JL1, A Novel Differentiation Antigen of Human Cortical Thymocyte

By Seong Hoe Park,*‡ Young Mee Bae,*‡ Hyung Joo Kwon,*‡
Tae Jin Kim,*‡ Joon Kim,‡ Sang Jong Lee,‡ and Sang Kook Lee*

From the *Department of Pathology, and ‡Division of Immunogenetics, Cancer Research
Institute, Seoul National University College of Medicine, Seoul, Korea 110-799

Summary

Expression of a novel thymocyte differentiation antigen, JL1, defined by a monoclonal antibody (mAb) developed against human thymocytes showed a specificity for stage II double positive (CD4$^+$CD8$^+$) human cortical thymocytes. This antigen was not expressed at detectable levels on medullary thymocytes, mature peripheral leukocytes, bone marrow cells or on other types of tissues elsewhere in the human body. Immunohistologic analysis revealed that JL1 had a clear pattern of distribution on cortical thymocytes. Immunoprecipitation of ^{125}I-labeled cell lysates from human thymocytes and Molt-4 leukemic cell line with anti-JL1 mAb yielded a 120–130-kD single chain glycoprotein. When immunoprecipitation of cell lysate was done after endoglycosidase F treatment, JL1 antigen was still detected by antibody but the band showed a reduction in apparent molecular mass of ~5 kD. This suggests that, although JL1 molecule contains carbohydrate group, this does not form a critical part of the antigenic determinant for anti-JL1 antibody. JL1 antigen appears to be the first double positive, stage-specific differentiation antigen of human thymocyte reported so far. This antigen would be a useful marker for lymphoblastic malignancy of stage II thymocyte origin and it may be involved in the thymocyte education process.

Intrathymic T cell development entails a complex series of proliferation, differentiation, and selection events (1, 2). This sequence of events has been mapped according to the expression of specific combinations of cell surface differentiation antigens. However, the major cell surface molecules to compartmentalize the subpopulations of human thymocytes, including CD1, CD3, CD4, and CD8, do not specifically define discrete stages in T cell differentiation (3). The involvement of the TCR–CD3 complex is imperative for selection events, and there are several key steps in T cell development, such as lineage commitment, induction and expression of TCR gene rearrangements, and education events for positive and negative selections (2). Clearly, for a better understanding of the mechanisms controlling T cell differentiation, it is important to identify new molecules on the surface of these cells as a prelude to determining their functional relevance. In this report, we introduce a novel stage II–specific human thymocyte differentiation antigen, JL1, that was detected by a murine mAb developed against human thymocytes. This specificity for stage II thymocytes was shown by flow cytometry and immunohistochemical analysis. Immunoprecipitation and SDS-PAGE analysis demonstrated that anti-JL1 mAb recognized a single chain glycoprotein with a molecular mass of 120–130 kD with relatively short carbohydrate residues determined by endoglycosidase F (endo-F) treatment of lysates from human thymocytes and Molt-4 T leukemic cells. Given the unique T cell stage specificity of the JL1 molecule, it is likely that this antigen is involved in a T cell education program during thymic ontogeny.

Materials and Methods

Cells and Tissues. Human thymocytes were collected from fragments of thymuses that had been removed for the exposure of the heart during corrective cardiac surgery of 1–12-mo-old patients. Single cell suspensions were prepared, washed, and used for immunization and screening. Human leukemic cell lines used for screening included Molt-4, Molt-3, CCRF-CEM, H9, Hut-78, Daudi, KG-1, U937, and THP-1. PBMC were isolated from peripheral blood of normal human volunteers. Lymphocytes were incubated in RPMI-1640 containing 10% fetal bovine serum with or without PHA (GIBCO-BRL, Gaithersburg, MD), PWM (Boehringer Mannheim Biochemicals, Mannheim, Germany), or anti-CD3 mAb (OKT3; American Type Culture Collection, Rockville, MD). Cultures were collected on day 3 for PHA and anti-CD3 mAb and on day 5 for PWM. Activation of cells was confirmed by [^{3}H]thymidine incorporation, expression of activation-related molecules such as HLA-DR and IL-2R, and morphological identification of blast formation under light microscopic examination (data not shown). Normal bone marrow samples were obtained from Seoul National University Children's Hospital and were also used for screening. Mouse L cells transfected with full-length cDNA of CD1a, CD1b, and CD1c were kind gifts of C. Terhorst (Beth Israel Hospital, Boston, MA) (4).

