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Immunology

Molecular cloning of Lyt-2, a membrane glycoprotein marking a subset of mouse T lymphocytes: Molecular homology to its human counterpart, Leu-2/T8, and to immunoglobulin variable regions

(cytotoxic/suppressor/differentiation antigen/transfectants/fluorescence-activated cell sorter)

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ABSTRACT **The sequence of Lyt-2 cDNA shows that it is a new member of the immunoglobulin super gene family. Analysis of the predicted amino acid sequence indicates that the Lyt-2 polypeptide is synthesized with a 27-amino acid leader, and that the mature protein has an immunoglobulin variable region (Ig V)-related sequence of ≈100 amino acids, an extracellular spacer of 43, a transmembrane region of 38, and an intracytoplasmic region of 27 amino acids. Lyt-2 and its human analogue Leu-2 are 56% homologous; analysis indicates that the Ig V-related domains of the two molecules have evolved away from each other faster than the carboxyl-terminal half of the proteins.**

Suppressor and cytotoxic T lymphocytes (CTLs) of mice are distinguished by the expression of the lymphocyte differentiation molecule Lyt-2,3 (1, 2). Human T lymphocytes with analogous functions express the molecule Leu-2 (T8, CD8) (3–5). We had postulated that these molecules are not only functionally but also structurally homologous (6). Monoclonal antibodies against the Lyt-2,3 or Leu-2 molecules in the two species block cytotoxic activity of most CTLs that bear them (7–9). This is apparently because Lyt-2,3 and Leu-2 serve an accessory function in the binding of CTL to target major histocompatibility complex class I molecules (9). The role of Lyt-2 (or Leu-2) is indicated to be binding rather than killing, because CTLs bound to target cells with a lectin such as concanavalin A can kill even in the presence of anti-Lyt-2 or anti-Leu-2 antibody (10).

The Lyt-2 antigen is found on 34-kDa and 38-kDa polypeptides disulfide-linked to Lyt-3 primarily as heteromultimers (11). On peripheral T cells and some T lymphomas, a small fraction of Lyt-2 exist also as homodimers (unpublished observations). The Leu-2 antigen is similarly found on a 32-kDa chain, which is disulfide-linked with another polypeptide (CD1 or T6) on thymocytes or with another Leu-2 peptide on peripheral blood lymphocytes to form heterodimers or homodimers (12, 13).

To determine the detailed structure of Leu-2 (and subsequently Lyt-2), we isolated molecular clones of cDNA and genomic DNA coding for Leu-2 (14) by using a subtractive cDNA approach with L-cell transfectants selected with the fluorescence-activated cell sorter (FACS) after DNA-mediated gene transfer (14, 15). In this paper, we describe the isolation of Lyt-2 cDNA and genomic clones, present the predicted amino acid sequence and the general structure of the Lyt-2 polypeptide, and compare the Lyt-2 sequence with that of Leu-2 as described (16).

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METHODS

Transfection and Cell Staining. The thymidine kinase deficient (TK^-) L cells were transfected with 19 μg of BALB/c liver cellular DNA, and 1 μg of pBR322 containing chicken *TK* gene (per 10^6 cells) by a calcium phosphate procedure (17, 18). Some of the dishes received 1 μg of pBR322 or phage DNA containing the putative Lyt-2 genomic clone. After 2 weeks of selection in hypoxanthine/aminopterin/thymidine (HAT) medium, TK^+ L cells were stained with rat monoclonal anti-Lyt-2 antibody (53-6.7) (19). Positive cells were selected by sorting, using FACS as described (15).

Immunoprecipitation and $NaDodSO_4$ Gel Electrophoresis. BALB/c thymocytes or Lyt-2 transformants were labeled with ^{125}I by the lactoperoxidase method. Immunoprecipitation and $NaDodSO_4$ gel electrophoresis were carried out as described by Jones (20).

