

DIFFERENT CLASSES OF T LYMPHOCYTES HAVE  
DIFFERENT mRNAs FOR THE LEUKOCYTE-COMMON  
ANTIGEN, T200

BY LEO LEFRANCOIS,\* MATTHEW L. THOMAS,† MICHAEL J. BEVAN,\*  
AND IAN S. TROWBRIDGE†

*From the \*Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037; and the †Department of Cancer Biology, The Salk Institute for Biological Studies, San Diego, California 92138*

The leukocyte common antigen (L-CA in the rat, T200 in the mouse) is a family of high  $M_r$  glycoproteins that are present exclusively on cells of the hematopoietic lineage (1, 2), excluding mature erythroid cells (3). Functional studies using mAbs have implicated L-CA in a number of immunologic processes, including NK- (4) and CTL-induced cytolysis (5), B cell differentiation (6), and regulation of IL-2 receptor expression (7). In addition, L-CA-specific mAbs provided the first means to distinguish between functional subpopulations of helper T cell ( $T$ ) in the rat (8). Although L-CA is expressed on all leukocytes, different classes of leukocytes express different forms of the molecule, and this pattern is conserved between species. For example, thymocyte L-CA appears to consist predominantly of a single major component, whereas peripheral T cell L-CA can be resolved into three or four species, and B cell L-CA constitutes an additional distinct high molecular weight form (9).

Recently, cDNA clones encoding rat L-CA from thymus and purified B cells have been isolated, and from the sequence of the thymocyte cDNA clones, a partial primary structure of 1,073 amino acids has been deduced (10). The region likely to be the membrane spanning segment was found at residues 347–368, and the sequence amino-terminal to this was found to be on the extracellular side. This suggests that there is a cytoplasmic domain of 705 amino acids. The isolated B cell cDNA clones encompassed the region from the membrane-spanning segment to the polyadenylation signal. The partial sequence of the B cell cDNA clones indicated complete identity between the 3' regions of thymocyte and B cell L-CA, implying that they have identical cytoplasmic domains and that these two forms of L-CA are encoded by the same gene. However, Northern blot analysis shows thymocyte mRNA to be 4.8 kb, whereas B cell mRNA is 5.5 kb. This suggested that differential RNA processing may contribute to the heterogeneity in the forms of L-CA expressed on the surface of different classes of lymphocytes. In the mouse, mAbs have been described that are reactive only with the T200 glycoprotein expressed on cytotoxic T cells (11, 12). The determinants recognized by these antibodies, termed CT antigens, are dependent upon carbohydrate moieties displayed on the molecule (13). This finding estab-

lishes that during T cell development, changes occur in the oligosaccharides found on the T200 glycoprotein. However, this does not exclude alterations in protein structure. In fact, the different patterns of glycosylation may be determined by such modifications. Here we show that cloned murine cytotoxic and helper T cell lines express T200 mRNAs that differ in size, and that differences in  $M_r$  of the newly synthesized T200 glycoprotein in the two cell types can be detected. Together, these data suggest that the primary structure of T200 glycoprotein on activated cytotoxic T cells differs from that found on activated Th cells.

### Materials and Methods

**Northern Blot Analysis of L-CA mRNA.** Total RNA was isolated from cells using guanidium isothiocyanate lysis and centrifugation over a cesium chloride cushion (14). Poly-A<sup>+</sup> RNA was selected by a single passage over oligo(dT)-cellulose. RNA was electrophoresed on a 0.5% Agarose gel containing formaldehyde and blotted onto Zetaprobe (BioRad Laboratories, Richmond, CA) as previously described (10). Prehybridization and hybridization were by the method of Gatti et al. (15). The probe used was a 3.7 kb Eco RI fragment from the rat thymocyte cDNA clone pLC-2 or a single-stranded rat thymocyte cDNA transcribed from a M13 clone and corresponding to position 1,110–1,621 (10). The filter was washed in 30 mM sodium citrate, pH 7, 0.3 M NaCl, 0.1% SDS at 52°C, and used to expose Kodak X-OMAT AR-5 film.

