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Short Communication

Galectin-3, a β-Galactoside-Binding Animal Lectin, Is a Marker of Anaplastic Large-Cell Lymphoma

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Galectin-3 is a member of a newly named family of β-galactoside-binding animal lectins, which has been described with a number of possible important biological functions, including the regulation of cell growth and association with tumor transformation. This protein has a wide tissue distribution but is notably not expressed by normal lymphocytes. We have previously shown that galectin-3 is markedly up-regulated in HTLV-I-infected T cells, most likely mediated by the viral transactivating protein Tax. In this study, we surveyed various lymphomas by immunohistochemistry and found the expression of galectin-3 in all of the 8 cases of Ki-1$^+$ anaplastic large-cell lymphoma (ALCL). Immunoreactivity for galectin-3 was found in a majority of the neoplastic cells in the ALCLs studied. In contrast, only 2 of the 35 cases of other types of lymphoma, including various Hodgkin's and non-Hodgkin's lymphomas, were positive. Unlike the cases of ALCL, immunoreactivity for galectin-3 in these 3 cases was found only sporadically in a small number of neoplastic cells. Thus, galectin-3 may prove to be a useful marker for ALCL and its expression in neoplastic cells in ALCL may contribute to the biological behavior of this specific type of lymphoma. (Am J Pathol 1996, 148:25–30)

Our understanding of the biology of lymphoma and its classification continues to benefit from identification of molecular markers that are selectively expressed by specific subtypes of lymphoma. We report in this communication the finding that the Ki-1$^+$ anaplastic large-cell lymphoma (ALCL) can be differentiated from other lymphomas by its expression of an endogenous lectin, galectin-3.

Animal lectins are being recognized as molecules that play important roles in a variety of biological processes through binding to glycoconjugates.[1,2] Galectins are members of a newly named growing family of β-galactoside-binding animal lectins[3,4] with galectin-3 being the most extensively studied member, which has been previously designated as εBP,[5–7] Mac-2,[8,9] CBP35,[10,11] CBP30,[12] L-29,[13] and L-34.[14] This M_r 30,000 protein has been cloned and sequenced from different animal species and is apparently composed of two domains; the amino-terminal half consists primarily of tandem repeats and the carboxyl-terminal half represents the carbohydrate-recognition domain.[15] Studies from a number of laboratories have suggested that galectin-3 might have a wide range of functions, including regulation of cell growth,[16] promotion of cell adhesion,[17,18] and amplification of inflammatory responses.[19,20]

The relationship between galectin-3 expression and neoplastic transformation is particularly noteworthy. Although this protein has a wide tissue distribution and is expressed constitutively by a variety of cells, examples have been accumulated that its

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expression is up-regulated in neoplastic transformation. It has been found that galectin-3 is expressed in elevated levels in various neoplastic cells[21] and that virally transformed mouse 3T3 cells express much higher levels of galectin-3 as compared with the untransformed cells.[16]

We have recently found that, although galectin-3 is lowly expressed in normal lymphocytes and not detected in a number of lymphoma cell lines, its expression is markedly up-regulated in HTLV-I-infected T cell lines (D. K. Hsu, S. R. Hammes, W. C. Greene, and F.-T. Liu, manuscript submitted for publication). This finding prompted us to survey the expression of galectin-3 in various lymphomas by immunohistochemistry and we report here that, among a variety of human lymphomas tested, only ALCL was found to consistently express this lectin in the neoplastic cells.

Table 1. *Galectin-3 Immunoreactivity in Lymphoproliferative Disorders*

Lymphoma type	Number of cases	
	Total	Galectin-3$^+$ (neoplastic cells)
CD30/Ki-1$^+$ ALCL	8	8
CTCL/mycosis fungoides	7	0
Peripheral T-cell lymphoma	2	1*
B cell lymphoma	17	1*
Small lymphocytic	2	0
Small cleaved	3	0
Follicular mixed	1	0
Follicular large cell	2	1*
Diffuse large cell	4	0
B-immunoblastic	5	0
Hodgkin's disease	7	0
Lymphocyte predominant	1	0
Mixed cellular	3	0
Nodular sclerosing	3	0
Lymphomatoid papulosis	1	0
Hairy cell leukemia	1	0

*Only a small percentage of the larger neoplastic cells were galectin-3 positive. CTCL, cutaneous T-cell lymphoma.