Production of Anti-JL1 mAb. After BALB/c mice were immunized with 10^7 human thymocytes at 2-wk intervals for 2 mo, the spleen was removed, and 10^8 spleen cells were fused with 10^7 SP2/0-Ag14 mouse myeloma cells using polyethylene glycol (PEG 4000; Rahway, NJ) (5). One of the resulting hybridoma clones, whose supernatants were reactive to human thymocytes, was named anti-JL1. The isotype of anti-JL1 mAb determined by enzyme immunoassay using ScreenType™ (Boehringer Mannheim Biochemicals) was IgG1.

Immunohistochemical Study. Snap-frozen sections of fetal and postnatal thymus, lymph node, tonsil, spleen, cerebrum, skin, and other tissues were incubated with anti-JL1 mAb, followed by purified biotinylated goat anti-mouse IgG, IgA, and IgM, and then by streptavidin-horseradish peroxidase conjugate after washing. The staining pattern was analyzed based on serial hematoxylin-eosin stained sections.

Immunofluorescence Assay. Fresh cell suspensions of human fetal and postnatal thymocytes, PBL, bone marrow cells, and tumor cell lines were prepared, stained by indirect immunofluorescence method with anti-JL1 mAb followed by FITC-conjugated goat anti-mouse Ig, and analyzed by a flow cytometer (FACScan®, Becton Dickinson & Co., Mountain View, CA).

Comparative analysis of JL-1 positive and negative thymocytes with those expressing and nonexpressing CD1a was performed along with their expression pattern of CD4 and CD8. Thymocytes were fractionated on the basis of the reactivity with anti-JL1 mAb and anti-CD1a by panning procedure. Enriched JL1+/- and CD1a+/- cells were analyzed by double-fluorescence FACS® analysis. Both types of cells were first stained with FITC-conjugated anti-CD4 mAb and subsequently stained with PE-conjugated anti-CD8 mAb (Becton Dickinson & Co.).

Immunoprecipitation of JL1 Antigen. Human thymocytes and Molt-4 tumor cells were surface labeled with Na^{125}I (1 mCi/4 × 10^7 cells) using lactoperoxidase-catalyzed iodination. The cells were then lysed in 4 ml of lysis buffer containing 0.5% (wt/vol) NP-40. The lysates were precleared several times by using an irrelevant ascites of IgG1-type antibody conjugated to protein A-Sepharose CL4B beads. Immunoprecipitation was performed by mixing of radiolabeled cell lysate with anti-JL1 mAb-conjugated protein A-Sepharose CL4B beads and incubating at 4°C overnight. For endo-F (Boehringer Mannheim Biochemicals) treatments, the washed beads were resuspended in 20 mM potassium phosphate buffer, pH 7.4, containing 0.2% SDS and incubated with endo-F 2 unit overnight at 37°C. The supernatant eluted from the beads was collected and SDS-PAGE was carried out on 8 or 10% polyacrylamide gels.

Results and Discussion

In this report, we describe a novel 120–130-kD human T cell surface molecule, designated JL1, which was strongly and specifically expressed on stage II cortical thymocytes of human origin and some leukemic T cell lines.

