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Structure and Expression of the Rat Homologue of CD5

Toshiya MURAKAMI and Akihiro MATSUURA
Department of Pathology, Sapporo Medical College, Sapporo, 060, Japan
(Chief : Prof. K. KIKUCHI)

ABSTRACT **The CD5 molecule is a lymphocyte differentiating antigen with mw. of 67,000, expressed on a majority of thymocytes, peripheral T cells and a subpopulation of B cells. We have isolated and characterized rat CD5 cDNA clones from the Wistar rat thymus cDNA library. A clone R5c64 was found to contain a 2.1 kb full-length cDNA insert. The deduced amino acid sequence was comprised of an amino-terminal extracellular domain of 344 residues, a transmembrane region of 31 residues, and a carboxyl-terminal domain of 93 residues with a leader peptide of 23 residues. The extracellular domain contained 22 cysteine residues, all of which were highly conserved among rat, mouse and human in position and in number, and were divided into two homologous subregions by a threonine/proline-rich region. The rat CD5 had a 85% amino acid identity with the murine CD5 and a 64% identity with its human counterpart for the whole peptide. In contrast, the cytoplasmic domain was 97% and 87% identical with those of mouse and human homologues, respectively. This implied an important functional property of this domain. A mAb R1-3B3 was shown to recognize rat CD5 antigens expressed on COS cells transfected with the insert DNA. We could also demonstrate rat CD5 expression on a subset of B cells. Analysis of RFLP of rat genomes showed allelic variants in some strains.** (Received December 26, 1991 and accepted January 24, 1992)

Key words: Expression, Nucleotide sequence, CD5

1. Introduction

The lymphocyte cell surface molecule CD5 (T1, Leu1, Tp67 in the human: Ly1 in the mouse) of relative molecular mass 67,000 is expressed on the majority of thymocytes, peripheral T cells[1] and a small population of B cells[2]. Although an exact role of CD5 is still unknown, studies using monoclonal antibodies against CD5 have demonstrated the involvement of CD5 in T-cell proliferation[3] and in T-cell helper function to immunoglobulin production[4]; T-cell proliferation mediated by CD5 stimuli depended on the expression of the TCR/CD3 complex and resulted in a rise in intracellular calcium level[5], accompanied by IL-2R expression and by IL-2 synthesis. It seemed costimulatory effect like IL-1 did on T cells[6]. Furthermore, $CD5^+$B cells were primarily responsible for the production of polyreactive autoantibodies such as rheumatoid factor[7], whilst CD5-B cells produced non-self reactive antibodies[8]. Most recently, the natural ligand for CD5 has turned out to be the B-cell surface antigen CD72 (Lyb-2 in the mouse)[9], a glycoprotein of mw. 45-kd, expressed as a disulfide-linked homodimer on pre-B and

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The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases with the following accession number D10728

mature B cells. With this respect, it is suggested that CD5 may play an important role as a ligand or as a receptor in T-B cognate interactions and in induction of autoimmunity in many immunological aspects. We have been studied the function and structure of CD5 in the immune responses[10] and interested in the mechanism by which the gene expression is regulated not only in T cells but also in B cells. The present study was aimed to elucidate the complete structure of rat CD5 to investigate whether or not previously unidentified ligand and T cell activating property of rat CD5 are similar to those of mouse and human. We have isolated and characterized the cDNA clones encoding rat CD5 by screening of rat thymus cDNA libraries. Formal proof that mAb R1-3B3[10] recognized rat CD5 was also provided by detection of CD5 on the COS-cell transfectants.

2. Materials and Methods

2. 1 Animals, antibodies and probe

Inbred 6-to 8-week old Wistar/Crj and Fischer/Crj (F344) rats were purchased from Charles-River Japan (Tokyo). Monoclonal antibody (mAb) against rat CD5, R1-3B3 and control mAb Y1 were developed in our laboratory and described previously[10][11]. RLN-9D3 mAb specific for rat B cells was kindly provided by Dr. T. Takami (Gifu university, Gifu). Biotinization of mAb was performed as described[12]. Avidin-PE and goat anti-mouse Ig conjugated with FITC were obtained from Funakoshi Inc. (Tokyo). Mouse CD5 cDNA clone, MD-10[13] was kindly provided by Dr, H. Nakauchi (Riken, Tsukuba, Ibaragi).

