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Cloned murine T200 (Ly-5) cDNA reveals multiple transcripts within B- and T-lymphocyte lineages

(subtractive hybridization/lymphocyte differentiation/regulated gene expression/T200 mutant)

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ABSTRACT **The murine T200 family of cell-surface glycoproteins is expressed on hematopoietic lineage cell types in a developmentally regulated manner with different lymphocyte subpopulations expressing cross-reactive but structurally distinct forms. To investigate the differences between these forms and the regulation of these differences, murine T200 cDNA clones were isolated by using a probe obtained by subtractive hybridization. This procedure made use of a T200$^+$ L-cell transfectant and the parent Ltk$^-$ cell line. The 1.9-kilobase (kb) cDNA clone was sequenced and found to contain the coding region of the COOH-terminal 331 amino acids and 0.9 kb of 3′ untranslated region. Where the sequence overlapped with the rat sequence, 80–90% homology was observed. RNA blot analysis revealed that B-lineage cell lines express either a 5.6-kb or a 6.5-kb mRNA correlating to the size difference of the T200 glycoprotein synthesized. Similarly, in the T-cell lineage a helper T-cell clone and a cytotoxic T-cell clone express T200 transcripts of 5.6 kb and 5.9 kb, respectively, which correlate with the distinct sizes of their T200 glycoproteins. A T200-negative mutant of a T-cell line was found to express full-length T200 mRNA, although at a diminished level.**

The T200 family of cell-surface glycoproteins, which bear the Ly-5 alloantigenic determinant (1), is restricted in expression to cells of hematopoietic lineages (2). An estimated 50,000–100,000 T200 molecules are present on the surface of a lymphocyte, making this component one of the major membrane glycoproteins of these cells (3). This family of structurally related cross-reactive glycoproteins may well be an important element in determining how these cells interact with their environment.

Defined roles of T200 family members is implied from their developmentally regulated distribution among the cell types expressing them. Most T cells, for example, express a 190-kDa form, which unglycosylated has a size of 150 kDa (4, 5). In contrast, most B cells express a 220-kDa T200 molecule, which unglycosylated is 175 kDa (4, 5). Other forms intermediate in size can also be detected (5, 6). Thus, the expression of T200 family members appears tightly regulated during differentiation. The observation that this pattern of regulation is similar in different species, including humans, further implies a fundamental importance that has been conserved during evolution.

To study in greater detail the regulation of expression of T200, we recently reported the transfection of murine lymphoma DNA into L cells, which normally do not express T200, and the selection of a T200$^+$ L-cell line (4). The T200 expressed by the transfectant has the same antigenic and structural properties as the form of T200 expressed by the donor lymphoma with the exception of glycosylation differences. In the study reported here, the T200$^+$ L-cell line is used to select T200 cDNA clones. These clones reveal multiple sizes of T200 mRNA in both the T- and B-cell lineages.

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MATERIALS AND METHODS

Cell Lines. Ltk$^-$ and B10 (a transfected L cell that is T200 positive and thymidine kinase positive) have been reported (4). The properties of the lymphomas (BW5147, BW5147 T200$^-$, WEHI279, X16, 38C13, A20, 18-81, 18-48, 70Z/3, K46, CH1, and BCL_1) and the plasmacytomas (P3, MOPC104E, MOPC315, MPC11, S107, and S194) have been described (4, 5, 7, 8). BB5, a C57BL/6 anti-DBA/2 Mls-reactive helper T-cell clone, was provided by Andrew Glasebrook (Eli Lilly). H7, a C57BL/6 anti-BALB/B cytotoxic T-cell clone specific for minor histocompatibility antigens associated with H-2^b was provided by Michael Bevan (Scripps Clinic and Research Foundation). Lipopolysaccharide and ConA blasts are 2-day cultures of BALB/c spleen cells at 10^6 cells per ml in RPMI 1640 medium, 10% fetal calf serum, and 50 μM 2-mercaptoethanol containing lipopolysaccharide (20 μg/ml) or Con A (2 μg/ml).