To study the detailed pattern of expression of JL1 antigen on lymphoid and nonlymphoid tissues, we performed immunohistochemical analysis using fresh tissues obtained from surgically removed materials including tonsil, lymph node, spleen, cerebrum, cerebellum, skin, lung, esophagus, stomach, small and large intestine, appendix, liver, pancreas, kidney, urinary bladder, testis, ovary, uterus, and adrenal gland (data not shown). JL1 antigen was absent in all types of tissues examined other than human thymocytes, indicating that JL1
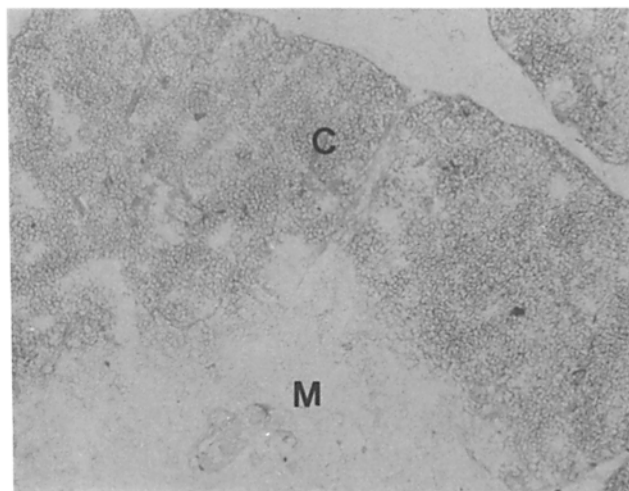
Figure 1. Immunohistologic localization of JL1 antigen in the thymus. Snap-frozen section of human thymus from a 5-mo-old patient was incubated with anti-JL1 mAb, then with biotinylated anti-mouse Ig, and streptavidin-peroxidase conjugate followed by freshly prepared 3,3-diaminobenzidine solution. Cortex (C, cortical thymocytes) shows intense positive staining in contrast with the medulla (M). ×40.

antigen has an extreme specificity for human thymocytes. Even in the thymus, localization of this antigen was confined to cortical thymocytes (Fig. 1). In addition to investigating the thymic reactivity of anti-JL1 mAb, we also looked at the expression of this antigen on various hematopoietic cells by indirect immunofluorescence staining and flow cytometry. As with mature medullary thymocytes, PBMC from blood and

Table 1. *Distribution of JL1 Antigen on Normal Hematopoietic Cells Determined by Indirect Immunofluorescence and Flow Cytometric Analysis*

Cells	Number of cases	Proportion of JL1$^+$ cells
		%
Thymocytes		
Prenatal 16–21 wk	4	82 ± 9*
22–27 wk	5	90 ± 5
28–33 wk	2	96 ± 3
Postnatal	6	91 ± 6
Spleen cells	4	-‡
Bone marrow cells	6	-
PBMC	54	-
Activated PBMC		
PHA (10 µg/ml)	5	-
PWM (3 µg/ml)	5	-
anti-CD3	5	-
(0.5 µg/ml)		

