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## ***Brief Definitive Report***

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### EXPRESSION OF Lyt-1 ANTIGEN ON CERTAIN MURINE B CELL LYMPHOMAS\*

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The Lyt-1, Lyt-2, and Lyt-3 antigens, as originally defined by alloantisera, are expressed exclusively on T lymphocytes (1). Functional analysis of cells expressing these antigens has suggested that Lyt-1 is associated with helper/amplifier T cells, whereas Lyt-2 and Lyt-3 are markers of cytotoxic and suppressor T lymphocytes (1). Several recent findings suggest, however, that the situation may be more complex. Although these subpopulations can often be distinguished by cytotoxic depletion, flow cytometry (FCM) analysis has shown that the suppressor/cytotoxic T cell subset has Lyt-1, although in clearly lower amounts per cell than the Lyt-2<sup>-</sup>, 3<sup>-</sup> cells (2-4).

Recently, a series of monoclonal antibodies (M.Abs.) has been generated against murine cell surface antigens that react with the Thy-1, Lyt-1, Lyt-2, and Lyt-3.2 antigens (3, 4). Using these M.Abs., we have examined normal mouse lymphoid tissues and reported that a minor subpopulation of lymphocytes exists that is strongly Lyt-1 positive, but Thy-1 negative. When tissue sections of lymphoid tissues were examined by two-color immunofluorescence, cells expressing this phenotype were found in the germinal centers and primary follicles of the lymph nodes and spleen. Because these areas are typically considered to be the sites of B lymphocytes, this finding raised the question of whether a subpopulation of B cells exists that expresses Lyt-1 (4).

In this study, a series of murine B cell lymphomas was screened with various anti-T cell M.Abs., including anti-Lyt-1, using the fluorescence-activated cell sorter (FACS). These tumors, in general, appear to represent normal subpopulations of lymphoid cells arrested in discrete stages of differentiation (5). Thus, they provide the opportunity to examine the expression of antigens usually present on only minor subpopulations of cells in heterogeneous lymphoid tissues. As a result of these studies, we have discovered that otherwise typical B cell lymphomas may express the Lyt-1 antigen.

#### Materials and Methods

*Tumors.* A detailed characterization of the lymphomas used is described in detail elsewhere (6).<sup>1</sup>

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<sup>1</sup> Lanier, L. L., N. L. Warner, J. A. Ledbetter, and L. A. Herzenberg. Flow cytometry analysis of murine B cell lymphomas and plasmacytomas with xenogenic monoclonal antibodies. Manuscript in preparation.

*Antisera and M.Abs.* Rat anti-mouse M.Abs. directed against Thy-1.2 (30-H12 hybridoma), Lyt-1 (53-7.3 hybridoma cell line), Lyt-2 (53-6.7 hybridoma), and Lyt-3.2 (53-5.8 hybridoma) were described previously (3, 4). Fluorescein isothiocyanate (FITC) goat anti-mouse  $\mu$  heavy chain serum was obtained from Meloy Laboratories, Inc., Springfield, Va. FITC mouse (SJL) anti-rat Ig serum was prepared as described elsewhere.<sup>1</sup>

*Immunofluorescence and FCM.* Immunofluorescence was performed as described in detail elsewhere (7). Fluorescence was measured by FCM analysis using a FACS III (B-D FACS Systems, Mountain View, Calif.) (7, 8).

*Trypsin Stripping of Surface Antigens.* 10 million cells were suspended in 30 ml RPMI-1640 containing 10 mg/ml trypsin (type IX, from porcine pancreas, Sigma Chemical Co., St. Louis, Mo.). After incubation for 1 h at 37°C, the digestion was halted by the addition of 3 ml heat-inactivated fetal calf serum (HIFCS). The cells were washed twice in medium with 20% HIFCS and placed in medium with 20% HIFCS,  $10^{-4}$  2-Mercaptoethanol, 2 mM glutamine, 100  $\mu$ /ml penicillin, and 100  $\mu$ g/ml streptomycin. Cells were examined by FCM immediately after enzyme treatment and again after overnight culture. Earlier reports have suggested that Lyt-1 is trypsin resistant (3). However, by using high concentration of enzyme (>5 mg/ml), it was possible to remove completely surface Lyt-1 without loss of cell viability.