mRNA Preparation. The method of mRNA preparation from all other cells utilized the ethanol perchlorate method and is a combination of procedures described by others (9, 10). Exponential phase cells were washed once with cold Hanks' balanced salt solution containing cycloheximide (50 μg/ml) and resuspended in ice-cold lysis buffer [0.14 M NaCl/1.5 mM $MgCl_2$/10 mM Tris·HCl, pH 8.6/cycloheximide (50 μg/ml)]. The suspension was made 10 mM with vanadyl–ribonucleotide complexes (11) and 0.5% (vol/vol) with Nonidet P-40, mixed in a Vortex for 20 sec, kept on ice for 1 min, underlayed with 25% (wt/vol) RNase-free sucrose (Bethesda Research Laboratories) in 10 mM Tris, pH 7.5/1 mM EDTA/1% Nonidet P-40, and spun at 6000 × *g* for 5 min. The turbid upper layer (cytoplasmic fraction) was combined with an equal volume of 2× proteinase K buffer (0.2 M Tris·HCl, pH 7.5/25 mM EDTA/0.3 M NaCl), 2% (wt/vol) $NaDodSO_4$, and proteinase K to give a final concentration of 200 μg/ml. After incubation at 37°C for 10 min, 0.75 vol of 3.5 M $NaClO_4$/50 mM Tris·HCl, pH 7.5, was added and the mixture was heated at 65°C for 5 min. RNA was then precipitated with 10 vol of ethanol perchlorate solution. Ethanol perchlorate was prepared by mixing solution A (200 g of $NaClO_4$ dissolved in 100 ml of water at 65°C and filtered through Whatman no. 1 paper) and solution B (55 g of $NaClO_4$ dissolved in 400 ml of absolute ethanol at 65°C and filtered through Whatman no. 1 paper) and stored at room temperature. After precipitation overnight at 4°C, the RNA was collected by centrifugation at 10,000 × *g* and reprecipitated in isopropanol and ethanol, respectively. Poly(A)$^+$ RNA was selected with oligo(dT)-cellulose. Poly(A)$^+$ RNAs from the

Abbreviation: kb, kilobase(s).

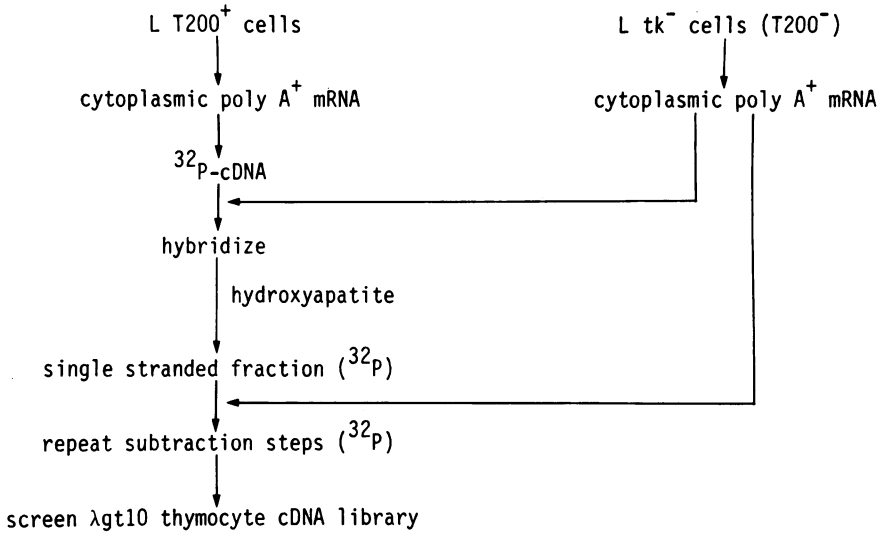


FIG. 1. Subtractive hybridization strategy for obtaining T200 cDNA clones.

BB5 and H7 lines were prepared and generously provided by Leo LaFrancois (Scripps Clinic and Research Foundation).

Subtractive Hybridization and cDNA Library Screening. ^{32}P-labeled single-stranded cDNA was prepared from B10 poly(A)$^+$ RNA using a random primer (Pharmacia) and reverse transcriptase. Two cycles of subtractive hybridization with L-cell poly(A)$^+$ RNA were performed (see Fig. 1) as described by Davis and coworkers (12). A BALB/c thymus cDNA library was generously provided by Howard Gershenfeld and Irving Weissman (Stanford University). The library was prepared by oligo(dT)-primed cDNA synthesis, standard second-strand synthesis, addition of *Eco*RI linkers, insertion into *Eco*RI λgt10 phage arms, and packaging by standard procedures. The "subtracted" single-stranded ^{32}P-labeled cDNA obtained after the final hydroxyapatite separation was used to screen the BALB/c thymocyte λgt10 library as described by Maniatis *et al.* (11).

DNA Sequencing. The dideoxy method was used to obtain the cDNA sequence. Restriction fragments were ligated into M13mp18 and M13mp19 at the appropriate polylinker sites. A series of deletions were prepared from the inserts in M13 by the method described by Dale *et al.* (13).