* Mean ± SD.
‡ <5%.
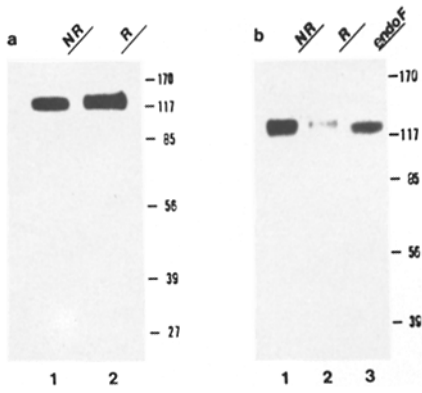
Figure 2. SDS-PAGE analysis of surface molecules immunoprecipitated by anti-JL1 mAb from thymocytes (a) and Molt-4 tumor cells (b). Cells (5 × 10⁶) were surface labeled with ¹²⁵I- using lactoperoxidase technique, and lysed in buffer containing 0.5% NP-40. Immunoprecipitation was performed by anti-JL1 mAb coupled to protein A–Sepharose beads. SDS-PAGE analysis was performed under reducing (R) and nonreducing (NR) conditions using 10% (a) or 8% (b) polyacrylamide gels. SDS-PAGE analysis was also done under reducing and nonreducing conditions after endo-F treatment of antigen-bound beads (endo-F).
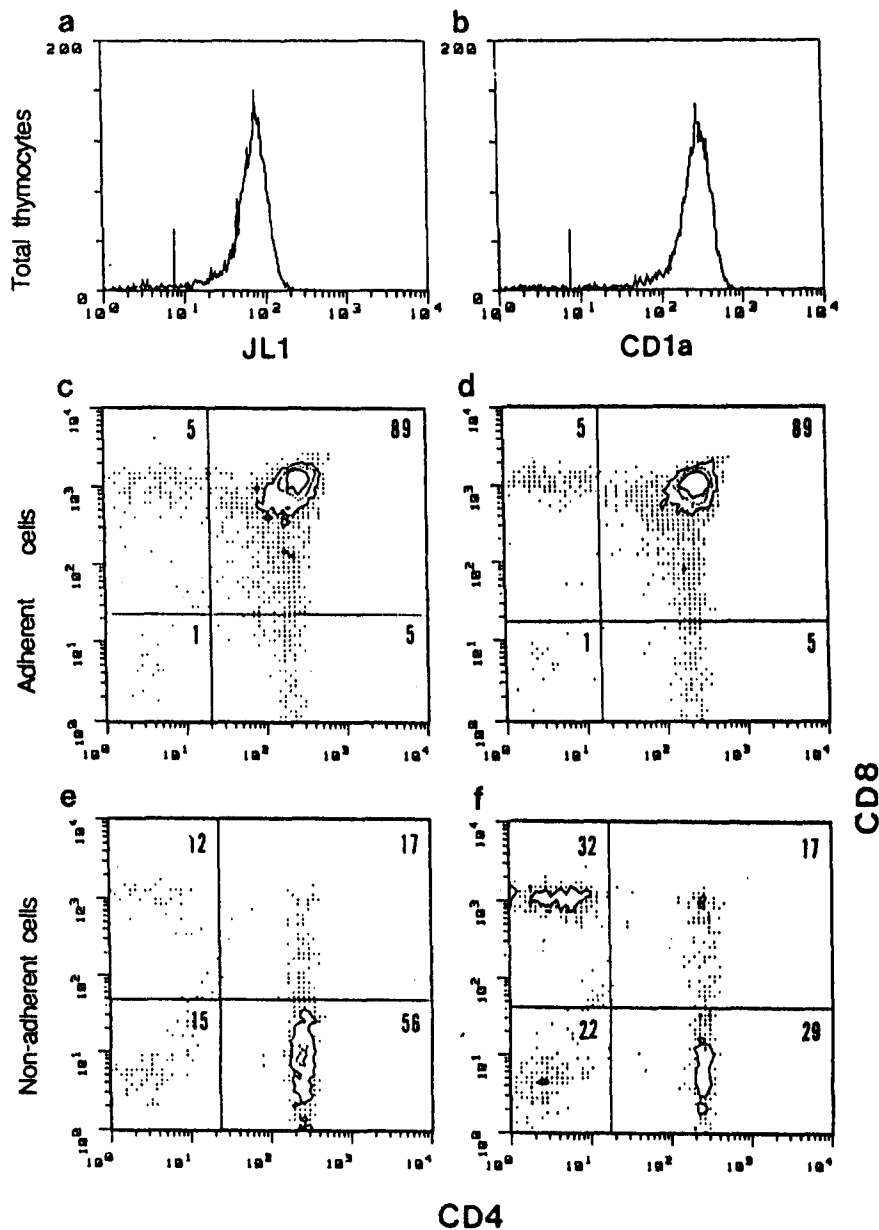
Figure 3. FACS® analysis of human thymocytes shows the single histogram of JL1 (a) and CD1a (b) from total thymocytes and the distribution profiles of CD4 and CD8 on thymocytes selected for the expression of JL1 (c and e) and CD1a (d and f) antigens using panning procedure. The adherent (c and d) and nonadherent cells (e and f) were stained with FITC-conjugated anti-CD4 mAb and PE-conjugated anti-CD8 mAb.

1449   Park et al.   Brief Definitive Report

Table 2. *Comparison of Expression of CD1 Antigens and JL1 Antigen on Several Human Hematopoietic Cell Lines*

Category	Cell line	Percent positive cells			
		CD1a	CD1b	CD1c	JL1
T cell	Molt-3	93	100	97	100
	Molt-4	98	74	95	100
	CCRF-CEM	–*	–	–	62
	H-9	–	–	–	–
	Hut-78	–	–	–	–
B cell	Daudi	–	–	–	–
Monocyte	U937	NT‡	NT	NT	–
	THP-1	NT	NT	NT	–
Myelocyte	KG-1	NT	NT	NT	–

* <5%.
‡ NT, not tested.

spleen were JL1 negative. Even when PBL were activated with PHA, PWM, or anti-CD3 mAb, there was no clear JL-1 positivity suggesting that this marker is no longer expressed on peripheral lymphoid cells (Table 1). JL1 appears to be specific to immature cortical thymocyte and distinct from other well-known cortical thymocyte differentiation antigens and activation molecules reported so far.