Cloning of Lyt-2 cDNA and Genomic Gene. A BALB/c thymocyte cDNA library (kindly provided by C. Benoit) was plated on *Escherichia coli* C600/HFL. Low stringency hybridization was in 6× SSPE with 0.1% $NaDodSO_4$/4× SSPE washings at 65°C (1× SSPE = 180 mM NaCl/10 mM $NaPO_4$, pH 7.7/1 mM EDTA). A confirmed Lyt-2 cDNA clone (see *Results*) was then used to pick full-length cDNA clones from a C57BL/6 thymocyte cDNA library in λgt10 cloning vector (kindly provided by H. Gershenfeld and I. Weissman) and a genomic clone from a B10.A liver genomic library in λj1 cloning vector (21), with washing done at high stringency (0.1% $NaDodSO_4$/0.1× SSPE).

DNA and RNA Hybridization. DNA was digested and analyzed by the Southern blot technique (22). RNA was prepared from cells or tissue by the guanidium thiocyanate procedure (23) and analyzed by RNA blot hybridization (24).

Sequencing. DNA nucleotide sequence was determined by the method of Sanger *et al.* (25) after subcloning restriction endonuclease fragments into M13 mp18 and mp19 phage vectors.

Computer Analysis. Sequences were analyzed on a VAX 11/780 computer using the programs described by Staden (26, 27). Alignment of cDNA, translation of cDNA, and amino acid alignment of Lyt-2 and Leu-2 sequence was done using the BIONET system (National Institutes of Health Grant 1 U41 RR01681-01) and the DB system (28). The Dayhoff Protein sequence bank was searched by the program described by Lipman and Pearson (29).

RESULTS

Establishment of Lyt-2 Transfectants. We found the frequency of Lyt-2 transformants after 2 weeks of HAT selec-

Abbreviations: FACS, fluorescence-activated cell sorter; TK, thymidine kinase; CTL, cytotoxic T lymphocyte; Ig V, immunoglobulin variable region; kb, kilobase(s); bp, base pair(s).

tion to be 10^{-4} to 10^{-3} of the TK^+ cells. Interestingly, as we found with Leu-2 transfection, some of the transfectants had higher amounts of Lyt-2 on the cell surface than others. These transformants, therefore, resembled the amplifying transformants we found in 25–50% of Leu-2 transformants (30). After several more rounds of selection for the most brightly staining cells, we established two cloned Lyt-2 amplified cell lines (S2K9, S2Q9). The mean fluorescence of these cells was >10 times greater than that of the original transfectants. The presence of Lyt-2 molecules on these amplified transformants was confirmed by immunoprecipitation and two-dimensional gel analysis (31). We have not detected Lyt-3 determinants on transformants obtained with either total cellular DNA or cloned Lyt-2 coding DNA fragments.

Screening of a Mouse Thymocyte cDNA Library with a Leu-2 cDNA Probe. We screened a BALB/c mouse thymocyte cDNA library (C. Benoit) using our 1.7-kilobase (kb) Leu-2 cDNA as a probe under conditions of low stringency hybridization. Of 152,000 plaques screened, 11 positive phage clones were isolated. After *Eco*RI digestion, each cDNA insert was purified and labeled with ^{32}P by nick-translation. The probe was hybridized to genomic DNA from L cells, and from the two amplified Lyt-2 cell lines, S2K9 and S2Q9 (whose isolation was described above). The cDNA inserts ranged in size from 300 to 1000 base pairs (bp); four were 650 bp long. Only these four gave very intense bands with the DNA obtained from the two amplified lines and a band of the same size (5.2 kb when digested with *Hin*dIII) at single copy intensity with L-cell DNA (Fig. 1*a*). These 650-bp inserts also hybridized strongly with RNA from the amplified transformants and not with L-cell RNA on an RNA blot (data not shown). These four hybridized with each other and not with any of the remaining cDNA inserts. We designated a pSP65 plasmid with this insert in the RI site pLY2C-1 (Fig. 2*b*).

