saline, pH 7.2, and Tris-buffered saline containing 10 mmol/L EDTA, pH 7.5, and lysed in 125 μl of 0.25 mol/L Tris-HCl, pH 8.0, by three freeze-thaw cycles in liquid nitrogen. CAT activity was determined by the phase transfer method[52] using [dichloroacetyl-1,2-^{14}C]-chloramphenicol, (>50 mCi/mmol, New England Nuclear, Boston, MA) and *n*-butyl-coenzyme A (Sigma Chemical Co., St. Louis, MO). The basal activity of cells transfected with the pCAT-basic vector was subtracted from CAT activities of all other transfections.

Analysis of DNA Sequences

The promoter region of the murine galectin-3 gene was analyzed with the GCG program,[53] and the motifs corresponding to transcription factors were derived from the transcription factor database.[54]

Activation of Jurkat E6-1 Cells and Assays for IL-2

Recombinant human galectin-3 (4 to 100 μg/ml) was added to Jurkat E6-1 cells and incubated for 24 hours at 37°C. IL-2 released from treated cells into the media was quantified by an assay based on the proliferation of an IL-2-dependent cell line, CTLL-2, determined by the formazan method using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-^{2}H-tetrazolium, inner salt; substrate (Promega). Recombinant IL-2 was used as standard in the assay. As a positive control, Jurkat cells were also treated with phytohemagglutinin (Murex, Dartford, UK) and phorbol myristate acetate (PMA; Sigma) as described.[43]

Results

Expression of Galectin-3 in Human T Cells Infected with HTLV-I

To determine whether galectin-3 expression in T cells correlates with HTLV-I infection, we surveyed a