For biochemical characterization of cell surface antigen recognized by anti-JL1 mAb, Molt-4 leukemic cells and human thymocytes were iodinated using lactoperoxidase, and immunoprecipitates of NP-40 solubilized cell lysates were analyzed by SDS-PAGE. As shown in Fig. 2, *a* and *b*, anti-JL1 mAb precipitated a single chain glycoprotein of apparent molecular mass 120–130 kD with short carbohydrate residues determined by endo-F treatment (Fig. 2). The reduced form migrated more slowly than the nonreduced form, suggesting the presence of intramolecular disulfide bridge. The molecular weight of JL1 determined from Molt-4 was slightly greater than that from thymocytes, suggesting some differences in the extent of glycosylation. The epitope recognized by anti-JL1 mAb seemed not to be a carbohydrate moiety since immunoprecipitation of Molt-4 cell lysate pretreated with endo-F yielded an identical band to that of immunoprecipitate digested with endo-F after antigen elution (data not shown).

There are a number of findings that JL1 seems to define a novel differentiation antigen of immature thymocytes distinct from thymocyte differentiation antigens of CD1 family. First, the biochemical nature of JL1 is entirely different from that of CD1 antigens, the latter being composed of a H chain of 43–49 kD and β_2-microglobulin (β_2m) of 12 kD. Second, cell and tissue distribution of JL1 is highly specific. JL1 antigen is expressed on the cell surface of a majority of cortical thymocytes, whereas antigens of CD1 family are expressed on a subset of B cells, dendritic cells of lymph nodes and spleen, and Langerhans cells of epidermis as well as cortical thymocytes (6–8). Third, anti-JL1 antibody did not react with

mouse L cells transfected with full-length cDNA of CD1a, CD1b, and CD1c (data not shown).

It is also very unlikely that JL1 belongs to known activation molecules since there are no reactivities in activated peripheral lymphocytes stimulated with PHA, PWM, and anti-CD3 antibody (Table 1), and the JL1 antigen expression pattern is different from that of activation antigens on thymocytes reported elsewhere, such as CD25, CD30, CD69, and CD71 (9–11). JL1 antigen was also clearly different from Thy-1, peanut agglutinin receptor, or nonclassical MHC class I antigen, in view of the expression pattern within the thymus and molecular size (12–14). Although non-β_2m–associated thymocyte differentiation antigens were described by using alloantisera procured from multiparous women (15), it was very difficult to compare JL1 with them because of a quantitative limit of the alloantisera.

Specificity for stage II cortical thymocytes was further supported by results of the comparative analysis with two-color flow cytometry between JL1+/– and CD1a+/– thymocytes (Fig. 3) and by results of single-color flow cytometric analysis of a few leukemic T cell lines which are individually representative of the corresponding stages of thymocyte development (Table 2; 16). On two-color FACS® analysis, nearly all the thymocytes that were selected by the reactivity to anti-JL1 mAb coexpressed CD4 and CD8, and some populations were single positive. Further, when screened on several tumor cell lines, the most prominent expression was seen in Molt-4 and Molt-3 cell lines which coexpress both CD4 and CD8 differentiation antigens, whereas mature T cell lines, Hut-78 and H9, were negative for JL1 antigen. CCRF-CEM, a T cell line representative of later stage II, also expressed JL1 antigen, but proportions of JL1+ cells and mean fluorescence intensity were lower than those of earlier stage II tumor cell lines (Molt-3 and Molt-4). These data indicated that JL1 antigen was confined to stage II thymocytes and seemed to encompass a wider range of thymic stage II than CD1a, CD1b,

and CD1c, at least in leukemic cell lines. In normal thymoctyes, however, the expression of both types of antigens largely overlapped and was mostly present in double positive thymocytes, except for the slightly different pattern of expression in the CD8 single positive subset. This finding was confirmed with a flow cytometric analysis of JL1- and CD1a-negative subsets which were obtained after panning with both types of mAbs. Populations of CD8 single positive subsets were smaller in

JL-1 negative thymocytes than that of CD1a negative cells in number.

In summary, JL1 antigen appears to be a novel thymic stage II T cell differentiation marker with the approximate molecular mass of 120–130 kD. Further studies including more detailed biochemical and molecular characterization and functional aspects of this molecule are currently underway.