*Biosynthetic Labeling, Immunoprecipitation, and Gel Electrophoresis.* 200 million WEHI-55 cells were labeled with <sup>35</sup>S-methionine for 30 min in methionine-deficient RPMI-1640 containing 10% dialyzed HIFCS and 1 mCi <sup>35</sup>S-methionine (Amersham Corp., Arlington Heights, Ill.). The cells were chased in 200 ml medium for 5 h, then extracted with 4 ml lysis buffer (0.5% Nonidet-P40, Particle Data, Inc., Elmhurst, Ill.; 50 mM Tris; 150 mM NaCl; 0.02% NaN<sub>3</sub>; 5 mM EDTA; 50 mM phenylmethylsulfonyl fluoride; 0.2 trypsin inhibiting units per ml Aprotinin (Sigma Chemical Co.), 1  $\mu$ g/ml pepstatin A (Sigma Chemical Co.), 50 mM iodoacetamide, pH 8.0) for 30 min at 4°C. Nuclei were removed by centrifugation at 5,000 g for 20 min. Proteins, immunoprecipitated with rabbit anti-mouse Ig, anti-Lyt-1 M.Ab., and fixed *Staphylococcus aureus* Cowan I strain (9), were run on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels as previously described (10). Gels were treated with Enhance (New England Nuclear, Boston, Mass.) for fluorography and autoradiographs were made with Kodak X-Omat R film (Eastman Kodak Co., Rochester, N. Y.) at -70°C. Molecular weight standards (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) were run in a parallel gel lane and were visualized by staining with Coomassie brilliant blue (Bio-Rad Laboratories, Richmond, Calif.).

## Results

*Analysis of B Cell Lymphomas by FCM.* Recently, we examined a series of B cell lymphomas for the presence of T cell differentiation antigens. The most striking finding was that three surface Ig<sup>+</sup>, Ia<sup>+</sup> B lymphomas unequivocally expressed the Lyt-1 antigen, whereas none expressed the Thy-1.2 or Lyt-2 determinants (Table I). The CH5 and WEHI-259 splenic tumors clearly demonstrate the presence of surface IgM and Lyt-1, but not the Thy-1.2, Lyt-2, or Lyt-3.2 antigens. CH5 was held in culture for 5 d, and was still found to express IgM and Lyt-1. Trace amounts of Lyt-1 were also detected on CH4, CH6, and WEHI-5. The WEHI-55 B lymphoma, which has been maintained in continuous cell culture over the past 12 mo, also expresses both surface IgM/IgD and the Lyt-1 antigen, but not Thy-1.2, Lyt-2, or Lyt-3.2 (Fig. 1).

*Trypsin Stripping and Regeneration of Lyt-1 in Culture.* Because WEHI-259 and CH5 have not been established in permanent cell culture as yet, it is possible that the Lyt-1 antigen was passively acquired in vivo. Hence, the splenic tumor cells were treated with trypsin, analyzed for removal of Lyt-1, and placed in tissue culture. After overnight culture, the trypsin-stripped cells, as well as untreated CH5 and WEHI-259 cells, were again examined for presence of Lyt-1. As shown in Fig. 2, enzymatic treatment with trypsin successfully removed the Lyt-1 antigen. However, within 18 h,

TABLE I  
*Analysis of a Series of Murine B Cell Lymphomas on Expression of T Cell Antigens*

Tumor	Strain	Surface IgM	Anti-Thy-1.2 M.Ab	Anti-Lyt-1 M.Ab	Anti-Lyt-2 M.Ab
CH1	B10H2 <sup>a</sup> H4 <sup>b</sup> p/Wts	+	-	-	-
CH2	B10H2 <sup>a</sup> H4 <sup>b</sup> p/Wts	+	-	-	-
CH4	B10H2 <sup>a</sup> H4 <sup>b</sup> p/Wts	+	-	trace	-
CH5	B10H2 <sup>a</sup> H4 <sup>b</sup> p/Wts	+	-	+	-
CH6	B10H2 <sup>a</sup> H4 <sup>b</sup> p/Wts	+	-	trace	-
CH7	B10H2 <sup>a</sup> H4 <sup>b</sup> p/Wts	+	-	-	-
CH8	B10H2 <sup>a</sup> H4 <sup>b</sup> p/Wts	+	-	-	-
WEHI-5	(BALB/c × NZB)F <sub>1</sub>	+	-	trace	-
WEHI-55	(BALB/c × NZB)F <sub>1</sub>	+	-	+	-
WEHI-231	(BALB/c × NZB)F <sub>1</sub>	+	-	-	-
WEHI-259	(BALB/c × NZB)F <sub>1</sub>	+	-	+	-

Tumor cells were stained for surface IgM with FITC goat anti-mouse  $\mu$  chain serum. For T cell antigens, cells were treated with M.Ab or medium (control), followed by FITC-SJL anti-rat Ig. Analysis was performed using a B-D FACS III system. Analysis of the CH series lymphomas and the WEHI-259 B lymphoma was performed on in vivo derived tumor cells. In all cases where splenic or ascites tumors were used, >90% of the analyzed cells were of tumor origin, as determined by characteristic light scatter profiles and by reaction of >90% of the cells with a monoclonal anti-mouse  $\mu$  antibody.