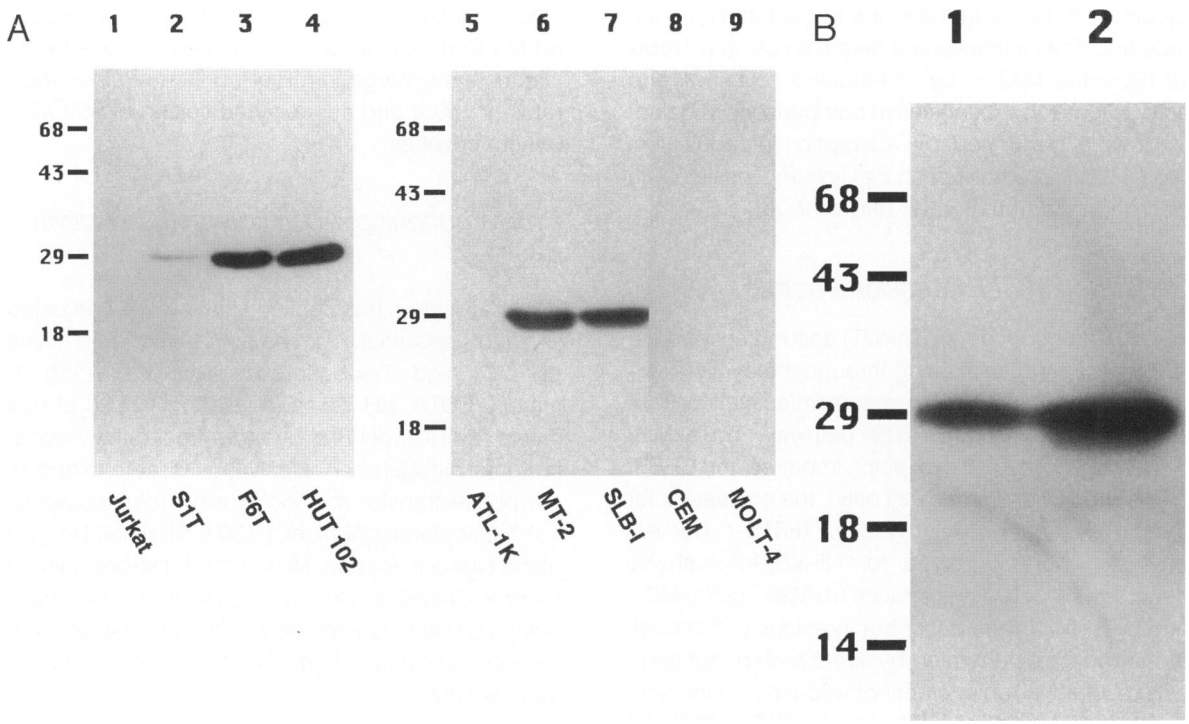

Figure 1. *Expression of galectin-3 in human T cells infected with HTLV-I. Expression of galectin-3 in cells was measured by immunobloting of cell lysates using a rabbit anti-galectin-3 antibody and chemiluminescent or ^{125}I autoradiographic detection. Lysates containing 100 μg of protein were adsorbed on lactosyl-Sepharose 4B, resolved on 12.5% polyacrylamide, and transferred to polyvinylidene difluoride membranes. Galectin-3 was detected by serial incubations with rabbit anti-galectin-3, alkaline phosphatase, or ^{125}I-labeled secondary antibody and developed with AMPPD or autoradiography, respectively.* **A:** *Galectin-3 expression in human T cell lines.* **Lanes 1** *to* **9** *correspond to cell lines Jurkat, S1T, F6T, HUT 102, ATL-1K, MT-2, SLB-I, CEM, and MOLT 4, respectively.* **Lanes 1, 8,** *and* **9,** *uninfected cell extracts;* **lanes 2** *and* **5,** *nonproductive HTLV-I-infected cell extracts;* **lanes 3, 4, 6,** *and* **7,** *productive HTLV-I-infected cell extracts.* **B:** *Expression of galectin-3 in human thymocytes after infection with HTLV-I.* **Lanes 1** *and* **2** *correspond to uninfected and HTLV-I-infected thymocytes, respectively. Each* **lane** *represents the galectin-3 present in 100 μg of total protein from each lysate. These results are representative of more than two experiments.*

number of uninfected and HTLV-I-infected T lymphocytic cell lines for galectin-3 expression by immunoblot. As shown in Figure 1A, significant amounts of galectin-3 were present in HTLV-I-infected F6T, HUT 102, MT-2, and SLB-I cell lines. In contrast, galectin-3 was not detectable in the uninfected cell lines Jurkat, CEM, and MOLT-4 (Figure 1A) nor in Jurkat E6-1 and CEM cells activated by phytohemagglutinin and phorbol ester (data not shown). Isolated peripheral blood lymphocytes also contained only low levels of galectin-3 (data not shown).

The expression of galectin-3 appeared to generally correlate with the level of the HTLV-I viral gene product, as shown by almost undetectable or no galectin-3 in the nonproductively infected lines S1T and ATL-1K, respectively, which are infected with HTLV-I but do not express viral proteins.[38,39] Correlation of HTLV-I infection with galectin-3 expression was further demonstrated by significantly enhanced galectin-3 expression in human thymocytes after infection. The level of galectin-3 in HTLV-I-infected thymocytes was 4.7-fold greater than uninfected thymocytes, as determined by immunoblotting and densitometric analysis (Figure 1B).

Influence of HTLV-I Tax Expression Vectors on Activity of the Murine Galectin-3 Promoter

Productive infection of cells with HTLV-I results in the synthesis of the transactivating factor Tax, which up-regulates a number of cellular genes via effects on both CREB/ATF and NF-κB/Rel transcription factor pathways.[2,3] Each pathway is independent of the other and can be differentially activated by various mutants of HTLV-I Tax.[48] The roles of CREB/ATF and NF-κB/Rel in regulation of the galectin-3 gene can, therefore, be studied by co-transfection of the appropriate HTLV-I Tax expression vector and the galectin-3 promoter coupled to a CAT reporter gene. The influence of the HTLV-I Tax protein on the galectin-3 promoter was studied using the murine B cell line M12.4.5, which does not express galectin-3. As

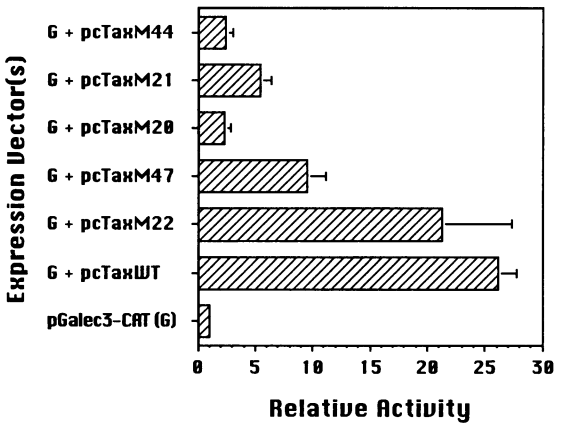

Figure 2. *Activation of the galectin-3 promoter by co-transfection with HTLV-I Tax expression vectors in murine B cells. The murine galectin-3 promoter was introduced upstream of a bacterial CAT gene to form the pGalec3-CAT expression vector. Co-transfection of M12.4.5 cells were performed by electroporation with pGalec3-CAT and HTLV-I Tax expression vectors pcTaxWT (wild type), pcTaxM22 (induces the CREB/ATF pathway), pcTaxM47 (induces the NF-κB/Rel pathway), and control vectors pcTaxM20, pcTaxM21, and pcTaxM44 that retain neither CREB/ATF nor NF-κB/Rel transactivating pathways. CAT activity was measured on day 2 by differential phase transfer using [dichloroacetyl-1,2-^{14}C]-chloramphenicol and n-butylcoenzyme A. A 20-μg amount of each vector was used to transfect 4 × 10^6 cells. All values were corrected for basal CAT activity reflective of transfection with the promoter-less CAT expression vector and are expressed relative to pGalec3-CAT alone (unity). Data are means of duplicate transfections, shown with error bars indicating the high value. These results are representative of two experiments.*