RNA Blot Analysis. Poly(A)$^+$ RNA (5 μg per lane) was incubated at 65°C for 5 min in 50% formamide/2.2 M formaldehyde/0.02 M morpholinopropanesulfonic acid/5 mM sodium acetate/1 mM EDTA, pH 7.0, and electrophoresed in agarose gels containing 2 M formaldehyde. RNA was blotted onto nitrocellulose paper and hybridized to nick-translated probes (14). RNA sizes were estimated by using eukaryotic and prokaryotic ribosomal RNAs.

RESULTS

Cloning Murine T200 cDNA. The B10 cell line was obtained from the cotransfection of high molecular mass DNA from the BW5147 thymic lymphoma and plasmid DNA containing the herpes simplex thymidine kinase gene into Ltk$^-$ cells (4). The subtractive hybridization regimen (Fig. 1) was chosen to make use of the mRNA differences between the B10 cell line (Ltk$^+$ T200$^+$) and the parent Ltk$^-$ (T200$^-$) line. The subtracted single-stranded ^{32}P-labeled cDNA (Ltk$^+$ T200$^+$ cDNA minus most homologous Ltk$^-$ T200$^-$ mRNA sequences) was used to screen ≈200,000 plaques from the BALB/c thymocyte cDNA library. DNA from positive phage was prepared, nick-translated, and used to probe RNA blots containing mRNA from Ltk$^-$ cells, BW5147 cells, and B10 (Ltk$^+$ T200$^+$) cells. From the first 10 positive plaques, three gave the pattern expected for T200 cDNA by hybridizing with an mRNA species of >5 kilobases (kb) from BW5147 and B10 cells, but giving no signal with Ltk$^-$ mRNA (Fig. 2*A*). Furthermore, the B-cell line WEHI279, which expresses a high molecular mass 220 kDa form of T200, has a larger mRNA that hybridizes to these probes. Interestingly, the B10 line expresses a T200 mRNA intermediate in size, even though it expresses a protein the same size as that in BW5147 cells. The T200 mRNA levels in these positive cell lines parallel the amount of T200 protein produced, with BW5147 synthesizing the most and B10 synthesizing the least (4).

The sizes of the BW5147 and WEHI279 transcripts, as measured by extrapolation using ribosomal RNA standards,

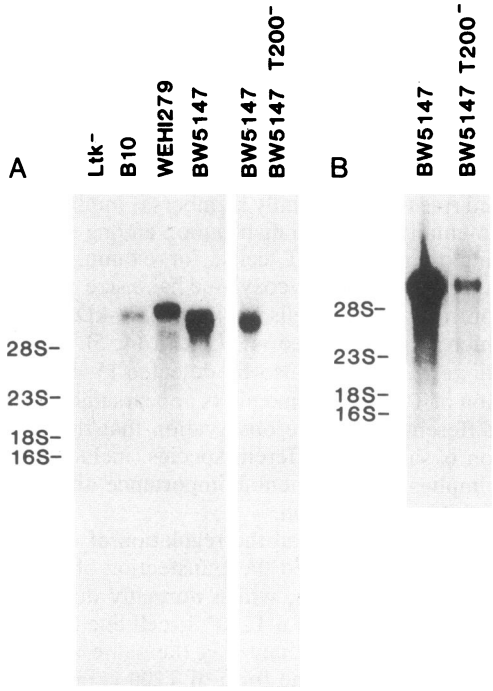
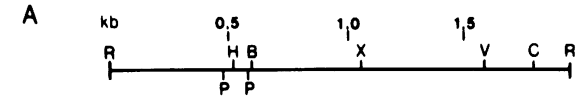


FIG. 2. RNA blot analysis of poly(A)$^+$ mRNA (5 μg) separated on a 1% agarose/formaldehyde gel. (*A*) Total phage DNA from the initial isolation of MT4 was nick-translated and used as the probe. The faint band for BW5147 T200$^-$ is more visible in Figs. 2*B* and 4. (*B*) The presence of T200 mRNA in the BW5147 T200$^-$ mutant is demonstrated with the *Eco*RI fragment of MT4 as the probe.