2. 2 Construction of rat cDNA library and screening

Total RNA was prepared from 6- to 8-week-old Wistar rat thymus by the guanidinium thiocyanate/CsCl method[14], and poly(A)$^+$fraction was selected by oligo (dT)-cellulose column chromatography by standard technique. The cDNA was synthesized by the method of Gübler and Hoffman[15]. Reverse transcriptase was purchased from Seikagaku Kogyo (Tokyo). After addition of the *Eco*RI/*Not*I adaptor (5'-d[AATTCGCGGCCGC]-3'/3'-[GCGCCGGCG]p-5', Pharmacia, Tokyo), cDNA ranging 1.1 kb to 7 kb in length was recovered from agarose gel (Seakem GTG, FMC Bioproducts, ME, U. S. A), ligated with *Eco*RI-digested λ ZAPII vector (Stratagene, CA, U. S. A), then introduced into *E. coli* XL-1 blue competent cells to construct the cDNA library. Aliquots of the library were plated out on 10 cm dishes containing 1.2% LB agar and the plates were incubated upside down at 37℃ overnight to grow colonies. More than 10^6 independent colonies were obtained. Colony lifting was performed using the colony/plaque screen filters (NEN, MA, U. S. A) by standard technique and the replica filters were denatured in 0.5 N NaOH for 2 min. twice, neutralized in 1 M Tris/HCl for 2 min. twice and rinsed in 2×SSC, 0.1% SDS for 10 min.

The resulting filters were hybridized with a radiolabelled probe containing about 550-bp long 5' *Eco* RI-*Hind* III fragment prepared from the murine CD5 (Ly-1) cDNA clone MD-10. This fragment contains the 5' coding sequence from the first amino acid extending to 151 residues of about a-third portion of the whole cDNA. Labeling of the probe with ^{32}P was carried out using the random hexamer priming method[16]. Plaque hybridization of the blots was performed at 42℃ in 40% deionized formamide, 4× SSC, 20 mM Tris/HCI pH 7.5, 10% Denhardt's solution, 0.1% SDS, 10% dextran sulfate, and 20 μg/m*l* salmon sperm DNA overnight. The filters were washed at room temperature in 2×SSC, 0.1% SDS for 20 min followed by 0.2×SSC, 0.1% SDS at 42℃ for 30-60 min, and exposed to X-ray film overnight.

2. 3 Isolation of cDNA clones and nucleotide sequencing

Positive clones were plaque-purified after two cycles of re-cloning steps and the plasmids containing inserts were rescued from the λ ZAP II vector with the helper phage R408 by using the *in vivo* excision protocol (Stratagene, La Jolla, Ca). These DNAs were prepared directly or subcloned into M13, and

then subjected to make series of deletion mutants. DNA sequence analysis was performed on both orientations by the method of Sanger *et al.*[17]

2. 4 Polymerase chain reaction (PCR)

PCR was carried out as described by Maniatis *et. al.*[14] Briefly, 1 μl of phage stock solution was used for template. Both the forward primer (5'-CCATGGACTCCCACGAAGTG-3') containing the first methionine codon and the reverse primer (5'-TCTAGGCAGATCAGGCTCA-3') which are highly conserved in mouse[13] and human[18] were added at a concentration of 1 μM each in 50 mM KCl, 10 mM Tris pH 8.3, 1.5 mM $MgCl_2$, 200 μM each dATP, dCTP, dGTP, and dTTP, 0.1% gelatin, 40 units of Taq DNA polymerase per ml. The 50 μl of reaction mixture was subjected to the amplification step by 30 cycles of 1 min at 94℃, 2 min at 55℃ and 4 min at 72℃ in a DNA thermal cycler (Perkin-Elmer, Cetus, CA).

The reaction mixture was electrophoresed on a 1.5% low-melting-point agarose gel, then the gel was stained with ethidium bromide.

2. 5 Computer analysis

Nucleotides and the predicted amino acids sequence analysis were performed by using Genetics sequence analysis programs (SDC, Tokyo). Hydrophobicity analysis of the amino acids sequence was done according to the method of Kyte and Doolittle[19].