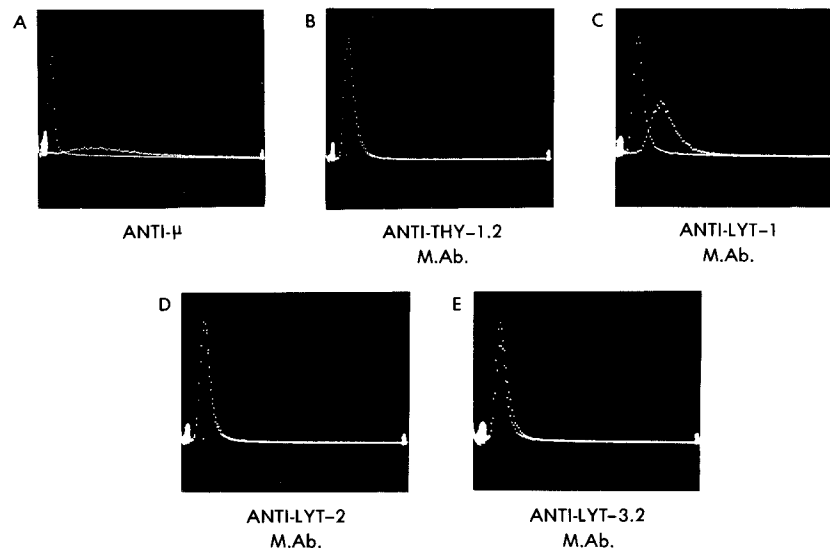


FIG. 1. Expression of the Lyt-1 antigen on the WEHI-55 B cell lymphoma. WEHI-55, an established B lymphoma cell line, was analyzed by FCM for the presence of surface Ig and T cell differentiation antigens. Cells were incubated with FITC goat anti-mouse  $\mu$  chain serum (A), rat anti-Thy-1.2 M.Ab. (B), rat anti-Lyt-1 M.Ab. (C), rat anti-Lyt-2 M.Ab. (D), and rat anti-Lyt-3.2 M.Ab. (E). 15,000 cells were analyzed using a B-D FACS III system. In the above figures, the fluorescence histogram of the stained populations was superimposed over the histogram of the autofluorescence control (X axis = fluorescence intensity increasing left to right, linear scale, 128 channels; Y axis = number of cells).

>90% of the trypsin-stripped cells again expressed Lyt-1 in the same quantity as the untreated control cells.

*Immunoprecipitation of Immunoglobulin and Lyt-1 from WEHI-55.* Lyt-1 has been immunoprecipitated from normal thymocytes after surface-labeling with <sup>125</sup>I and lactoperoxidase, and has been shown to be a glycoprotein of ~67,000–70,000 mol wt (3, 11). In addition, a higher molecular weight form of Lyt-1 was identified by immu-