B

```
          Asn Ser Asn Val Val Pro Tyr Asp Phe Asn Arg Val Pro Leu Lys His Glu Leu   18
Mouse G AAT TCT AAT GTT GTT CCA TAT GAC TTT AAC AGA GTG CCA CTT AAG CAT GAA CTG   55
Rat   - -G- --- --C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A
          Ser

Glu Met Ser Lys Glu Ser Glu Pro Glu Ser Asp Glu Ser Ser Asp Asp Asp Ser Asp Ser   38
GAG ATG AGC AAA GAG AGT GAG CCT GAA TCA GAT GAG TCT TCA GAT GAT GAC AGT GAC TCA  115
--- --- --- --- --- --C --- G-- --- --C --C --A --- --- --- --G --- --- --- --G
                          Ala                                 Glu

Glu Glu Thr Ser Lys Tyr Ile Asn Ala Ser Phe Val Met Ser Tyr Trp Lys Pro Glu Met   58
GAA GAA ACC AGC AAA TAC ATT AAT GCA TCC TTT GTG ATG AGT TAC TGG AAA CCA GAA ATG  175
--- --- --- --- --- --- --- --- --G --A --- --- --- --- --- --- --- --- --- ---

Met Ile Ala Ala Gln Gly Pro Leu Lys Glu Thr Ile Gly Asp Phe Trp Gln Met Ile Phe   78
ATG ATT GCT GCT CAG GGG CCA CTA AAA GAA ACG ATC GGT GAC TTT TGG CAG ATG ATA TTC  235
--- --- --- --C --- --T --- --- --- --G --T --T --- --- --- --- --- --- --- ---

Gln Arg Lys Val Lys Val Ile Val Met Leu Thr Glu Leu Val Asn Gly Asp Gln Glu Val   98
CAA AGA AAA GTC AAA GTT ATT GTG ATG TTG ACA GAG TTA GTG AAT GGA GAC CAG GAA GTC  295
--- --- --- --- --G --- --- --- --- --- --- --- --- A-- -G- --- --- --- --- ---
                                                    Met Ser

Cys Ala Gln Tyr Trp Gly Glu Gly Lys Gln Thr Tyr Gly Asp Met Glu Val Glu Met Lys  118
TGT GCT CAG TAC TGG GGC GAA GGA AAG CAG ACT TAT GGA GAC ATG GAA GTG GAG ATG AAA  355
--- --- --A --- --- --A --- --- --- --- --- --- --- --- --- --- --- --A AT- T-- ---
                                                                Met Leu

Asp Thr Asn Arg Ala Ser Ala Tyr Thr Leu Arg Thr Phe Glu Leu Arg His Ser Lys Arg  138
GAC ACA AAC AGA GCC TCA GCC TAC ACT CTC CGA ACT TTT GAG CTG AGA CAT TCC AAG AGG  415
--- --G --- -A- T-- --- --- --T -T- --G --- G-A --- --A --- --- --- --- --- --A
          Lys Ser              Ile         Ala

Lys Glu Pro Arg Thr Val Tyr Gln Tyr Gln Cys Thr Thr Trp Lys Gly Glu Glu Leu Pro  158
AAG GAG CCC AGA ACT GTG TAC CAG TAC CAG TGT ACC ACA TGG AAA GGG GAA GAG CTG CCT  475
--- --- --T --- --- --- --- --- --- --- --- --- --- --- --- --G --- --C ---

Ala Glu Pro Lys Asp Leu Val Ser Met Ile Gln Asp Leu Lys Gln Lys Leu Pro Lys Ala  178
GCA GAA CCC AAA GAC CTG GTG TCT ATG ATT CAG GAC CTC AAA CAG AAG CTT CCC AAG GCT  535
--- --- --- --- --T --- --- A-- C-- --- --- A-- A-- --- --- --- --- --- --- AG-
                          Thr Leu         Asn Ile                             Ser

Ser Pro Glu Gly Met Lys Tyr His Lys His Ala Ser Ile Leu Val His Cys Arg Asp Gly  198
TCC CCA GAA GGG ATG AAG TAT CAC AAG CAT GCA TCC ATC CTC GTC CAC TGC AGA GAT GGA  595
GG- T-- --- --- --- --- --C --- --- --- --- --A --- --A --- --- --- --G --- ---
Gly Ser

Ser Gln Gln Thr Gly Leu Phe Cys Ala Leu Phe Asn Leu Leu Glu Ser Ala Glu Thr Glu  218
TCC CAG CAG ACA GGG TTG TTC TGT GCC TTG TTC AAT CTC TTG GAA AGT GCA GAA ACA GAA  655
--- --- --- --G --- --- --- --- --T C-- --- --- --- C-- --- --- --- --- --- ---

Asp Val Val Asp Val Phe Gln Val Val Lys Ser Leu Arg Lys Ala Arg Pro Gly Val Val  238
GAT GTG GTT GAT GTT TTC CAA GTG GTA AAG TCT CTA CGC AAA GCA CGG CCT GGG GTG GTG  715
--- --- --- --- --- --T --- --- --- --- --- --- --- --- --- --- --G --- A-- ---
                                                                      Met

Cys Ser Tyr Glu Gln Tyr Gln Phe Leu Tyr Asp Ile Ile Ala Ser Ile Tyr Pro Ala Gln  258
TGC AGC TAT GAG CAA TAC CAG TTC CTC TAT GAC ATC ATC GCC AGC ATC TAT CCC GCC CAG  775