2. 6 DEAE-dextran transfection of COS cell

R5c64 DNA was double-digested with both *Hind* III and *Xba* I, then the fargment containig the entire coding region of the rat CD5 cDNA was subcloned unidirectionally into the expression vector CDM8 allowing that the insert DNA could be properly controled under the promoter. The recombinant clone termed R5c64/CDM8 was prepared and purified by the alkaline lysis procedure for transfection. COS7 cells (1.5×10^6) were plated on 10 cm dishes and grown overnight, then transfected by the DEAE-dextran method, as follows. Serum-free Modified Eagle's medium (MEM from GIBCO) containing 400 $\mu g/ml$ DEAE-dextran, 200 μM chloroquine and 20 μg of R5c64/CDM8 was added to the cells. After the cells were incubated at 37℃ for 4 hr. (earlier if necessary), the DNA mixture was removed and the cells were washed with phosphate buffered saline (1×PBS). The cells were then fed with MEM/10% fetal calf serum for 48-72 hr. to allow expression of transfected DNA.

2. 7 FACS analysis

Expression of CD5 was examined by indirect immunofluorescence. Forty to seventy-two hr. after transfection, the cells were washed once with 1×PBS and exposed for 30 min. at 37℃ with 8 ml PBS/0.5 mMEDTA/0.02% azide per 10 cm dish. The detached cells were centrifuged and incubated for 60 min. at 4℃ with a mouse anti-rat CD5 mAb (R1-3B3) or with a mAb against CD45 on Jurkat cells (Y1) as a negative control followed by another incubation with FITC-conjugated goat anti-mouse lg as a second antibody. Analysis of these cells was carried out on a fluorescence activated cell sorter (FACStar, Becton Dickinson, Sunnyvale, CA). The data were expressed as a histogram in which the intensity of fluorescence was plotted on an abscissa against the relative number of cells on the ordinate. The analysis of each sample was carried out with 10,000 cells. Two color staining was performed as follows; cells were simultaneously stained with two antibodies. First, cells were treated with 10 $\mu g/ml$ of biotinized anti-CD5 mAb (R1-3B3) at 4℃ for 30 min. After washing, cells were incubated with an appropriate dilution of PE-conjugated avidin (Avidin-PE; Funakoshi, Tokyo) at 4℃ for 30 min. After washing, cells were then incubated with FITC-conjugated RLN-9D3 mAb. Samples were analyzed by FACStar. Green fluorescence (515-530 nm wave length) from FITC and red fluorescence (630 nm wave length) from PE were detected independently and displayed as contour maps in the logarithmic scale of fluorescence intensity.

2. 8 Southern blot analysis

Cellular DNA was prepared from rat liver as standard protocol[20]. Inbred rat strains, LEW/Hkm, TO/Hkm, LEJ/Hkm, BUF/HKm, ACI/Hkm, WN/Hkm and WKAH/Hkm were kindly provided by Dr. N. Kasai (Hokkaido University, School of Medicine, Sapporo). NIG III/Hok, SDJ/Hok, ALB/Hok and BN/Hok were gift of Dr. M. Yoshida (Hokkaido University, Faculty of Science, Sapporo). F344/Crj was purchased from Charles-River Japan (Tokyo). Fifteen μg of each cellular DNA was digested with *Pst* I and fractionated in 0.7% agarose gel and transferred to the Gene Screen Plus blotting membrane (NEN, MA) with capillary transfer metheod. Blots hybridization was done according to manufacturer's protocol.

3 Results

3. 1 Isolation and characterization of rat CD5 cDNA clones

A λ ZAPII cDNA library constructed from the Wistar rat thymus was screened with a probe containing 550 bp *Eco*RI-*Hind* III digested DNA fragment of murine CD5 cDNA clone MD-10 which corresponded to a-third portion of the 5' coding sequence. Of 60,000 plaques screened. two clones were obtained and characterized in detail (Fig. 1). Restriction mapping revealed that two clones denoted R5c52 and R5c64 were overlapping and contained cDNA inserts having the same or similar 3' ends but variable 5' ends. Because we have only isolated 5' truncated cDNA clones in several rounds of repeated screenings (data not shown), we decided to use PCR amplification of 5' region to identify full-length cDNA clones. According to PCR analysis R5c52 lacks a partial open reading frame including a initiation codon (ATG). However, R5c64 has a longer insert (2.1 kb) and contained a initiation codon. It overlaps whole of R5c52 and extends to both 5' and 3' ends. Therefore, the nucleotide sequence of R5c64 was determined on both strands.