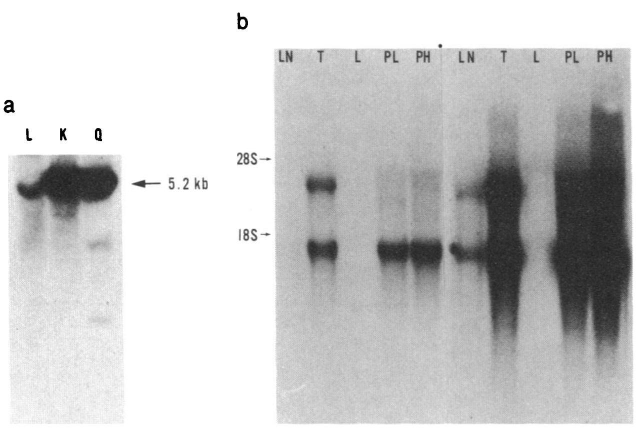

FIG. 1. (*a*) Autoradiogram of Southern blot showing that the Lyt-2 gene is greatly amplified in the genomic DNA obtained from two Lyt-2 amplified transformants. Fifteen micrograms of DNA from L cells (lane L) and 10 μg from the two Lyt-2 amplified lines (lanes K and Q) were digested with *Hin*dIII, electrophoresed through a 0.8% agarose gel, transferred to nitrocellulose filter paper, and hybridized with a ^{32}P-labeled insert of pLY2C-1. (*b*) Autoradiogram of RNA blot from different sources hybridized to a nick-translated Lyt-2 cDNA (pLY2C-1). Fifteen micrograms of total RNA obtained from lymph node cells (lanes LN), thymocytes (lanes T), L cells (lanes L), and two Lyt-2 transfectants (lanes PL and PH), which were transfected with p6CCA plasmid or L-6CCA phage DNA containing Lyt-2 genomic insert, respectively, were loaded in each lane. Two different sized bands are seen in lanes LN and T (1.7 and 3.0 kb), but only the 1.7-kb band is seen in lanes PL and PH. Longer exposure of the same filter (shown on right) allowed visualization of the bands in lane LN as well as multiple distinct bands from 2.5 to 3.8 kb in lanes PL and PH.

Lyt-2 transformants, thymocytes, and lymph node cells revealed two mRNA species, of ≈1.7 and 3.0 kb, which hybridize to nick-translated pLY2C-1. We screened a second thymocyte cDNA library, from a C57BL/6 thymocyte (H.

a

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                                                                         GGAGAGCACACC

-27                         -20                                         -10
ATG GCC TCA CCG TTG ACC CGC TTT CTG TCG CTG AAC CTG CTG CTG CTG GGT GAG TCG ATT
MET Ala Ser Pro Leu Thr Arg Phe Leu Ser Leu Asn Leu Leu Leu Leu Gly Glu Ser Ile

                        -1  1                                       10
ATC CTG GGG AGT GGA GAA GCT AAG CCA CAG GCA CCC GAA CTC CGA ATC TTT CCA AAG AAA
Ile Leu Gly Ser Gly Glu Ala Lys Pro Gln Ala Pro Glu Leu Arg Ile Phe Pro Lys Lys

                        20                          ↓               30
ATG GAC GCC GAA CTT GGT CAG AAG GTG GAC CTG GTA TGT GAA GTG TTG GGG TCC GTT TCG
MET Asp Ala Glu Leu Gly Gln Lys Val Asp Leu Val Cys Glu Val Leu Gly Ser Val Ser

                        40      ***                                 50
CAA GGA TGC TCT TGG CTC TTC CAG AAC TCC AGC TCC AAA CTC CCC CAG CCC ACC TTC GTT
Gln Gly Cys Ser Trp Leu Phe Gln Asn Ser Ser Ser Lys Leu Pro Gln Pro Thr Phe Val

                        60                                          ***
GTC TAT ATG GCT TCA TCC CAC AAC AAG ATA ACG TGG GAC GAG AAG CTG AAT TCG TCG AAA
Val Tyr MET Ala Ser Ser His Asn Lys Ile Thr Trp Asp Glu Lys Leu Asn Ser Ser Lys

                        80                                          90
CTG TTT TCT GCC ATG AGG GAC ACG AAT AAT AAG TAC GTT CTC ACC CTG AAC AAG TTC AGC
Leu Phe Ser Ala MET Arg Asp Thr Asn Asn Lys Tyr Val Leu Thr Leu Asn Lys Phe Ser