Materials and Methods

Patients

Normal and pathological tissues were retrieved from the Department of Pathology, Scripps Clinic. For immunohistochemistry with the polyclonal antibody, formalin-fixed and paraffin-embedded specimens were used and for immunohistochemistry with the monoclonal antibody, B5-fixed ones were used. All lymphoma cases were diagnosed according to the established criteria and were immunohistochemically characterized with a panel of appropriate monoclonal antibodies. Benign tissue sections used as controls for immunohistochemical stains included histologically normal tonsils, lymph nodes, thymus, and skin tissues. Cases studied, as listed in Table 1, included CD30/Ki-1$^+$ ALCLs, cutaneous T-cell lymphomas, peripheral T cell lymphomas, B cell non-Hodgkin's lymphomas, Hodgkin's disease, lymphomatoid papulosis, and hairy cell leukemia. The description of all cases of ALCL is summarized in Table 2.

Antibodies

Polyclonal rabbit anti-galectin-3 antibody was raised against recombinant human galectin-3[22] and affinity purified on galectin-3-coupled Sepharose 4B, using a previously described procedure.[18] The monoclonal anti-galectin-3 antibody B2C10 is described elsewhere (F.-T. Liu, D. K. Hsu, R. I. Zuberi, A. Shanhav, P. N. Hill, and I. Kuwabara, manuscript submitted for publication).

Immunohistochemistry

Immunohistochemical staining for galectin-3 was performed by the biotin-streptavidin method as previously described.[23] Briefly, sections were deparaffinized and treated with phosphate-buffered saline solution (pH 7.35). Endogenous peroxidase was blocked with 3% H_2O_2. Sections were pretreated with normal goat serum and incubated for 1 hour at room temperature with the primary antibody (rabbit polyclonal or mouse monoclonal) at a concentration of 10 μg/ml. Immunohistochemical staining was performed with a biotin-streptavidin-amplified detection system (Stravigen, BioGenex Laboratories, San Ramon, CA). Diaminobenzidine was used as chromogen substrate. Control slides were stained through substitution of the primary antibody with the IgG fraction of normal rabbit serum or isotype-matched irrelevant monoclonal antibody or incubation of the sections with the antibodies of the amplifying system alone.

Table 2. *Clinical Characterization of Patients with ALCL*

Age	Sex	Location	Phenotype
77	M	Soft tissue of the arm	Null*
43	F	Inguinal lymph node	Null*
40	F	Inguinal lymph node	T cell
37	M	Lymph node	T cell
39	M	Skin	T cell
53	M	Skin	T cell
76	M	Lymph node	B cell
71	M	Axillary lymph node	Mixed T and B cells

*No evidence of T or B cell differentiation on paraffin section immunohistochemistry.