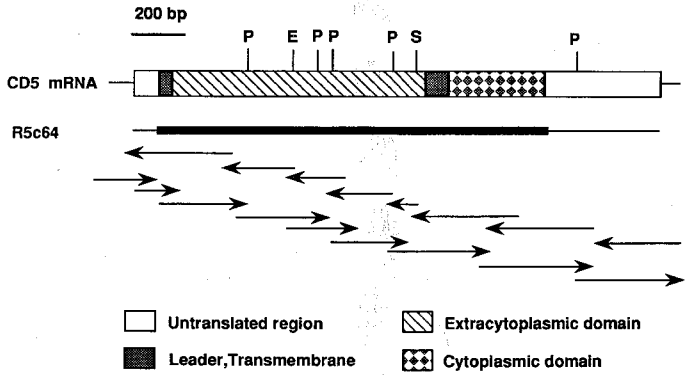

Fig. 1 Schematic representation and restricton map of the cDNA clone.
The box represents the open reading frame and both 5' and 3' untranslated region. The cleavage sites for restriction enzymes are shown as follows: *Pst* I(P), *Eco* RI(E), *Sma* I(S). The corresponding cDNA is indicated by a line beneath the box, the bold line shows the coding sequence. Arrows indicate the sequence strategy (direction and extent of sequence).

3. 2 Nucleotide and deduced amino acids sequence of the rat CD5

The R5c64 insert is 2059 bp long (Fig. 2). An open reading frame of 1473 bases (positions 90-1562) begins with an ATG methionine codon at 90 and ends with the termination codon TAA at 1565 and is flanked by 89 nucleotides of 5' untranslated sequence and 497 nucleotides of 3' untranslated sequence. The ATG at 90 is assigned as the initiation site because it is flanked by the initiation consensus sequence proposed by Kozak[21]. There is no canonical poly(A)-additional signal (AATAAA), but exists a related sequence, AATAAT, at nearly 3' end as shown in the cases of murine or human CD72[22),23)]. The first 23 amino acids (from methionine to glycine) are characteristic of a classical signal peptide in agreement with von Heijne[24].

Cleavage of a precursor peptide between the 23 th Gly and 24 th Gln evokes a typical integrated