                        100         ↓                               110
AAG GAA AAC GAA GGC TAC TAT TTC TGC TCA GTC ATC AGC AAC TCG GTG ATG TAC TTC AGT
Lys Glu Asn Glu Gly Tyr Tyr Phe Cys Ser Val Ile Ser Asn Ser Val MET Tyr Phe Ser

                        120         ***                             130
TCT GTC GTG CCA GTC CTT CAG AAA GTG AAC TCT ACT ACT ACC AAG CCA GTG CTG CGA ACT
Ser Val Val Pro Val Leu Gln Lys Val Asn Ser Thr Thr Thr Lys Pro Val Leu Arg Thr

                        140                                         150
CCC TCA CCT GTG CAC CCT ACC GGG ACA TCT CAG CCC CAG AGA CCA GAA GAT TGT CGG CCC
Pro Ser Pro Val His Pro Thr Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro

                        160                                         170
CGT GGC TCA GTG AAG GGG ACC GGA TTG GAC TTC GCC TGT GAT ATT TAC ATC TGG GCA CCC
Arg Gly Ser Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro

                        180                                         190
TTG GCC GGA ATC TGC GTG GCC CTT CTG CTG TCC TTG ATC ATC ACT CTC ATC TGC TAC CAC
Leu Ala Gly Ile Cys Val Ala Leu Leu Leu Ser Leu Ile Ile Thr Leu Ile Cys Tyr His

                        200                                         210
AGG AGC CGA AAG CGT GTT TGC AAA TGT CCC AGG CCG CTA GTC AGA CAG GAA GGC AAG CCC
Arg Ser Arg Lys Arg Val Cys Lys Cys Pro Arg Pro Leu Val Arg Gln Glu Gly Lys Pro

                        220
AGA CCT TCA GAG AAA ATT GTG TAA AATGGCACCGCCAGGAAGCTACAACTACTACATGACTTCAGAGATCT
Arg Pro Ser Glu Lys Ile Val
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b

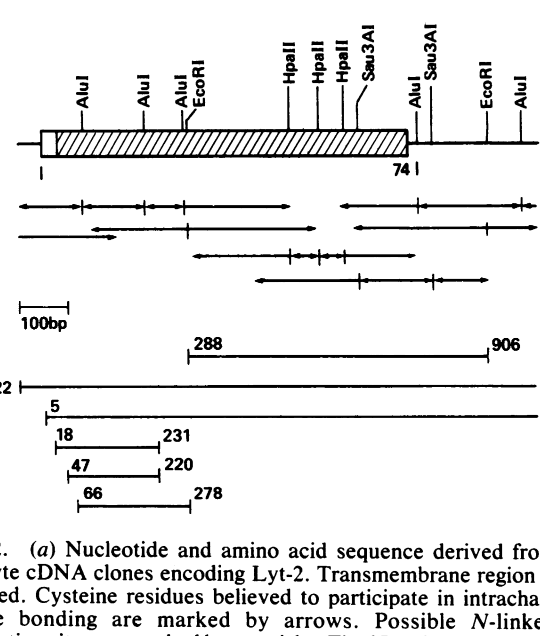

FIG. 2. (*a*) Nucleotide and amino acid sequence derived from thymocyte cDNA clones encoding Lyt-2. Transmembrane region is underlined. Cysteine residues believed to participate in intrachain disulfide bonding are marked by arrows. Possible *N*-linked glycosylation sites are marked by asterisks. The 27 amino acid leader peptide is marked as −27 to −1. Mature peptide is marked from 1 to 220. (*b*) Restriction endonuclease maps of Lyt-2 cDNA clones. Protein coding region is boxed. Shaded region is mature protein after cleavage of leader peptide. DNA sequence strategy is presented below the map. Numbering refers to nucleotide sequence within each clone relative to the ATG of NH_2-terminal methionine.

Gershenfeld and I. Weissman), using pLY2C-1 as a probe, and we obtained the clone pLY2C-22. This clone contains the entire coding sequence plus 12 bp upstream of the ATG start codon.