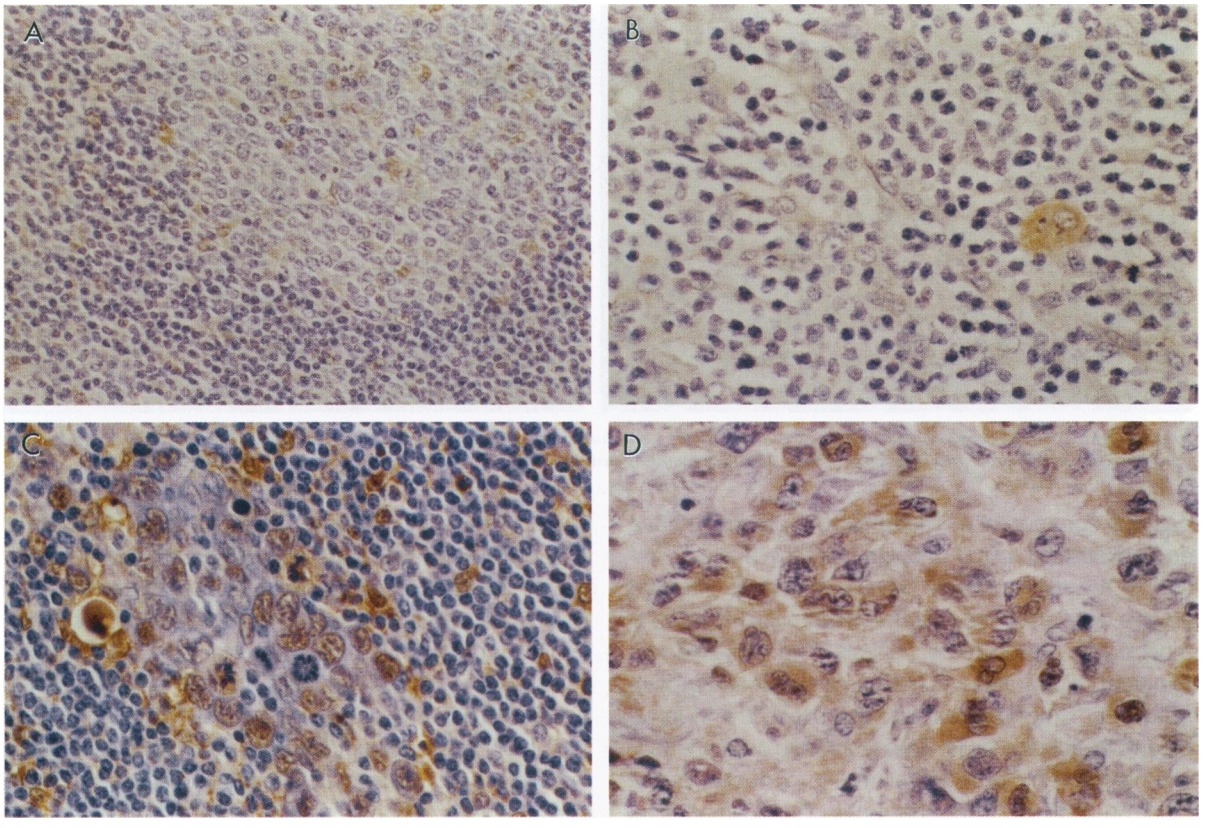

Figure 1. *Immunohistochemistry of normal tissues and lymphomas with anti-galectin-3 polyclonal antibodies.* **A:** *Normal tonsil.* **B:** *Follicular mixed lymphoma.* **C** *and* **D:** *ALCL. Macrophages and dendritic cells in the tonsil, macrophages in follicular mixed lymphoma, and neoplastic cells in ALCL are positively stained.*

Results

We found galectin-3 immunoreactivity in macrophages and dendritic cells in tonsils (Figure 1A). Both cytoplasmic and nuclear staining was present in positive cells. Epithelial cells in tonsils also showed positive staining (data not shown). Lymphocytes were predominantly negative, with only rare small lymphocytes exhibiting nuclear staining for galectin-3.

Of the various lymphomas surveyed, we found galectin-3 immunoreactivity in neoplastic cells in all of the 8 cases of ALCL studied. None of the 7 cases of Hodgkin's disease was positive and only 2 of the 26 cases of non-Hodgkin's lymphomas were positive, including 1 of the 2 cases of peripheral T-cell lymphoma and 1 of the 2 cases of follicular large cell lymphoma (Table 1). In contrast to immunoreactivity found in ALCL, in which a majority of neoplastic cells were positive for galectin-3, immunoreactivity to galectin-3 in the other 2 cases was seen in only a small percentage of the neoplastic cells. Figure 1B shows an example of galectin-3-negative lymphomas, in which only macrophages were stained positively. In all of the cases of ALCL studied, the immunoreactivity was found in the characteristic large neoplastic cells as well as scattered histiocytes (Figure 1, C and D). There was heterogeneity in staining intensity among neoplastic cells and prominent nuclear staining as well as cytoplasmic staining was observed in many of the tumor cells. The cells positive for galectin-3 were also positive for CD30 (data not shown).