G-- --- -T- --- --- --- --- --- --- --- --- --- --G --- --- --- --- --- A-- ---
Gly     Phe                                     Met                     Thr

Asn Gly Gln Val Lys Lys Thr Asn Ser Gln Asp Lys Ile Glu Phe His Asn Glu Val Asp  278
AAT GGA CAA GTC AAG AAA ACA AAC AGC CAA GAC AAA ATT GAA TTT CAT AAT GAA GTG GAT  835
--- --- --- --G --- --- G-- --- --- --- --- --- --- --- --- --- --C --- --- --C
                          Ala

Gly Gly Lys Gln Asp Ala Asn Cys Val Arg Pro Asp Gly Pro Leu Asn Lys Ala Gln Glu  298
GGA GGC AAG CAG GAT GCT AAC TGT GTC CGT CCA GAT GGT CCT CTG AAT AAA GCC CAG GAA  895
--- -C- --- --- --C --A --- --- --T -AG --- -C- -A- --- --- --C --- --- --- ---
    Ala                           Gln     Ala Asp

Asp Ser Arg Gly Val Gly Thr Pro Glu Pro Thr Asn Ser Ala Glu Glu Pro Glu His Ala  318
GAC AGC AGA GGG GTG GGA ACC CCG GAG CCT ACC AAT AGT GCT GAG GAA CCA GAA CAT GCT  955
--- --- -A- -AA --T --- G-T T-A --- --- G-A -G- G-- T-- --- --G --- --- --- T--
          Lys Glu         Ala Ser         Ala Ser Gly Ser                     Ser

Ala Asn Gly Ser Ala Ser Pro Ala Pro Thr Gln Ser Ser ***                        331
GCC AAT GGT TCT GCG AGC CCA GCT CCA ACC CAG AGT TCA TAGGAAAGGAGTCATGTGGGACAACGC 1021
--A --- --- C-C AT- --- --- --- -T- --- -C- --C --- ------C---T--GA------------
            Pro Met             Leu     Pro

AGACTCTCACATTAGTTCTTTCTATTTTTCTAGACCTAATGAAAGAACATGGCTGTGCAGTGGTTTATGGAATCTGTGT 1000
-A-A-G--A-------G---------------------------A--T--------A-------CCG------------

TCACCTTTGCCACTGTATAAAAATATTTAAGTTTGTCAAAACATTTTGTACAGTTTTATGCTTATTT AAAAGTGTATC 1178
-------------------------------------------GG-------------------C-----T----A------

TATGTCATTCAGCAGGAATGTATATGTGAGAGAGGTGTCTGTGTGTGTGAGAGTGTGTTTATGTATGAGTGACTGTGTG 1257
--A------T----A--G---G-G-AG----- TA----------A----------GCT-C-G-T----TG---T-

TGTGCATGTTTGTGCGTGTGTATGACATCTAAATGTGATTGGAGAATACTTTCAAGCCATTTCAAATGCTTTCGAGAAA 1336

CAGTGTGCCTTTTCTCCTCTTGAGGAAACTATACATTTTATATCTAAACTGTTAATTTGTTTGAGGGATTAATTTTTTA 1415

AAATCCCATTGAAAGTGGATTCAGTTGTAAGAATAACAATGTGTACCATTCTGGAATGACCTCAAGGTGTCCTCCTTGT 1494

CCTGTTGATGATCTTGTAGTTTAAGATGCTCTTTTTGGATATAGATAAGCGTATGTAAGAGTGCTGTGGTGTGTACAGC 1573

TGATCTGGGACGTGAACAAAATCAACATGTGAGACTTATGTTCCATATACTGTCATTTCATCACTATCTCTTAATGCAT 1652

ATTTAATCAAACATGAAAATCTCAAGGGAGACTATTTTTGTATCCACATGGGAAGTAGAACATTGCAAGTCAGTTGCTG 1731

TCTACACAATAGATAAAAATTACTAGTTAATGCTCTTGGTCATATCGATATATGCTATGAACCTAAATAATTGCCCTTA 1810

GCCAAATATAATGTATGTTAAAAACACATAGAATAAAAACAGGGGCATGAAAACTTGTTTGTACTGAATATTTACATAG 1889

GTAACCTCGTACAGTTAGTTCTGTTATGGAATTC                                              1923
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are 5.6 kb and 6.5 kb, respectively. These values differ substantially from those reported by others [4.8 and 5.5 kb for rat (15) and 4.7 and 5.0 kb for mouse (16)]. The discrepancy is most likely due to the technical difficulty inherent in measuring accurate mRNA sizes in this range. However, from the 150-kDa size of the nonglycosylated BW5147 T200 protein (4.5 kb) and the length of the 3′ untranslated region sequenced, 926 base pairs (see Fig. 3), the BW5147 T200 mRNA can be estimated to be a minimum of 5.0 kb, exclusive of the remaining 3′ untranslated region and probable 5′ leader and untranslated regions. The larger form of WEHI279 is 30–40 kDa larger (4, 5) and would require a transcript with an additional 1.0 kb of coding sequence.