**Radiolabeling and Immunoprecipitation of T200 Proteins.** These procedures are essentially as previously described (15). Briefly, cells were labelled with [<sup>35</sup>S]methionine for 5 min and subsequently lysed. After centrifugation and preclearing, the resulting supernatant was incubated with anti-T200 mAb 13/2.3 coupled to Sepharose 4B. For treatment with endoglycosidase H (Miles Laboratories, Inc., Naperville, IL), the washed precipitates were boiled in 50 mM Tris-HCl, pH 6.8, and 0.1% SDS, followed by the addition of 0.15 M sodium citrate, pH 5.5, with or without the addition of the enzyme. Digestion was carried out for 16 h at 37°C. The precipitates were then analyzed by SDS-PAGE.

### Results and Discussion

To compare the T200 mRNA made by activated CTL and Th cells, poly-A<sup>+</sup> RNA was isolated from several cloned murine CTL and Th cell lines and subjected to Northern blot analysis using a rat L-CA cDNA probe (10). As shown in Fig. 1a, a small but consistent difference in the size of T200 mRNA isolated from the CTL and Th cell lines was detected. The mRNA encoding T200 from three CTL lines was estimated to be 5.2 kb, and was larger than that from each of four Th cell lines, which was 4.8 kb. This difference in size was observed for all of the CTL and Th clones analyzed, regardless of their strain of origin or antigen specificity. For comparison, mRNA from thymus, lymph node T cells, and a pre-B cell line, 70Z/3 (16), were also analyzed. B cell T200 was encoded by a 5.5 kb mRNA and was significantly larger than thymocyte T200 mRNA, as previously reported for L-CA mRNA isolated from rat B lymphocytes (10). These findings are highly reproducible, and the results of a second experiment are shown in Fig. 1b. Northern blot analysis of the same gels using a probe for the  $\beta$ -chain of the T cell receptor revealed no variation in size of the mRNA from the various T cell types (data not shown). It is concluded that there are at least three different T200 mRNAs, and that CTL, Th cells, and B cells each express a form of T200 mRNA characteristic of that cell type.