Since Walker *et al.* reported a major and a minor NH_2-terminal amino acid sequence for Lyt-2 (32), we searched for evidence of more than one Lyt-2 gene. Low stringency Southern hybridization analysis of BALB/c liver genomic DNA using as probe a subclone that lacks the COOH-terminal portion of the protein and 3′ untranslated region of clone pLY2C-16 (bases 992–1393) showed only a single band with *Pst* I and *Hin*dIII digests, consistent with a single gene coding for Lyt-2. Furthermore, we found identical sequences for the NH_2-terminal coding portion of 4 other cDNA clones (C-16, C-27, C-23, C-20) obtained from the C57BL/6 thymocyte cDNA library (Fig. 2*b*).

We screened a B10.A genomic library (21) with the insert of the pLY2C-1 cDNA clone and isolated a λj1 phage (L-6CCA) containing a 15-kb fragment with the Lyt-2 gene. We obtained a subclone in pBR322 (p6CCA) with a 5.2-kb *Hin*dIII fragment, which hybridizes to pLY2C-1. About 25% of TK^+ L cells transfected with either the L-6CCA or p6CCA DNA stained positive for Lyt-2. The presence of the Lyt-2 glycoprotein on cloned transformants was confirmed by immunoprecipitation, showing 38-kDa and 34-kDa polypeptides with multiple charge differences on two-dimensional gel electrophoresis (Fig. 3).

Primary Protein Structure. The nucleotide sequence and predicted amino acid sequence of the Lyt-2 cDNA is presented in Fig. 2*a*. Comparison of the amino acids predicted from the cDNA sequence with the "major" amino acid sequence of purified Lyt-2 protein (32) shows complete correspondence for 16 residues beginning 27 amino acids downstream from the NH_2-terminal methionine (Fig. 2*a*). Hydrophobicity analysis of the protein (data not shown) showed that residues 1–27 have a profile characteristic of hydrophobic leader sequences. These data, taken together with the NH_2-terminal microsequencing data, lead us to conclude that the mature peptide is 220 amino acids (predicted size, 24.7 kDa) and has a leader peptide of 27 residues.

Analysis of the hydrophobicity plot also reveals a region of strong hydrophobicity near the COOH terminus (residues 158–193) followed by a hydrophilic sequence (residues 194–220); these regions we predict to be the transmembrane region and cytoplasmic tail, respectively. Three possible *N*-linked glycosylation sites are present at positions 42, 70, and 123 (Fig. 2*a*). The two different sizes (34 kDa and 38 kDa) of Lyt-2 are reported to be due to differential glycosylation of the same polypeptide sequence (33). Given the observed sizes of Lyt-2 as 34 kDa and 38 kDa, *O*-linked and/or *N*-linked glycosylation must account for 9–14 kDa.

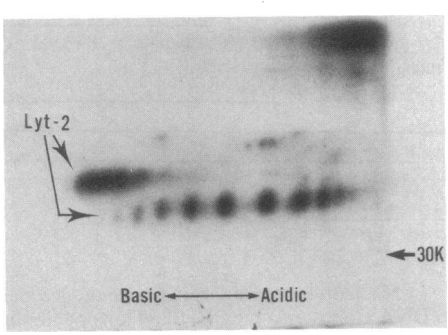

FIG. 3. Two-dimensional gel electrophoresis of the Lyt-2 molecule. A detergent lysate from surface ^{125}I-labeled transfectants generated by using p6CCA DNA was immunoprecipitated with anti-Lyt-2 antibody (53-6.7). The first dimension was a charge separation with the acidic side on the right and the basic side on the left. The second dimension was $NaDodSO_4$/10% polyacrylamide slab gels from the top to the bottom under reducing conditions. 30K, 30 kDa.

Comparison of Lyt-2 and Leu-2. Comparison of Lyt-2 and Leu-2 at the amino acid level (Fig. 4) shows that they are 56% identical after computer alignment of both sequences. The distribution of homology across the two molecules reveals that the variable-like regions (identities, counting gaps as mismatches, are 42%) of the proteins have diverged more than the COOH termini (identities are 64%).