To confirm the findings made with the polyclonal antibody, we performed immunohistochemistry using a newly developed anti-galectin-3 monoclonal antibody on selected cases of ALCL. Specific staining of macrophages and dendritic cells in tonsils was observed (Figure 2A). The results with ALCL samples were similar to those obtained using the polyclonal antibodies, with the neoplastic cells showing strong staining (Figure 2B). Heterogeneity in staining intensity of the neoplastic cells was also observed.

Discussion

ALCL is a recently described lymphoma subtype (reviewed in Ref. 24). The clinical features of this lymphoma are unusual and include a young median age and frequent extranodal manifestation with skin

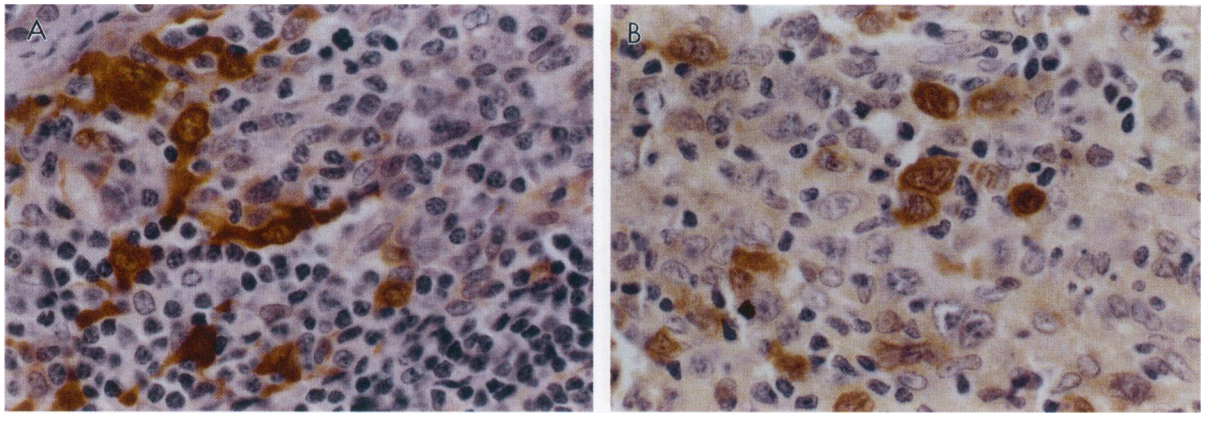

Figure 2. *Immunohistochemistry of normal tonsil and ALCL with monoclonal anti-galectin-3 antibody.* **A:** *Normal tonsil.* **B:** *ALCL. Macrophages and dendritic cells in the tonsil and neoplastic cells in ALCL are positively stained.*

being a common site. These tumors generally have an aggressive clinical behavior, but the disease limited to the skin tends to have a slower, protracted course, tendency to spontaneous remissions, and, in most cases, favorable prognosis. The classical histological appearance of ALCL is a sinusoidal growth pattern in lymph nodes and the presence of large bizarre anaplastic cells. The neoplastic cells are usually pleomorphic and contain reniform nuclei, prominent nucleoli, and abundant cytoplasm (anaplastic cytology). Multinucleated cells are also commonly observed and some tumor cells may resemble Reed-Sternberg cells. Because of its pleomorphic appearance, sinus distribution, and frequent reactivity with epithelial membrane antigen, ALCL is often mistaken for other diseases such as metastatic carcinoma and malignant histiocytosis. Immunophenotypically, most ALCLs have an aberrant $CD4^+$ T cell phenotype with variable loss of pan-T cell antigens. In addition to epithelial membrane antigen, ALCL consistently reacts strongly with antibodies Ki-1 and Ber-H2, which recognize the CD30 antigen, hence the designation CD30/$Ki\text{-}1^+$ ALCL.

One possible explanation for the preferential expression of galectin-3 in ALCL is that this lymphoma contains histiocytic features and thus the expression of galectin-3, which is known to be expressed by macrophages.[9] Indeed, Carbone et al[25] studied 20 cases of $Ki\text{-}1^+$ ALCL and found 2 cases in which histiocyte-associated antigens were also expressed. They concluded that this group of neoplasms have immunophenotypic heterogeneity. In our study, however, we found that all of the 8 cases of ALCL studied were positive for galectin-3. It thus seems unlikely that in all of these cases the neoplastic cells have histiocytic features.