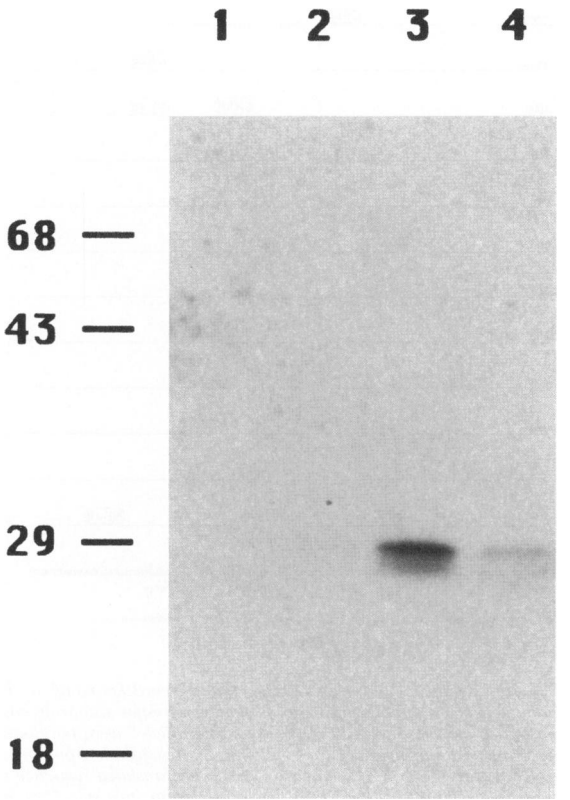

Figure 3. *Induction of galectin-3 expression in U-937 cells by transfection with an HTLV-I Tax expression vector. The human promonocytic cell line U-937 was transfected with the wild-type HTLV-I Tax expression vector (pcTaxWT) or control Tax expression vector and assessed for galectin-3 expression 2 days later. Galectin-3 in the cell lysates was detected as described in Figure 1.* Lane 1, *extracts from untransfected U-937 cells;* lane 2, *extracts from cells that were sham transfected;* lane 3, *extracts from cells transfected with pcTaxWT;* lane 4, *extracts from cells transfected with control vector. In each transfection, 4 × 10^6 cells were used and electroporations were performed with 40 μg of DNA. These results are representative of two experiments.*

demonstrated by the CAT activity in Figure 2, the galectin-3 promoter was considerably up-regulated by the wild-type Tax expression vector (pcTaxWT), which is known to stimulate both CREB/ATF and NF-κB/Rel transcription factor pathways.[48] In contrast, control vectors pcTaxM20, pcTaxM21, and pcTaxM44, which fail to induce through either pathway, induced only low levels of CAT activity. The pcTaxM22 and pcTaxM47 mutant vectors, containing CREB/ATF-inducible and NF-κB/Rel-inducible activities, respectively,[48] were also able to up-regulate the galectin-3 promoter. pcTaxM22 was almost as effective as pcTaxWT in up-regulating the galectin-3 promoter, whereas pcTaxM47 was less effective.

Induction of Galectin-3 Expression in Human Promonocytic U-937 Cells

In an effort to investigate the influence of Tax on the galectin-3 promoter under permissive conditions in the native state, the wild-type HTLV-I Tax expression vector was transfected into the human promonocytic line U-937. These cell types are known to express galectin-3 upon differentiation and are thus suitable for this purpose.[55] As shown in Figure 3, galectin-3 expression was initiated in U-937 cells with the wild-type Tax expression vector. The control Tax expression vector pcTaxM44, in which both CREB/ATF and NF-κB/Rel pathways are inactive, induced galectin-3 expression only marginally over the sham transfection in U-937 cells. The negligible leakiness in galectin-3 expression relative to untransfected cells may be due to unrelated stimuli imparted to U-937 cells merely by introduction of the DNA.