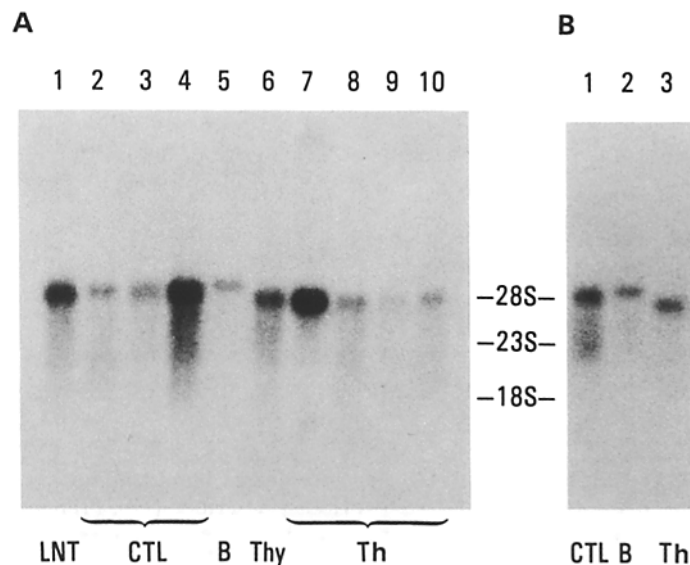


FIGURE 1. Northern blot analysis of L-CA mRNA from various mouse leukocytes. RNA was fractionated on a 0.5% agarose formaldehyde gel and blotted onto Zetaprobe (Biorad Laboratories). The filter was hybridized with a 3.7 kb Eco RI probe from the rat thymocyte cDNA clone pHc-2 (*a*) or a single-stranded rat thymocyte cDNA transcribed from a M13 clone and corresponding to position 1,110–1,621 (*b*) (10). In *a*, lane 1, RNA was from lymph node T cells isolated by depleting B cells with the mAb J11D plus complement (25); lanes 2–4 RNA from the CTL clones B3, 8.2, and H7, respectively; lane 5 RNA from the pre-B cell line 70Z/3; lane 6, from thymocyte RNA; lanes 7–10, Th RNA from K3, BB5, K31C7, and K321C9, respectively (Th lines were generously provided by Dr. Andrew Glasebrook, The Salk Institute, and Dr. Susan Webb, Scipps Clinic and Research Foundation). In *b*, RNA was from: lane 1, CTL clone H7; lane 2, pre-B cell line 70Z/3; lane 3, Th clone K3.3.

The T200 glycoprotein expressed on the surface of CTL is larger than that found on Th cells, thymocytes, and lymph node T cells, and this is consistent with the difference in the size of T200 glycoprotein mRNA found in these cell types. However, the mature glycoprotein is heavily glycosylated, and there is compelling evidence that the oligosaccharide structures displayed by the T200 glycoproteins on the surface of CTL and Th cells are not identical and contribute to the heterogeneity in size observed (13). To determine whether the precursors of the T200 glycoproteins in CTL and Th cells at the earliest time detectable after synthesis differ in size, cells were pulse-labeled for 5 min with [<sup>35</sup>S]-methionine and the T200 proteins were isolated by immunoprecipitation. Various precursor forms of T200 glycoprotein characteristic of each lymphocyte population are evident as shown in Fig. 2. Even at this early time point there are at least three species of T200 for CTLs and for lymph node T cells. The B cell T200 is identified by the presence of a protein of high *M<sub>r</sub>* in unseparated lymph node cells that is absent after removal of B cells (Fig. 2, compare lanes 3 and 5 and 6). Treatment of the isolated proteins with endoglycosidase H (endo H) showed that all of the precursor molecules contain high mannose *N*-linked oligosaccharides (17). However, even after removal of the high-mannose oligosaccharides, the same heterogeneous pattern of T200 species is seen. In the case of CTL, the same pattern was previously observed after tunicamycin treatment

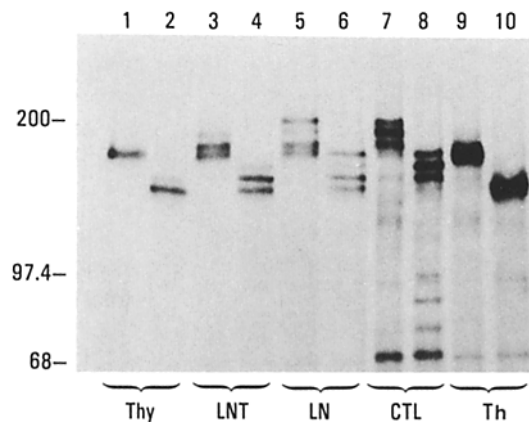


FIGURE 2. Immunoprecipitation of T200 precursor proteins. Thymocytes (lanes 1 and 2), lymph node T cells (lanes 3 and 4), unseparated lymph node cells (lanes 5 and 6), CTL clone H7 (lanes 7 and 8), and Th clone BB5 (lanes 9 and 10) were pulse-labeled with [ $^{35}$ S]methionine for 5 min. The cells were lysed and immunoprecipitation was carried out using I3/2.3 (anti-T200) coupled to Sepharose 4 B. Immunoprecipitates from each lysate were divided in half and treated with endo H (lanes 2, 4, 6, 8, 10) or mock-treated (lanes 1, 3, 5, 7, 9) for 16 h at 37°C, followed by analysis on a 7.5% SDS-polyacrylamide gel.

(13). It is also unlikely that the addition of *O*-linked oligosaccharides contributes to the heterogeneity of the T200 precursors, as this is known to occur as a relatively late event in the biosynthesis of membrane glycoproteins (18).