For additional evidence that these clones and the mRNA they detect contain T200 sequences, use was made of the $T200^-$ mutant of BW5147, which expresses no detectable T200 protein (7). A dramatically reduced but detectable level of T200 mRNA was found in the BW5147 mutant (Fig. 2 *A* and *B*). Quantitation by excising and counting the radioactive bands revealed the T200 mRNA level in the mutant to be 11 times less abundant than in the wild-type BW5147 line.

T200 cDNA Partial Sequence. The longest of the three initial T200 cDNA clones, MT4, completely overlaps the regions contained in the other two (650 and 1200 base pairs). The 1.9-kb *Eco*RI insert of MT4 was placed into M13 and sequenced. The sequence of the 1.9-kb fragment is given in Fig. 3, as are the differences with the rat sequence (15).

The similarity of the mouse and rat sequences is evident. The amino acid sequences are 88% homologous for this COOH-terminal region with 90% nucleic acid homology through the termination codon. Interestingly, the 3′ untranslated region is nearly as conserved (84% homology) between rat and mouse as the portion of the coding sequence thus far analyzed. Termination occurs at the same site and one possible site of serine phosphorylation at amino acid residues 27–40 is conserved with only an aspartate/glutamate exchange at position 34. No hydrophobic sequence that could represent a transmembrane region is apparent, as anticipated from the predicted location of this region in the rat, ≈1 kb 5′ to the sequence in Fig. 3 (15). The restriction map for this region is similar to that reported for another mouse T200 cDNA (16). The partial sequence of that clone shares no homology with the sequence given here and presumably represents a more 5′ region.

Multiple Forms of T200 mRNA Expression in B-Cell Lines. The availability of cDNA clones makes possible the investigation of the molecular mechanisms responsible for T200 size differences in lymphocytes. Previous studies showed that B-cell lines express different sizes of T200 protein (6). In general, immature B lymphomas and mature mIg^+ B lymphomas synthesize a 220-kDa form of T200, although some express a smaller 190-kDa molecule. Plasmacytomas, however, if they express detectable T200 at all, synthesize

FIG. 3. (*A*) Restriction map and (*B*) nucleotide sequence of the *Eco*RI fragment of MT4 T200 cDNA clone. The restriction enzymes are indicated as follows: B, *Bam*HI; C, *Cla* I; H, *Hin*dIII; P, *Pst* I; R, *Eco*RI; V, *Pvu* II; X, *Xba* I. The comparison with nucleotides 2286–3541 of the rat cDNA sequence (15) is shown with shared sequence indicated by a dash. One-nucleotide gaps after position 1167 in the mouse sequence and at the position in the rat sequence corresponding to mouse residue 1211 are included to maximize homology. The termination codon is designated with asterisks. Even though *Eco*RI linkers were used in the construction of the cDNA library, the 5′ *Eco*RI site of this insert is authentic, since additional cDNA clones have now been selected that overlap and confirm this restriction site. The 3′ *Eco*RI site may be authentic as well based on the isolation of 16 more T200 cDNA clones from this library having the same *Eco*RI fragment as MT4. Thus, the total cDNA insert of MT4 probably extends short distances 3′ and 5′ from the fragment sequenced.

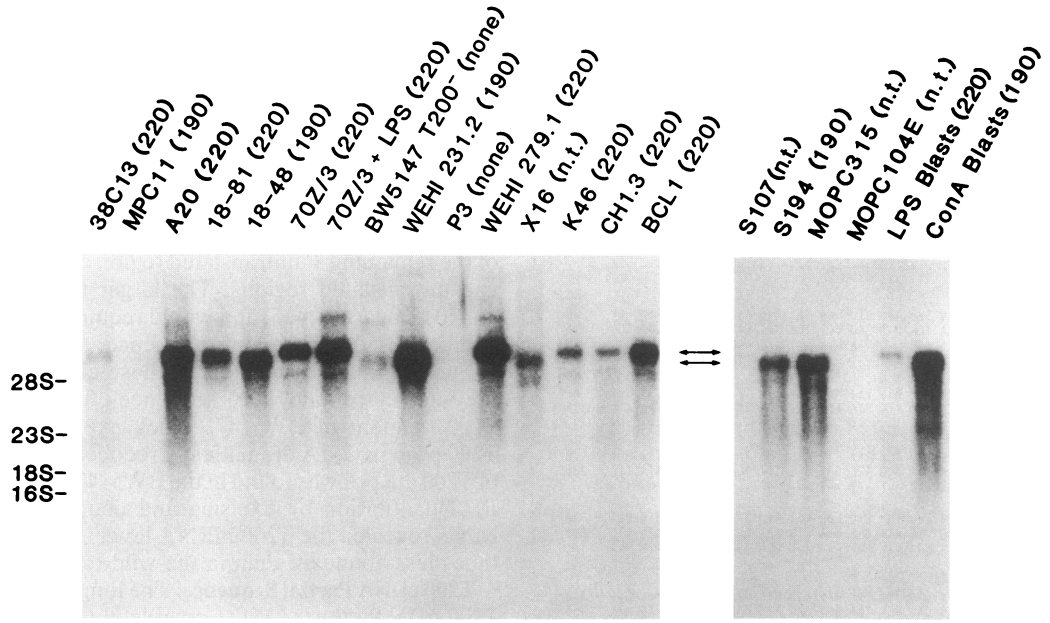

FIG. 4. T200 mRNA of B-cell lineage lines. The probe and gel conditions are the same as in Fig. 1*B*. The size of the T200 glycoprotein expressed by the cell line is given in parentheses in kilodaltons. "None" indicates no T200 glycoprotein is detectable, and "n.t." indicates not tested.

primarily a 190-kDa form of T200. Most normal B cells express the larger form.