Potential CREB/ATF and NF-κB/Rel Binding Sites in the Mouse Galectin-3 Promoter

Possible CREB/ATF and NF-κB/Rel binding sites within the mouse galectin-3 promoter[50] were identified by homology search (Figure 4). The exon 1 sequence depicted was obtained from the cDNA sequence of murine galectin-3,[16] and several appropriately positioned motifs relative to exon 1 appear in the promoter. Each site shown differs from the clas-

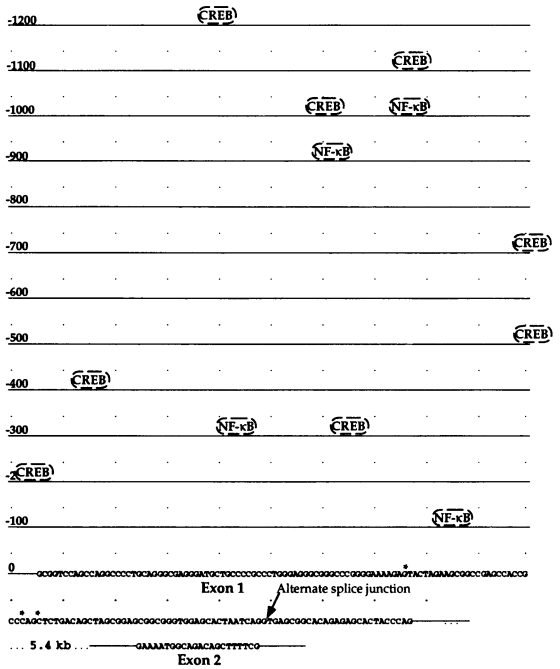

Figure 4. *Relative positions of CREB/ATF and NF-κB/Rel motifs in the promoter region of galectin-3. Motifs closely corresponding (one base mismatch) to those for CREB/ATF and NF-κB/Rel*[54] *were positioned according to murine galectin-3 genomic DNA sequence from Gritzmacher et al.*[50] *Exon 1 is based on the 5′-untranslated sequence of murine galectin-3 cDNA,*[16] *and the transcription start sites (*) were from Rosenberg et al.*[56] *This apparent divergence in observed transcription start sites may be due to the absence of a TATA consensus in this promoter.*[50,56] *The alternate splice junction in exon 1 was described in Gritzmacher et al.*[50]

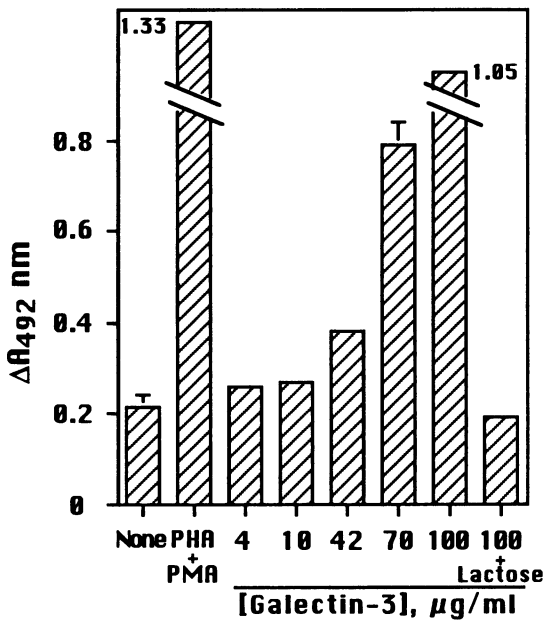

Figure 5. *Activation of Jurkat E6-1 cells by galectin-3. IL-2 secreted by Jurkat E6-1 cells incubated with various activation factors was determined with the IL-2-dependent murine cell line CTLL-2 as described in Materials and Methods using the formazan method to measure proliferation rates. phytohemagglutinin and PMA were used in cultures at 1 μg/ml and 50 ng/ml, respectively. The lectin activity of galectin-3 was inhibited with the specific saccharide lactose at 25 mmol/L. The data represent means ± SD of triplicate measurements. Similar results were obtained in two separate experiments.*

sical enhancers by a single mismatch. Also shown are likely transcription start sites[56] and an alternate exon 1/exon 2 splice junction.[50]

Activation of Jurkat E6-1 Cells by Galectin-3

A consequence of galectin-3 expression in T lymphocytes is that this lectin may activate the cells in an autocrine fashion by binding and cross-linking appropriately glycosylated cell surface receptors through mechanisms similar to mitogenic activities of plant lectins. As shown in Figure 5, galectin-3 activated Jurkat E6-1 cells in a dose-dependent manner, as determined by IL-2 secretion from treated cells. Significant activation was observed with galectin-3 at 42 μg/ml ($P \leq 0.0091$, paired *t*-test), and galectin-3 at 100 μg/ml induced activation to an extent similar to that observed with the combination of phytohemagglutinin and PMA. The activation was inhibited by lactose, suggesting that the action of galectin-3 is dependent on its lectin function. Unlike the plant lectin phytohemagglutinin, induction of IL-2 secretion resulting from activation of Jurkat cells by galectin-3 was not dependent on concurrent activation by PMA. No additional activation was observed when Jurkat cells were treated with both galectin-3 and PMA (data not shown). Galectin-3 was not mitogenic to responder CTLL-2 cells at concentrations tested for the activation of Jurkat E6-1 cells. This suggests that the responses observed in the CTLL-2 cells did not result from soluble galectin-3 transferred with medium from the Jurkat cells cultured with the lectin.