Our previous observation of the up-regulation of galectin-3 in HTLV-I-infected T cells (D. K. Hsu, S. R. Hammes, W. C. Greene, and F.-T. ILiu, manuscript submitted for publication) suggests another attractive possibility that the expression of galectin-3 in ALCL may be uniquely related to HTLV-I being an etiological agent for this particular type of lymphoma. Indeed, Anagnostopoulos et al[26] have detected HTLV-I proviral sequences in all of six cases of $CD30^+$ large-cell cutaneous T-cell lymphoma but in none of four cases of small-cell cutaneous T-cell lymphoma. Similarly, Takimoto et al[27] reported the detection of clonal integration of HTLV-I proviral DNA in a patient with ALCL. It is interesting that transformation of mycosis fungoides to $CD30^+$ large-cell lymphoma has been reported[28] and evidence is emerging that cutaneous T-cell lymphoma is an HTLV-I-associated disease.[29,30] It is to be noted, however, that the histological features were those of the immunoblastic variant. Nevertheless, a small percentage of cells did have the cytological features of ALCL.[28] A patient with mycosis fungoides who had $Ki\text{-}1^+$ cells in the skin lesions and enlarged lymph nodes with the histology of $Ki\text{-}1^+$ ALCL has also been described.[31]

Based on our knowledge of galectin-3, we can make certain speculations about the relationship between its expression and the biology of lymphomas. The expression of galectin-3 has been shown to be dramatically up-regulated in proliferating cells and the protein is concentrated in the nucleus in these cells.[16] Thus, galectin-3 may be involved in the regulation of cell growth. We have recently shown, by gene transfection experiments, that galectin-3 expression in leukemia T cells results in an enhanced growth rate of the transfected cells (R.-Y. Yang, D. K. Hsu, and F.-T. Liu, manuscript submitted for publication). It is interesting then that it is a recurrent finding in our immunohistochemical analysis that there is prominent staining of the nuclei in the neo-

plastic cells of ALCL. In addition, galectin-3 may be involved in neoplastic transformation and tumor metastasis; transfection of normal fibroblasts with galectin-3 cDNA resulted in acquisition of anchorage-independent growth and in morphological transformation.[32] Furthermore, transfection of weakly metastatic fibrosarcoma cells resulted in an increased incidence of experimental lung metastasis in experimental mice.[32]

The relationship between galectin-3 and CD30 is noteworthy. CD30, originally discovered as a surface antigen recognized by the Ki-1 monoclonal antibody on lymphoma cells and Reed-Sternberg cells in Hodgkin's disease, is considered to be an activation-associated lymphocyte antigen. *In vitro*, up-regulation of the CD30 antigen is observed in virus-transformed B and T lymphocytes (HTLV-I- or II-transformed T cells and Epstein-Barr virus-transformed B cells) or lectin-activated normal lymphocytes.[33] The coexpression of CD30 and galectin-3 in ALCL suggests that these two antigens may be under a similar control mechanism. In fact, as noted above, HTLV-I, which is one of the strongest inducers of CD30 expression in T cells,[34] also induces galectin-3, most likely through transactivation by viral Tax protein (D. K. Hsu, S. R. Hammes, W. C. Greene, and F.-T. Liu, manuscript submitted for publication). However, the expression patterns of galectin-3 and CD30 are not identical. For example, galectin-3 was not detected in Reed-Sternberg cells or variants in any of the Hodgkin's lymphomas we examined.

In summary, we have shown that, among a variety of lymphomas, ALCL neoplastic cells appear to be the only ones that consistently express galectin-3. Immunohistochemical detection of this lectin therefore appears to be useful for identification of this subtype of lymphoma. Additional studies may allow the development of a useful reagent for routine subtyping of lymphomas as well as a fuller understanding of the role of this particular animal lectin in the biological behavior of ALCL.

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