Most notably, there are nine cysteines in each protein which, after computer alignment of the amino acid sequences, with no bias for alignment of cysteines *per se*, all align with their putative homologue. Given that cysteine residues are important in the formation of inter-/intrachain disulfide bonds, the maintenance of these cysteine residues, and the amino acids immediately surrounding them, indicates their probable functional or structural importance. After computer alignment of the sequences, no Lyt-2 potential *N*-linked glycosylation site is located at exactly the one Leu-2 potential glycosylation site. Comparison of the hydrophobicity plots of Lyt-2 and Leu-2 (data not shown) confirms that the hydrophobic and hydrophilic regions are similar in the two proteins, the major difference being a hydrophilic segment just before the transmembrane region in Lyt-2, which is neutral in Leu-2.

Lyt-2 Is a Member of the Immunoglobulin Super Gene Family. A computer search of the Dayhoff protein sequence bank for sequences similar to Lyt-2 indicates that Lyt-2 is homologous to human and mouse κ light chain variable (V) regions (30% identity, requiring 18 gaps in Lyt-2 and κ sequence to give best alignment), heavy chain V regions (20% identity, requiring 9 gaps); clear but lesser homology was observed with mouse Thy-1 (34), λ light chain V regions, and T-cell receptor α- (35, 36), β- (37), and γ-chain V regions (38).

Alignment of the most significant homologies (Fig. 5) suggests that Lyt-2 has an Ig V-like domain. Residues regarded as important for heavy chain Ig domain structure/function such as the cysteines that form the intrachain disulfide bond as well as an invariant tryptophan are also found appropriately located, after alignment, in Lyt-2 and Leu-2 (Lyt-2 cysteine residues 26 and 102; tryptophan residue 38).

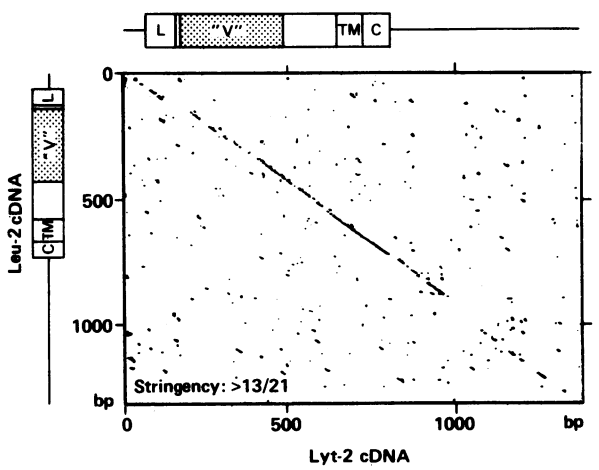

FIG. 4. DNA homology of Lyt-2 and Leu-2. A computer homology representation of the Lyt-2 cDNA sequence versus the Leu-2 cDNA (ref. 16 and unpublished data) using the DIAGON program of Staden (27). Stringency for plotting of homology was (% score = 13)/(odd span length = 21) = 61%. The general structures of the Lyt-2 and Leu-2 proteins in relation to their cDNA coding sequence are presented on the horizontal and vertical axes, respectively.