Discussion

We concluded from this study that HTLV-I infection of T cells leads to a marked increase in galectin-3 gene expression resulting from transactivation by the viral Tax protein. The results obtained with the mutant Tax expression vectors allowed us to conclude that the activation of the galectin-3 gene by the Tax protein independently involves primarily the CREB/ATF and, to a lesser extent, the NF-κB/Rel transcription factor pathways. Inspection of the 5′-flanking sequence of murine galectin-3 genomic DNA, which we previously cloned,[50] in fact revealed a number of potential enhancer elements quite similar to CREB/ATF and NF-κB/Rel binding sites (Figure 4). CREB/ATF alone appears to constitute full activity when compared

with the Tax wild-type expression vector, suggesting a major role for this transcription factor pathway. It is also possible that an additional pathway is recruited by CREB/ATF for activation of the galectin-3 promoter. Lower activation of the galectin-3 promoter by NF-κB/Rel suggests that this pathway may be of lesser significance.

Our finding that up-regulation of galectin-3 expression occurs in HTLV-I-infected T cells convincingly demonstrates that the expression of this protein can be influenced by viral infection. Of interest is the previous observation by Moutsatsos et al[19] that 3T3 cells transformed with the Kirsten murine sarcoma virus expressed much higher levels of galectin-3 when compared with uninfected cells. Induction of galectin-3 expression by viral influence is likely to be significant because existing information on galectin-3 suggests that expression of galectin-3 in some cells may in fact contribute to the transformed phenotype in tumor cells. It has been reported that transfection of 3T3 fibroblasts with galectin-3 cDNA resulted in cells exhibiting morphology of transformed cells, ie, loss of contact inhibition, anchorage-independent growth in agar, and disruption of structural microfilament organization.[21] In addition, fibrosarcoma cells transfected with the galectin-3 cDNA, and thus expressing elevated levels of this lectin, showed a significantly enhanced ability to colonize in lungs of mice infused with these cells, suggesting that this lectin is capable of conferring the metastatic phenotype.[21]

Galectin-3 expression may be closely related to cell growth. Increased levels of this lectin are found in proliferating fibroblasts, and the protein shows prominent nuclear localization in proliferating cells.[19] As infection with HTLV-I leads to spontaneous proliferation of T cells,[3] it is possible that elevated expression of galectin-3 may be linked to this proliferative response. A possible mechanism by which cells are induced to proliferate by galectin-3 is by autocrine stimulation of susceptible receptors on the cell surface. Although no conventional signal sequence is present in cDNA coding for galectin-3, secretion has been demonstrated from a variety of cell types,[6] and it would be expected that the lectin would be secreted from T cells in a similar manner. Galectin-3 is capable of activating monocytes/macrophages,[57] mast cells,[58] and neutrophils by interacting with cell surface glycoproteins.[59] This study now demonstrates that galectin-3 is also capable of activating T lymphocytes. As a factor able to participate in autocrine stimulation in T cells, galectin-3 may be compared to the up-regulation of IL-2/IL-2 receptor by Tax[60] as early events contributing to cell proliferation resulting from HTLV-I infection.[61,62] In fact, we have recently observed in gene transfection experiments that galectin-3 expression in leukemic T cells results in an enhanced rate of cell growth (R.-Y. Yang, D. K. Hsu, and F.-T. Liu, manuscript submitted). The beneficial roles of galectin-3 to tumor cell growth suggests that expression of this lectin may indeed contribute to pathogenesis in individuals infected with HTLV-I.

Significantly, the finding that up-regulation of galectin-3 expression may occur through CREB/ATF and NF-κB/Rel transcription factor pathways, or alternatively through activation of a secondary pathway resulting from activation of the above, provides insights into how expression of this member of the novel multifunctional animal lectins might be regulated. This information also suggests possible factors/conditions that lead to the induction of this protein. Other viruses may up-regulate galectin-3, as many viral transactivating factors are known to activate the NF-κB/Rel pathway.[63] In addition, NF-κB/Rel was proposed to be a signal transducer in the immediate early phases of immune responses,[64] and it can be induced by various stimuli, including ultraviolet radiation[65] and oxidative processes.[64] Thus, it is possible that galectin expression is influenced under these conditions.