RNA blot analysis of T200 mRNA from a variety of B-cell lines representing various differentiation states was performed and the results are shown in Fig. 4. The T200 mRNA sizes fall into two major groups. Those cell lines synthesizing a 200-kDa T200 glycoprotein express a larger mRNA (6.5 kb) than those that synthesize a 190-kDa T200 molecule (5.6 kb). [Protein data for some of the cell lines have been reported (6).] The surface IgM-positive B-cell lines BCL1, CH1.3, K46, WEHI279, A20, and 38C13 exhibit the larger T200 mRNA and a 220-kDa glycoprotein, whereas X16 and WEHI231 express the smaller mRNA and a 190-kDa glycoprotein. Among the pre-B-cell lines, 70Z/3 (cytoplasmic μ only) has the 6.5-kb mRNA, 18-48 (cytoplasmic κ only) the 5.6-kb mRNA, and 18-81 (cytoplasmic μ only) an apparently intermediate size T200 mRNA. 70Z/3 and 18-81 cells express a 220-kDa T200 glycoprotein form on the cell surface (6); 18-48 cells express the 190-kDa form. Also shown in Fig. 4 is the result of treatment of 70Z/3 cells with lipopolysaccharide for 24 hr, a procedure that induces synthesis of κ light chains in these cells (17). A moderate increase in 70Z/3 T200 mRNA results. The plasmacytomas P3 (κIgG1), MPC11 (κIgG2b), and S194 (κIgA), which express undetectable, very low, and moderate levels of 190-kDa T200, respectively, show corresponding amounts of 5.6-kb T200 mRNA relative to each other and the other cell lines. The MPC11 T200 mRNA band is barely detectable and is present at a substantially lower level than the BW5147 T200⁻ mutant (Fig. 4). P3 has no detectable T200 mRNA. Other plasmacytomas, S107 (κIgA) and MOPC104E (λIgM), also have undetectable mRNA, whereas MOPC315 (λIgA) expresses the 5.6-kb mRNA. Thus, the size of the transcript and the level of the transcript generally correlate with the size and the level of expression of the T200 glycoprotein. No correlation with immunoglobulin class is seen.

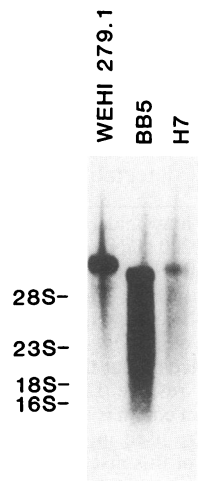

FIG. 5. T200 mRNAs from H7, a cytotoxic T-cell clone, and BB5, a helper T-cell clone. The probe and gel conditions are the same as in Fig. 1*B*.

Multiple Forms of T200 mRNA Expression in T-Cell Clones. Cytotoxic Ly-2,-3-positive T-cell clones have been reported to have a higher molecular mass T200 than Ly-1-positive T cell clones (18, 19). Since the monoclonal antibodies specific for the cytotoxic T-cell form of T200 block the cytolytic function of these cells, it has been proposed that the structural differences have functional significance (19). RNA blot analysis of mRNA from a helper T-cell clone and cytotoxic T-cell clone indicates different sizes of T200 mRNA in these lines, 5.6 kb and 5.9 kb, respectively, which correspond with the relative sizes of the T200 glycoprotein expressed (Fig. 5). Furthermore, the larger T200 mRNA of the cytotoxic clone is smaller than that of the B-cell lines as represented by WEHI279. The reported size of the glycosylated cytotoxic T-cell T200 molecule is 210–215 kDa (18, 19), which is consistent with the intermediate size of the transcript.

DISCUSSION

To clone T200 cDNA, use was made of an L-cell clone transfected with lymphoma DNA that had been selected for cell-surface expression of lymphocyte T200 glycoprotein (4). Since the first screening of the thymocyte cDNA library with the subtracted cDNA probe yielded T200 cDNA clones,