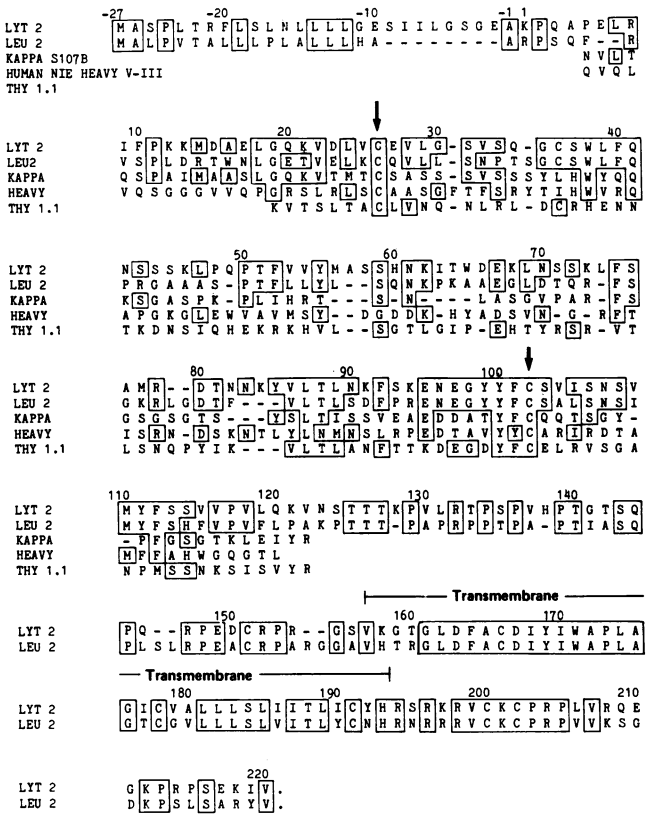

FIG. 5. Lyt-2 homology to other immunologically relevant proteins. Using the BIONET programs IFIND and ALIGN and the protein homology search program of Lipman and Pearson (29), the deduced Lyt-2 protein sequence was aligned against Leu-2 and the most significantly homologous V region sequence of mouse κ light chain, human heavy chain Ig, and the V-like region of Thy-1.1. Homologies of these proteins to Lyt-2 are boxed. The cysteines proposed to make the intrachain disulfide bond to form an Ig-like domain in Lyt-2 and Leu-2 are marked by arrows. The transmembrane region of Lyt-2 is indicated. Amino acids are identified by the single letter code.

Assuming an Ig V-like structure, Lyt-2 resembles a heavy-chain V region (V_H) most in the number of residues between the disulfide-linked cysteines (V_H, 73; V region κ chain, 65; Lyt-2, 75; Leu-2, 71) (39). Because of this observation, we mapped our alignment of Lyt-2 and the Nie Ig V_H region onto a two-dimensional representation of an Ig domain (Fig. 6) (40). Of the 23 residues that are shared between the two proteins, 7 are at positions considered invariant for Ig V regions (39). This can be compared with a total of 14 invariant residues in Ig V-region domains.

DISCUSSION

We have predicted the structure of the Lyt-2 molecule by deduction from DNA sequencing of the cDNA and gene for Lyt-2, and compare it with Leu-2 (T8), which had recently been cloned and sequenced using similar methods (14, 16). We previously suggested the homology of these mouse and human genes from consideration of FACS and $NaDodSO_4$ gel analyses (6). A number of other common properties suggest homology. For example, (*i*) they both are selectively expressed on suppressor/cytotoxic T cells (1–4), (*ii*) monoclonal antibodies against Lyt-2 or Leu-2 block cytotoxicity of most class I but not of class II restricted cytotoxic T cells (7–9), (*iii*) the genes that code for Lyt-2 or Leu-2 are closely linked to the immunoglobulin κ light chain locus in each species (41–43), (*iv*) they are composed of polypeptide subunits that are disulfide-bonded into a variety of multimeric