In summary, this study allowed identification of mechanisms by which galectin-3 expression is regulated and thus provided a basis for further delineation of regulatory mechanisms for expression of this lectin in various other cell types. The study also yielded new information on HTLV-I infection. Additional studies of the function of galectin-3 in HTLV-I-infected T lymphocytes, including the identification of cell surface glycoconjugate ligands, may provide important insights into the pathogenesis of HTLV-I infection.

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References

1. Masucci MG: Viral immunopathology of human tumors. Curr Opin Immunol 1993, 5:693–700
2. Smith MR, Greene WC: Molecular biology of the type I

human T-cell leukemia virus (HTLV-I) and adult T-cell leukemia. J Clin Invest 1991, 87:761–766
3. Hjelle B: Human T-cell leukemia/lymphoma viruses. Arch Pathol Lab Med 1991, 115:440–450
4. Gallo RC: Human retroviruses: a decade of discovery and link with human disease. J Infect Dis 1991, 164: 235–243
5. Barondes SH, Castronovo V, Cooper DNW, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai K, Leffler H, Liu F-T, Lotan R, Mercurio AM, Monsigny M, Pillai S, Poirer F, Raz A, Rigby PWJ, Rini JM, Wang JL: Galectins: a family of animal β-galactoside-binding lectins. Cell 1994, 76:597–598
6. Barondes SH, Cooper DNW, Gitt MA, Leffler H: Galectins: structure and function of a large family of animal lectins. J Biol Chem 1994, 269:20807–20810
7. Liu F-T, Albrandt K, Mendel E, Kulczycki A Jr, Orida NK: Identification of an IgE-binding protein by molecular cloning. Proc Natl Acad Sci USA 1985, 82:4100–4104
8. Albrandt K, Orida NK, Liu F-T: An IgE-binding protein with a distinctive repetitive sequence and homology with an IgG receptor. Proc Natl Acad Sci USA 1987, 84:6859–6863
9. Robertson MW, Albrandt K, Keller D, Liu F-T: Human IgE-binding protein: a soluble lectin exhibiting a highly conserved interspecies sequence and differential recognition of IgE glycoforms. Biochemistry 1990, 29: 8093–8100
10. Roff CF, Wang JL: Endogenous lectins from cultured cells: isolation and characterization of carbohydrate-binding proteins from 3T3 fibroblasts. J Biol Chem 1983, 258:10657–10663
11. Jia S, Wang JL: Carbohydrate binding protein 35: complementary DNA sequence reveals homology with proteins of the heterogeneous nuclear RNP. J Biol Chem 1988, 263:6009–6011
12. Mehul B, Bawumia S, Martin SR, Hughes RC: Structure of baby hamster kidney carbohydrate-binding protein CBP30, an S-type animal lectin. J Biol Chem 1994, 269:18250–18258
13. Cherayil BJ, Weiner SJ, Pillai S: The Mac-2 antigen is a galactose-specific lectin that binds IgE. J Exp Med 1989, 1972, 170:1959
14. Cherayil BJ, Chaitovitz S, Wong C, Pillai S: Molecular cloning of a human macrophage lectin specific for galactose. Proc Natl Acad Sci USA 1990, 87:7324–7329
15. Leffler H, Masiarz FR, Barondes SH: Soluble lactose-binding vertebrate lectins: a growing family. Biochemistry 1989, 28:9222–9229
16. Raz A, Pazerini G, Carmi P: Identification of the metastasis-associated, galactoside-binding lectin as a chimeric gene product with homology to an IgE-binding protein. Cancer Res 1989, 49:3489–3493
17. Raz A, Carmi P, Raz T, Hogan V, Mohamed A, Wolman SR: Molecular cloning and chromosomal mapping of a human galactoside-binding protein. Cancer Res 1991, 51:2173–2177
18. Liu F-T: Molecular biology of IgE-binding protein, IgE-binding factors and IgE receptors. CRC Crit Rev Immunol 1990, 10:289–306
19. Moutsatsos IK, Wade M, Schindler M, Wang JL: Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. Proc Natl Acad Sci USA 1987, 84: 6452–6456
20. Dagher SF, Wang JL, Patterson RJ: Identification of galectin-3 as a factor in pre-mRNA splicing. Proc Natl Acad Sci USA 1995, 92:1213–1217
21. Raz A, Zhu D, Hogan V, Shah N, Raz T, Karkash R, Pazerini G, Carmi P: Evidence for the role of 34-kDa galactoside-binding lectin in transformation and metastasis. Int J Cancer 1990, 46:871–877
22. Sato S, Hughes RC: Binding specificity of a baby hamster kidney lectin for H type I and II chains, polylactosamine glycans, and appropriately glycosylated forms of laminin and fibronectin. J Biol Chem 1992, 267:6983–6990
23. Woo H-J, Shaw LM, Messier JM, Mercurio AM: The major non-integrin laminin binding protein of macrophage is identical to carbohydrate binding protein 35 (Mac-2). J Biol Chem 1990, 265:7097–7101
24. Sato S, Burdett I, Hughes RC: Secretion of the baby hamster kidney 30-kDa galactose-binding lectin from polarized and nonpolarized cells: a pathway independent of the endoplasmic reticulum-Golgi complex. Exp Cell Res 1993, 207:8–18
25. Lindstedt R, Apodaca G, Barondes SH, Mostov KE, Leffler H: Apical secretion of a cytosolic protein by Madin-Darby canine kidney cells: evidence for polarized release of an endogenous lectin by a nonclassical secretory pathway. J Biol Chem 1993, 268:11750–11757
26. Liu F-T: S-type mammalian lectins in allergic inflammation. Immunol Today 1993, 14:486–490
27. Truong M-J, Gruart V, Kusnierz J-P, Papin J-P, Loiseau S, Capron A, Capron M: Human neutrophils express immunoglobulin E (IgE)-binding proteins (Mac-2/εBP) of the S-type lectin family: role in IgE-dependent activation. J Exp Med 1993, 177:243–248
28. Truong M-J, Gruart V, Liu F-T, Prin L, Capron A, Capron M: Human eosinophils express functional IgE-binding molecules (Mac-2/εBP) of the S-type lectin family. Eur J Immunol 1993, 23:3230–3235
29. Flotte TJ, Springer TA, Thorbecke GJ: Dendritic cell and macrophage staining by monoclonal antibodies in tissue section epidermal sheets. Am J Pathol 1983, 111:112–124
30. Hsu DK, Zuberi R, Liu F-T: Biochemical and biophysical characterization of human recombinant IgE-binding protein, an S-type animal lectin. J Biol Chem 1992, 267:14167–14174
31. Frigeri LG, Liu F-T: Surface expresson of functional IgE