There is a clear correlation between the size of the T200 mRNA transcripts made by the various cell types and the  $M_r$  of the newly synthesized T200 molecules. This supports the notion that the mRNAs encode T200 glycoproteins with a different primary structure. However, only a single mRNA species was detected in each cell type, whereas multiple precursor protein species are observed in some cells. It is possible that other differential posttranslational modifications of the newly synthesized glycoproteins, including proteolytic cleavage, may occur. Sequence analysis will be required to determine the precise differences. How the various forms of T200 mRNA are generated is unknown, but could be the result of alternate splicing of a common RNA precursor (19, 20) or use of alternate transcription initiation sites (21).

The precise role of the T200 glycoprotein in lymphocyte interactions is still obscure. It is possible that cell type-specific T200 determinants play a role in lymphocyte homing to particular immunologic sites. Previous results (13–15) indicate that certain oligosaccharide moieties (termed CT antigens) that are likely to be *O*-linked are added to the T200 proteins of activated CTL and not to the T200 proteins of activated Th or any other T200 proteins. A strong correlation was noted between the presence of the CT antigens and cytotoxic activity (11, 12, 22). A large portion (705 amino acids) of the T200 molecule is on the cytoplasmic side of the plasma membrane, suggesting involvement of T200 in transmembrane signaling perhaps via cytoskeletal interactions (23). Since, in the rat, the cDNAs from thymocytes and B cells encoding the intracellular domain of the molecule are identical (10), it is likely that any cell type-specific differences in primary structure of T200 glycoprotein will be in the extracellular domain. The programmed expression of different forms of T200 glycoprotein during B

and T cell development is highly conserved across species, and thus is likely to have important consequences for lymphocyte function.

A cDNA clone from the mouse has recently been reported (24), and consistent with the results reported here, Northern blot analysis showed size differences in the T200 mRNAs isolated from T and B leukemic cell lines.

### Summary

The leukocyte common antigen, T200, is expressed on all white blood cells but not on other differentiated cells. Within the hematopoietic lineage, specific cell types display characteristic structural forms of the molecule on their surface. We show that murine cytotoxic T lymphocyte clones and helper T cell clones contain different size mRNA for this molecule, and that the early precursors of T200 glycoprotein made in the helper and cytotoxic T cells differ in  $M_r$ . Thus, in addition to differences in posttranslational modifications, it is highly likely that a difference in protein structure contributes to the distinct forms of T200 glycoprotein found on these functional T cell subsets.

*Received for publication 19 December 1985 and in revised form 20 February 1986.*

### References

1. Trowbridge, I. S. 1978. Interspecies spleen-myeloma hybrid producing monoclonal antibodies against the mouse lymphocyte surface glycoprotein, T200. *J. Exp. Med.* 148:313.
2. Michaelson, J., M. Scheid, and E. A. Boyse. 1979. Biochemical features of Ly-5 alloantigen. *Immunogenetics.* 9:103.
3. Scheid, M. P., and D. Triglia. 1979. Further description of the Ly-5 system. *Immunogenetics.* 9:423.
4. Kasai, M., J. C. Leclerc, F. W. Shen, and H. Cantor. 1979. Identification of Ly-5 on the surface of natural killer cells in normal and athymic inbred mouse strains. *Immunogenetics.* 8:153.
5. Nakayama, E., H. Shiku, E. Stockert, H. F. Oettgen, and L. J. Old. 1979. Cytotoxic T cells: Lyt phenotype and blocking of killing activity by Lyt antisera. *Proc. Natl. Acad. Sci. USA.* 76:1977.
6. Yakura, H., F. W. Shen, E. Bourcet, and E. A. Boyse. 1983. On the function of Ly-5 in the regulation of antigen-driven B cell differentiation. *J. Exp. Med.* 157:1077.
7. Ledbetter, J. A., L. M. Rose, C. E. Spooner, P. G. Beatty, P. J. Martin, and E. A. Clark. 1985. Antibodies to common leukocyte antigen p220 influence human T cell proliferation by modifying IL-2 receptor expression. *J. Immunol.* 135:1819.
8. Spickett, G. P., M. R. Brandon, D. W. Mason, A. F. Williams, and G. R. Woollett. 1983. MRC OX-22, a monoclonal antibody that labels a new subset of T lymphocytes and reacts with the high molecular weight form of the leukocyte common antigen. *J. Exp. Med.* 158:795.
9. Woollett, G. R., A. N. Barclay, M. Puklavec, and A. F. Williams. 1985. Molecular and antigenic heterogeneity of the rat leukocyte-common antigen from thymocytes and T and B lymphocytes. *Eur. J. Immunol.* 15:168.
10. Thomas, M. L., A. N. Barclay, J. Gagnon, and A. F. Williams. 1985. Evidence from cDNA clones that the rat leukocyte-common antigen (T200) spans the lipid bilayer and contains a cytoplasmic domain of 80,000  $M_r$ . *Cell.* 41:83.
11. Lefrançois, L., and M. J. Bevan. 1985. Functional modifications of cytotoxic T-