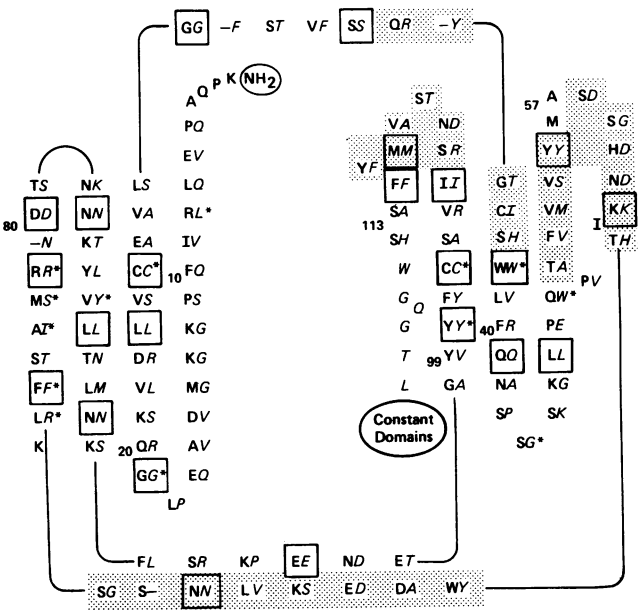

FIG. 6. The Lyt-2 sequence (bold lettering) was mapped, by homology to the human Nie heavy chain V region sequence (italic lettering), onto a two-dimensional representation of the β-pleated sheet structure of the Ig heavy chain V region domain (39). Numbering is from NH_2-terminal of Lyt-2 sequence. Boxed residues indicate homology. Asterisks mark invariant heavy chain Ig V region residues. Shaded regions cover the hypervariable region of heavy chain Ig V domain.

forms (11), (*v*) both genes amplify in a high proportion of transformants after DNA-mediated gene transfection (30, 31).

Here we have shown that by DNA and amino acid sequence comparison, Lyt-2 and Leu-2 are highly homologous to each other and less but clearly homologous with other members of the immunoglobulin super gene family (44). Lyt-2 has a typical leader sequence of 27 amino acids, followed by ≈110 amino acids that show clear homology to Ig V regions. This region also shows homology to Thy-1 (34), OX-2 (45), and T-cell receptor polypeptides (35–38) (data not shown). Thus, these molecules are all related evolutionarily and probably arose from a common ancestral gene. The cDNA sequence of Lyt-2 also codes for a peptide stretch of 43 amino acids COOH-terminal to the region of Ig V homology. The deduced COOH-terminal amino acid sequence has a 38 amino acid hydrophobic stretch, resembling a transmembrane domain, with an abrupt change to a hydrophilic region, probably corresponding to an intracytoplasmic domain of 27 amino acids.

The V region-like domains of Lyt-2 (residues 1–120) and Leu-2 have ≈18% less identity than the COOH-terminal portion of these proteins (Lyt-2 residues 121–220; Fig. 5). Since Lyt-2 and Leu-2 are related to Ig and Ig-like proteins that function in molecular recognition, and Lyt-2/Leu-2 restrict CTL recognition to class I expressing targets, it is interesting to speculate that this recognition of class I is mediated by the Ig-like structures of Lyt-2 and Leu-2. The greater divergence in the V-like regions of these two molecules could be due to their coevolution with their different major histocompatibility complex counterparts: Lyt-2 evolving to recognize H-2 class I, and Leu-2 evolving to recognize class I HLA. Although the finding that Lyt-2 cDNA has an Ig V-like sequence led us to investigate the possibility of DNA rearrangement in this gene, we could not find any evidence supporting this idea.

Comparison of cDNA sequences of Lyt-2 and Leu-2 revealed that the two cDNAs are most similar across those

regions corresponding to coding sequences (Fig. 4). However, the homology also extends into 5′ and 3′ untranslated cDNA, possibly reflecting regulatory sequences that have been evolutionarily maintained.

The origin of two mRNA species remains unknown. It is likely that 1.7-kb mRNA is sufficient for the expression of Lyt-2 molecule since the L-cell transformants receiving 5.2 kb or 15 kb Lyt-2 genomic fragments make abundant Lyt-2 protein even though the amount of 3.0-kb mRNA in these cells is greatly reduced or absent. It should also be noted that both 34-kDa and 38-kDa molecules of Lyt-2 can be immunoprecipitated from those L-cell transformants transfected with either this 5.2-kb (Fig. 3) or 15-kb genomic fragments (data not shown). Therefore, a single gene and the 1.7-kb mRNA seem to be responsible for both the 34-kDa and the 38-kDa glycoproteins; L cells are able to glycosylate Lyt-2 protein similarly to thymocytes.

Preliminary sequence data of the Lyt-2 genomic insert from p6CCA indicate that the Lyt-2 gene is composed of at least five exons. Unlike other Ig-like molecules, Lyt-2 does not have a separate leader exon. The leader peptide, fused to the V-like region, is encoded by one exon. The transmembrane portion is encoded entirely by its own exon; the cytoplasmic region is encoded by two exons.

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