binding protein, an endogenous lectin, on mast cells and macrophages. J Immunol 1992, 148:861–869
32. Karasuyama H, Melchers F: Establishment of mouse cell lines which constitutively secrete large quantities of interleukin 2, 3, 4 or 5, using modified cDNA expression vectors. Eur J Immunol 1988, 18:97–104
33. Arima N, Molitor JA, Smith MR, Kim JH, Daitoku Y, Greene WC: Human T-cell leukemia virus type I Tax induces expression of the Rel-related family of κ-B enhancer-binding proteins: evidence for a pretranslational component of regulation. J Virol 1991, 65:6892–6899
34. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC: Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 1980, 77:7415–7419
35. Miyoshi I, Kubonishi I, Yoshimoto S, Akagi T, Ohtsuki Y, Shiraichi Y, Nagata K, Hinuma Y: Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukaemic T cells. Nature 1981, 294:770–771
36. Koeffler HP, Chen ISY, Golde DW: Characterization of a novel HTLV-infected cell line. Blood 1984, 64:482–490
37. Nyborg JK, Matthews M-AH, Yucel J, Walls L, Golde WT, Dynan WS, Wachsman W: Interaction of host cell proteins with the human T-cell leukemia virus type I transcriptional control region. J Biol Chem 1990, 265: 8237–8242
38. Hoshino H, Esumi H, Miwa M, Shimoyama M, Minato K, Tobinai K, Hirose M, Watanabe S, Inada N, Kinoshita K, Kamihira S, Ichimaru M, Sugimura T: Establishment and characterization of 10 cell lines derived from patients with adult T-cell leukemia. Proc Natl Acad Sci USA 1983, 80:6061–6065
39. Mills GB, Arima N, May C, Hill M, Schmandt R, Li J, Miyamoto NG, Greene WC: Neither the LCK nor the FYN kinases are obligatory for IL-2-mediated signal transduction in HTLV-I-infected human T cells. Int Immunol 1992, 4:1233–1243
40. Hamano T, Kim JK, Leiserson WM, Asofsky R: Establishment of B cell hybridomas with B cell surface antigens. J Immunol 1982, 129:1403–1406
41. Sundström C, Nilsson K: Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int J Cancer 1976, 17:565–577
42. Grassmann R, Dengler C, Muller-Fleckenstein I, Fleckenstein B, McGuire K, Dokhelar M-C, Sodroski JG, Haseltine WA: Transformation to continuous growth of primary human T lymphocytes by human T-cell leukemia virus type I X-region genes transduced by a herpesvirus saimiri vector. Proc Natl Acad Sci USA 1989, 86:3351–3355
43. Weiss A, Wiskocil RL, Stobo JD: The role of T3 surface molecules in the activation of human T cells: a two-stimulus requirement for IL-2 production reflects events occurring at a pre-translational level. J Immunol 1984, 133:123–128
44. Liu F-T, Orida N: Synthesis of surface immunoglobulin E receptor in *Xenopus* oocytes by translation of mRNA from rat basophilic leukemia cells. J Biol Chem 1984, 259:10649–10652
45. Levi G, Teichberg VI: Isolation and physicochemical characterization of electrolectin, a β-D-galactoside binding lectin from the electric organ of *Electrophorus electricus*. J Biol Chem 1981, 256:5735–5740
46. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970, 227:680–684
47. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979, 76:4350–4354
48. Smith MR, Greene WC: Identification of HTLV-I tax trans-activator mutants exhibiting novel transcriptional phenotypes. Genes Dev 1990, 4:1875–1885
49. Smith MR, Greene WC: Erratum: identification of HTLV-I tax trans-activation mutants exhibiting novel transcriptional phenotypes. Genes Dev 1995, 9:2324
50. Gritzmacher CA, Mehl VS, Liu F-T: Genomic cloning of the gene for an IgE-binding lectin reveals unusual utilization of 5′ untranslated regions. Biochemistry 1992, 31:9533–9538
51. Kubiniec RT, Liang H, Hui SW: Effects of pulse length and pulse strength on transfection by electroporation. Biotechniques 1990, 8:16–20
52. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, Albright LM, Coen DM, Varki A, Janssen K: Current Protocols in Molecular Biology. New York, Current Protocols, John Wiley and Sons, 1994
53. Devereux J, Haeberli P, Smithies O: A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res 1984, 12:387–395
54. Ghosh D: Status of the transcription factors database (TFD). Nucleic Acids Res 1993, 21:3117–3118
55. Nangia-Makker P, Ochieng J, Christman JK, Raz A: Regulation of the expression of galactoside-binding lectin during human monocytic differentiation. Cancer Res 1993, 53:1–5
56. Rosenberg IM, Iyer R, Cherayil B, Chiodino C, Pillai S: Structure of the murine Mac-2 gene: splice variants encode proteins lacking functional signal peptides. J Biol Chem 1993, 268:12393–12400
57. Liu F-T, Hsu DK, Zuberi RI, Kuwabara I, Chi EY, Henderson WR Jr: Expression and function of galectin-3, a β-galactoside-binding lectin, in human monocytes and macrophages. Am J Pathol 1995, 147:1016–1029
58. Frigeri LG, Zuberi RI, Liu F-T: εBP, a β-galactoside-binding animal lectin, recognizes IgE receptor (FcεRI) and activates mast cells. Biochemistry 1993, 32:7644–7649
59. Yamaoka A, Kuwabara I, Frigeri LG, Liu F-T: Human soluble lectin εBP (IgE-binding protein) stimulates superoxide production by neutrophils. J Immunol 1995, 154:3479–3487
60. Siekevitz M, Feinberg MB, Holbrook N, Wong-Staal F,