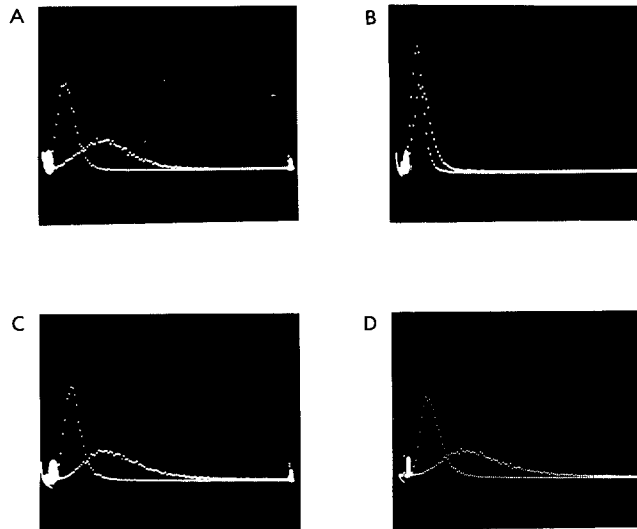


FIG. 2. Trypsin stripping and regeneration of Lyt-1 on the WEHI-259 B cell lymphoma. WEHI-259 splenic tumor cells were incubated with 10 mg/ml trypsin for 1 h at 37°C, analyzed by FCM for removal of Lyt-1, and placed in culture overnight. (A) Untreated WEHI-259 cells stained with rat anti-Lyt-1 M.Ab., followed by FITC SJL anti-rat Ig serum. (B) Trypsin-treated WEHI-259 cells stained for Lyt-1 immediately after enzyme treatment. (C) Untreated cells analyzed for Lyt-1 expression after overnight culture. (D) Trypsin-treated WEHI-259 cells stained for Lyt-1 after overnight culture. The data are presented as described in the legend to Fig. 1. Note the considerable reduction in Lyt-1 antigen expression immediately after trypsin treatment (B vs. A). After overnight culture, Lyt-1 is again expressed (D vs. B). Similar results were found with the CH5 tumor (not shown).

noprecipitation after surface-labeling of carbohydrates with [ $^3\text{H}$ ]borohydride (11). We used biosynthetic labeling of WEHI-55 cells with  $^{35}\text{S}$ -methionine, followed by immunoprecipitation with anti-Lyt-1, to see if the molecular form of Lyt-1 on this B lymphoma is similar to Lyt-1 from T cells. Fig. 3 shows the anti-Ig, anti-Lyt-1, and control immunoprecipitations from WEHI-55. The anti-Ig serum specifically immunoprecipitated a single light chain and two heavy chains that correspond in size to  $\delta$  chain and  $\mu$  chain (75,000 and 85,000 mol wt, respectively). Thus, the Lyt-1 glycoprotein expressed on WEHI-55 shows a similar structure to the Lyt-1 glycoprotein on thymocytes.

### Discussion

Previous studies have implied that Lyt-1 is only present on thymus-derived lymphocytes and that this molecule is associated with certain specific functional subsets of T cells (1, 2). However, using FCM analysis and M.Abs., we have conclusively demonstrated that certain B lymphomas may indeed express the Lyt-1 antigen. Of the 11 B lymphomas examined, three tumors expressed high levels of the Lyt-1 antigen. Furthermore, three other tumors gave weak but detectable reactions with the anti-Lyt-1 M.Ab. Although these tumors expressed this presumably "T cell specific" antigen, they were otherwise typical B lymphomas. All expressed surface Ig, Ia antigens, and Fc receptors. None expressed other T cell differentiation antigens such as Thy-1, Lyt-2, Lyt-3, or the T30 antigen (6).<sup>1</sup>

Because such a large percentage of these surface Ig<sup>+</sup> B lymphomas was found to express Lyt-1, it seems that expression of this antigen is probably not due to random

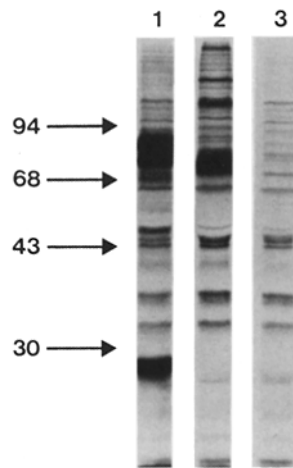


FIG. 3. Lyt-1 and immunoglobulin from WEHI-55 cells. WEHI-55 cells were biosynthetically labeled with  $^{35}\text{S}$ -methionine, extracted with lysis buffer containing 0.5% NP-40 and immunoprecipitated with (1) rabbit anti-mouse Ig; (2) rat anti-Lyt-1 (53-7.3); and (3) staph A alone (background). Immunoprecipitates were electrophoresed on 10% SDS-polyacrylamide gels under reducing conditions.

gene derepression in a transformed cell line. One possibility is that the small subset of B lymphocytes, which presumably express Lyt-1, is extremely susceptible to oncogenic transformation. At present, there is no evidence to support this hypothesis. It should be noted that with respect to other surface markers, these Lyt-1<sup>+</sup> tumors are quite heterogeneous (6).<sup>1</sup> Moreover, analysis of the expression of differentiation antigens on these tumors does not support the idea that they are from a homogeneous subpopulation of B cells. Another possibility is that leukemogenesis selectively includes expression of Lyt-1 on certain B lymphomas. Along these lines, it also is conceivable that normal B cells express Lyt-1 in undetectable amounts. However, the rapid proliferation and increase in cell size associated with transformation may increase expression of the existing antigen, therefore allowing detection.