References

1. Nikolic-Zugic, J. 1991. Phenotypic and functional stages in the intrathymic development of $\alpha\beta$ T cells. *Immunol. Today.* 12:65.
2. Boyd, R.L., and P. Hugo. 1991. Towards an integrated view of thymopoiesis. *Immunol. Today.* 12:71.
3. Petrie, H.T., P. Hugo, R. Scollay, and K. Shortman. 1990. Lineage relationship and developmental kinetics of immature thymocytes: CD3, CD4, and CD8 acquisition in vivo and in vitro. *J. Exp. Med.* 172:1583.
4. Balk, S.P., P.A. Bleicher, and C. Terhorst. 1989. Isolation and characterization of a cDNA and gene encoding for a fourth CD1 molecule. *Proc. Natl. Acad. Sci. USA.* 86:252.
5. Koehler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (Lond.).* 256:495.
6. Amiot, M., A. Bernard, B. Raynal, W. Knapp, C. Deschildre, and L. Boumsell. 1986. Heterogeneity of the first clusters of differentiation: characterization and epitope mapping of three CD1 molecules on normal human thymus cells. *J. Immunol.* 136:1752.
7. Martin, L.H., F. Calabi, F.A. Lefebvre, C.A.G. Bilsland, and C. Milstein. 1987. Structure and expression of the human thymocyte antigens, CD1a, CD1b, and CD1c. *Proc. Natl. Acad. Sci. USA.* 84:9189.
8. Cattoretti, G., E. Berti, C. Parravicini, M. Buscaglia, F. Cappio, R. Caputo, A. Cerri, L. Crosti, D. Delia, G. Gaiera, and N. Polli. 1989. Expression of CD1 molecules on dendritic cells: ontogeny, epitope analysis on normal and malignant cells, and tissue distribution. *In* Leucocyte Typing IV. White Cell Differentiation Antigens. W. Knapp, B. Doerken, W.R. Gilks, E.P. Rieber, R.E. Schmidt, H. Stein, and A.E.G. Kr von dem Borne, editors. Oxford University Press, Oxford, UK. 262–264.

9. Schuurman, H.J., D. van Wichen, and R.A. de Weger. 1989. Expression of activation antigens on thymocytes in the 'common thymocyte' stage of differentiation. *Thymus.* 14:43.
10. Ceredig, R., J.W. Lowenthal, M. Nabholz, and H.R. MacDonald. 1985. Expression of interleukin-2 receptors as a differentiation marker on intrathymic stem cells. *Nature (Lond.).* 314:98.
11. Sanchez-Madrid, F., M.L. Tobrio, F. Gambon, and M.O. Landazuri. 1985. Cell surface molecular changes on the activation of human thymocytes. *J. Immunol.* 135:3938.
12. Bonewald, L.F., J.M. Goust, R.M. Sade, and C.-C. Wang. 1985. Isolation and characterization of Thy-1 homologue from human thymus. *Thymus.* 4:199.
13. Rose, M.L., and F. Malchiodi. 1981. Binding of peanut lectin to thymic cortex and germinal centers of lymphoid tissue. *Immunology.* 42:583.
14. Pook, M.A., V. Woodcock, M. Tassabehji, R.D. Campbell, C.W. Summers, M. Taylor, and T. Strachan. 1991. Characterization of an expressible nonclassical class I HLA gene. *Hum. Immunol.* 32:102.
15. Matsuo, Y., T. Ariyasu, M.B. Brenner, J. Imanishi, M.M. Yokoyama, and J. Minowada. 1989. Expression and distribution of cytoplasmic CD3 antigen and TcR β-chain with or without expression of surface CD3 TcRα/β or TcRγ/δ complexes. *In* Leucotype Typing IV. White Cell Differentiation Antigens. W. Knapp, B. Doerken, W.R. Gilks, E.P. Rieber, R.E. Schmidt, H. Stein, and A.E.G. Kr von dem Borne, editors. Oxford University Press, Oxford, UK. 299–301.
16. Park, S.H., R.J. Mahoney, S.R. Given, M.A. Fajardo, D.P. Dubey, and E.J. Yunis. 1988. Serological identification of thymocyte differentiation antigens. *Hum. Immunol.* 22:151.