Greene WC: Activation of interleukin 2 and interleukin 2 receptor (Tac) promoter expression by the trans-activator (tat) gene product of human T-cell leukemia virus, type I. Proc Natl Acad Sci USA 1987, 84:5380–5393

61. Maeda M, Arima N, Daitoku Y, Kashihara M, Okamoto H, Uchiyama T, Shirono K, Matsuoka M, Hattori T, Takasuki K, Ikuta K, Shimizu A, Honjo T, Yodoi J: Evidence for the interleukin-2 dependent expansion of leukemic cells in adult T cell leukemia. Blood 1987, 70:1407–1411
62. Hollsberg P, Wucherpfennig KW, Ausubel LJ, Calvo V, Bierer BE, Hafler DA: Characterization of HTLV-I *in vivo* infected T cell clones: IL-2-independent growth of nontransformed T cells. J Immunol 1992, 148: 3256–3263
63. Baeuerle PA: The inducible transcription activator NF-kB: regulation by distinct protein subunits. Biochim Biophys Acta 1991, 1072:63–80
64. Schreck R, Albermann K, Baeuerle PA: Nuclear factor κ-B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). Free Radical Res Commun 1992, 17:221–237
65. Brach MA, Hass R, Sherman ML, Gunji H, Weichselbaum R, Kufe D: Ionizing radiation induces expression and binding activity of the nuclear factor kB. J Clin Invest 1991, 88:691–695