Of particular relevance are the recent reports that the human Leu-1 antigen is expressed on most human B chronic lymphocytic leukemias, but not on normal human B lymphocytes (9, 12). Immunochemical and functional studies have demonstrated that human Leu-1 is probably the homologue of the mouse Lyt-1 antigen (9). Thus, the finding that murine Lyt-1 and its putative human counterpart Leu-1 are usually expressed on normal T cells, but are also present on B cell malignancies, implies a common mechanism of gene regulation (9).

A final point is that both the Leu-1 and Lyt-1 molecules on T cells have molecular weights of ~67,000–70,000 (9, 12). Immunoprecipitation analysis of the Lyt-1 antigen on WEHI-55 has shown a single diffuse band of protein with approximately the same average molecular weight. This suggests that the B lymphoma Lyt-1 protein is similar to the T cell antigen, perhaps with glycosylation differences. Bases on these data, a more extensive examination of normal B lymphocytes is needed to resolve the apparent discrepancy of Lyt-1 expression of B lymphomas vs. normal lymphoid cells.

#### Summary

Although the Lyt-1 antigen has previously been considered a T cell-specific marker, recent evidence suggests that a population of Thy-1<sup>-</sup>, Lyt-1<sup>+</sup> cells exists in normal

lymphoid tissues. In this study, we have observed that the WEHI-55, WEHI-259, and CH5 B cell lymphomas express high levels of the Lyt-1 antigen, as detected by monoclonal antibodies using the fluorescence-activated cell sorter. Three other B cell lymphomas of the 11 examined also gave weak but detectable reactions with the anti-Lyt-1 monoclonal antibody. Except for the expression of the Lyt-1 antigen, these lymphomas are typical of cells in the B cell lineage with respect to surface phenotype. The Lyt-1 glycoprotein immunoprecipitated from metabolically labeled WEHI-55 cells is similar in structure to the Lyt-1 glycoprotein on thymocytes. These findings are similar to recent reports that B-type human lymphocytic leukemia cells express the putative human homologue of Lyt-1, the Leu-1 antigen.

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### References

1. Cantor, H., and E. A. Boyse. 1971. Lymphocytes as models for the study of mammalian cellular differentiation. *Immunol. Rev.* **33**:105.
2. Mathieson, B. J., S. O. Sharrow, P. S. Campbell, and R. Asofsky. 1979. An Lyt differentiated subpopulation of thymocytes detected by flow cytometry. *Nature (Lond.)* **277**:478.
3. Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* **47**:63.
4. Ledbetter, J. A., R. V. Rouse, H. S. Micklem, and L. A. Herzenberg. 1980. T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens: two-parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modified current views. *J. Exp. Med.* **152**:280.
5. Warner, N. L., M. J. Daley, J. Richey, and C. Spellman. 1979. Flow cytometry analysis of murine B cell lymphoma differentiation. *Immunol. Rev.* **48**:197.
6. Lanier, L. L., N. L. Warner, L. W. Arnold, R. B. Raybourne, and G. Haughton. 1981. B cell lymphomas in New Zealand and B10 H-2<sup>a</sup> H-4<sup>b</sup> Wts mice: antigenic analysis by flow cytometry. In *Proceedings of the Second International Conference on B Lymphocytes in the Immune Response*. N. Klinman, D. Mosier, I. Scher, and E. Vitetta, editors. Elsevier North-Holland, New York. In press.
7. Lanier, L. L., and N. L. Warner. 1981. Cell cycle related heterogeneity of Ia antigen expression on a murine B lymphoma cell line: analysis by flow cytometry. *J. Immunol.* **126**:626.
8. Herzenberg, L. A., and L. A. Herzenberg. 1978. Analysis and separation using the fluorescence activated cell sorter. In *Handbook of Experimental Immunology*. D. M. Weir, editor. Blackwell Scientific Publications, England. 221.
9. Ledbetter, J. A., R. L. Evans, M. Lipinski, C. Cunningham-Rundles, R. A. Good, and L. A. Herzenberg. 1981. Evolutionary conservation of surface molecules that distinguish T lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. *J. Exp. Med.* **153**:310.
10. Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature (Lond.)* **227**:680.
11. Durda, P. J., C. Shapiro, and P. D. Gottlieb. 1978. Partial molecular characterization of the Lyt-1 alloantigen from mouse thymocytes. *J. Immunol.* **120**:53.
12. Wang, C. Y., R. A. Good, P. Ammirato, G. Dymbort, and R. L. Evans. 1980. Identification of a p69/70 complex expressed on human T cells sharing determinants with B-type chronic lymphatic leukemia cells. *J. Exp. Med.* **151**:1539.