- lymphocyte T200 glycoprotein recognized by monoclonal antibodies. *Nature (Lond.)*. 314:449.
12. Lefrançois, L., and M. J. Bevan. 1985b. Novel antigenic determinants of the T200 glycoprotein expressed preferentially by activated cytotoxic T cells. *J. Immunol.* 135:374.
  13. Lefrançois, L., L. Puddington, C. E. Machamer, and M. J. Bevan. Acquisition of cytotoxic T lymphocyte-specific carbohydrate differentiation antigens. *J. Exp. Med.* 162:1275.
  14. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*. 18:5294.
  15. Gatti, R. A., P. Concannon, and W. Salser. 1984. *Biotechniques*. May/June:148.
  16. Paige, C. J., B. W. Kincade, and P. Ralph. 1978. Murine B cell leukemia line with inducible surface immunoglobulin expression. *J. Immunol.* 121:641.
  17. Tarentino, A. L., and F. Maley. 1974. Purification and properties of an endo- $\beta$ -acetylglucosaminidase from *Streptomyces griseus*. *J. Biol. Chem.* 249:811.
  18. Hanover, J. A., and W. J. Lennarz. 1981. Transmembrane assembly of membrane and secretory glycoproteins. *Arch. Biochem. Biophys.* 211:1.
  19. Early, P., J. Rogers, M. Davis, K. Calame, M. Bond, R. Wall, and L. Hood. 1980. Two mRNAs can be produced by a single immunoglobulin gene by alternative RNA splicing pathways. *Cell*. 20:313.
  20. Kornblihtt, A. R., K. Vibe-Petersen, and F. E. Baralle. 1984. Human fibronectin: Molecular cloning evidence for two mRNA species differing by an internal segment coding for a structural domain. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:221.
  21. Nabeshima, Y., Y. Fujii-Kuriyama, M. Muramatsu, and K. Ogata. 1984. Alternative transcription and two modes of splicing result in two myosin light chains from one gene. *Nature (Lond.)*. 308:333.
  22. Lefrançois, L., and O. Kanagawa. 1986. Coordinate expression of carbohydrate differentiation antigens and cytolytic activity in a T cell hybridoma. *J. Immunol.* 136:1171.
  23. Bourguignon, L. Y. W., S. J. Suchard, M. L. Nagpol, and J. R. Glenney. 1985. A T-lymphoma transmembrane glycoprotein (Gp180) is linked to the cytoskeletal protein, fodrin. *J. Cell. Biol.* 101:477.
  24. Shen, F. W., Y. Saga, G. Litman, G. Freeman, J. S. Tung, H. Canton, and E. A. Boyse. 1985. Cloning of Ly-5 cDNA. *Proc. Natl. Acad. Sci. USA.* 82:7360.
  25. Bruce, J., F. W. Symington, T. J. McKearn, and J. Sprint. 1981. A monoclonal antibody discriminating between subsets of T and B cells. *J. Immunol.* 127:2496.