which was the goal of the project, information concerning the complexity of the subtracted probe or the nature of the mRNA differences between the transfected B10 T200$^+$ line and the Ltk$^-$ line was not pursued. By rescreening the cDNA library with the MT4 T200 cDNA insert, the frequency of T200 clones was found to be high, $\approx$1 in 2–4 $\times$ 10^3 plaques. The level of T200 mRNA in the cell lines is also relatively high, with 0.5 μg of poly(A)$^+$ RNA giving a strong signal in RNA blot analysis in most cases. With T200 mRNA present as a moderately abundant message and a cDNA library reflecting this abundance, the system was favorable for selecting T200 clones. Although not specifically tested, the subtracted probe should have been greatly enriched for herpes thymidine kinase sequences. These viral sequences, however, would not be expected to find complementary sequences in the thymocyte cDNA library.

In the initial design of the cDNA cloning an alternative pair of cell lines, BW5147 and the mutant BW5147 T200$^-$ (7), was considered for the subtractive hybridization. Since the nature of the genetic defect in the BW5147 mutant was not known, the possibility existed that T200 mRNA could still be made and undermine the subtraction process. The Ltk$^-$ line was deemed most likely to be negative for T200 mRNA by nature of originating from a cell lineage that does not express the T200 protein. The finding that the T200-negative mutant of BW5147 synthesizes T200 mRNA substantiated the choice of the L-cell combination for the subtractive hybridization. However, in view of the results from cell-surface analysis and biosynthetic labeling studies on the BW5147 T200$^-$ mutant, which showed no detectable synthesis of T200 protein, the finding of a low level of full-length T200 mRNA is noteworthy. Clearly, the promoter and other transcription regulatory components are functional and full-sized T200 mRNA is transcribed in spite of the apparent lack of translation. Although a number of explanations are possible, the most likely include a point mutation in the coding sequence generating a termination codon or a small addition or deletion that alters the reading frame. The quantitative difference in T200 mRNA levels between the wild-type and mutant BW5147 lines is possibly due to different turnover rates.

At least three sizes of T200 transcripts have been detected in lymphoid cells that encode T200 proteins that differ in size and possibly function on distinct cell types. Cell lines of the B-cell lineage express either a 5.6-kb T200 mRNA and a 190-kDa T200 glycoprotein or a 6.5-kb mRNA and a 220-kDa glycoprotein. The T-cell line BW5147 and the helper T-cell clone express mRNA and glycoprotein of sizes similar to the smaller B-cell forms, whereas a cytotoxic T-cell clone expresses a 5.9-kb T200 mRNA and a 210-kDa T200 glycoprotein. These differences are demonstrated on the basis of size. Although the T200 mRNA and glycoprotein are similar in size in some B-lineage cells and in some T-lineage cells, the structures of these molecules in these lineages as well as their role may well be different.

Not only does the size of the T200 mRNA correlate with the size of the glycoprotein, but the level of expression of T200 mRNA also parallels the amount of T200 expressed at the cell surface (with the exception of the BW5147 T200$^-$ mutant). The mRNA size difference is most likely not due to multiple T200 genes, since restriction analysis of genomic DNA infers a single murine T200 gene (data not shown), a conclusion also reached for the rat (15). Thus, the findings of this study are consistent with the hypothesis that the form and the amount of T200 glycoprotein expressed is regulated at the mRNA level.

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