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CTCCAAGAG AGTAAGACAC TGAGCCAGAC ACCTTTCCTG GCTGGCCAAC TGAACTCACC TGAGGCTGAG GCAGCAGAAG GCCATTATCC
                                                                                               +1
ATG GAC TCT CAC GAA GTG CTG CTG GCT GCC ACA TAC CTG CTG GGA ACA CTG GCT GCT TTC TGC CTT GGA CAG TCT GGA AGG GGC TTC GTA   179
Met Asp Ser His Glu Val Leu Leu Ala Ala Thr Tyr Leu Leu Gly Thr Leu Ala Ala Phe Cys Leu Gly Gln Ser Gly Arg Gly Phe Val     7
                                             •                                                         •
GGT GCC CAG GTG ATG CTA AGT GGC TCC AAC TCC AAG TGT CAG GGT CTA GTG GAG GTC CAG ATG AAT GGA ATG AAA ACG GTG TGC AGT TCC   269
Gly Ala Gln Val Met Leu Ser Gly Ser Asn Ser Lys Cys Gln Gly Leu Val Glu Val Gln Met Asn Gly Met Lys Thr Val Cys Ser Ser    37
                                                                     •                   •
AGT TGG AGG CTG AGC CAG GAT CTC TGG AAA AAC GCC AAT GAG GCC TCT ACA GTG TGC CAG CAG CTG GGA TGT GGT AAC CCT CTG GCC CTT   359
Ser Trp Arg Leu Ser Gln Asp Leu Trp Lys Asn Ala Asn Glu Ala Ser Thr Val Cys Gln Gln Leu Gly Cys Gly Asn Pro Leu Ala Leu    67
                                                    •                                 * *  •
GGC CAT TTG ACC TTA TGG AAC AGA CCC AAG AAC CAG ATC CTC TGC CAA GGA CCC CCG TGG TCT TTC TCC AAC TGC AGC ACA AGT TCG CTG   449
Gly His Leu Thr Leu Trp Asn Arg Pro Lys Asn Gln Ile Leu Cys Gln Gly Pro Pro Trp Ser Phe Ser Asn Cys Ser Thr Ser Ser Leu    97
        •                   •
GGC CAG TGC CTT CCG CTG AGC CTG GTC TGC CTA GAG CCC CAG AAG ACG ACA CCT CTA CCC ACA ACC ACC CTA CCC ACC ACC ATG CCG GAG   539
Gly Gln Cys Leu Pro Leu Ser Leu Val Cys Leu Glu Pro Gln Lys Thr Thr Pro Leu Pro Thr Thr Thr Leu Pro Thr Thr Met Pro Glu   127
                                                            •                             * *
CCC ACA GCC CCT CCC AGA TTG CAG CTG GTG CCA GGA CAC GAG GGC CTG AGG TGC ACA GGT GTG GTG GAA TTC TAC AAT GGC AGC CGG GGT   629
Pro Thr Ala Pro Pro Arg Leu Gln Leu Val Pro Gly His Glu Gly Leu Arg Cys Thr Gly Val Val Glu Phe Tyr Asn Gly Ser Arg Gly   157
                                                                     •                   •
GGC ACC ATC CTC TAT AAG GCC AAG GCC AGG CCC GTG GAC CTG GGG AAC CTC ATC TGT AAG TCT CTG CAG TGT GGC TCT TTC CTG ACA CAT   719
Gly Thr Ile Leu Tyr Lys Ala Lys Ala Arg Pro Val Asp Leu Gly Asn Leu Ile Cys Lys Ser Leu Gln Cys Gly Ser Phe Leu Thr His   187
                                                                                                                  * *
CTG TCC CGG ATA GAG ACA GCA GGA ACT CCA GCT CCT GCA GAG CTG AGG GAC CCC AGG CCC TTG CCA ATT CGA TGG GAG GCC CAG AAC GGG   809
Leu Ser Arg Ile Glu Thr Ala Gly Thr Pro Ala Pro Ala Glu Leu Arg Asp Pro Arg Pro Leu Pro Ile Arg Trp Glu Ala Gln Asn Gly   217
    •                   •                                                                          •
AGC TGC ACC TCA CTA CAG CAA TGC TTC CAG AAA ACA ACG GTC CAG GAG GGC AGC CAG GCA CTC GCA GTC GTC TGC TCT GAT TTC CAG CCC   899
Ser Cys Thr Ser Leu Gln Gln Cys Phe Gln Lys Thr Thr Val Gln Glu Gly Ser Gln Ala Leu Ala Val Val Cys Ser Asp Phe Gln Pro   247
                                             •                                                              •
AAG GTT CAG AGC CGC CTG GTG GGG GGC AGC AGC GTG TGT GAG GGC ATC GCT GAA GTT CGC CAG AGG TCA CAG TGG GCT GCC CTG TGT GAC   989
Lys Val Gln Ser Arg Leu Val Gly Gly Ser Ser Val Cys Glu Gly Ile Ala Glu Val Arg Gln Arg Ser Gln Trp Ala Ala Leu Cys Asp   277
                                                 •                   •
AGT TCT GCA GCC AGG GGT CCA GGA CGG TGG GAG GAG CTA TGC CAA GAG CAG CAG TGT GGC AAC CTC ATC TCC TTC CAT GTG ATG GAT GCT  1079
Ser Ser Ala Ala Arg Gly Pro Gly Arg Trp Glu Glu Leu Cys Gln Glu Gln Gln Cys Gly Asn Leu Ile Ser Phe His Val Met Asp Ala   307
                                •                             •                             •
GAC AGG ACC TCC CCG GGG GTC CTC TGT ACC CAG GAG AAG CTG TCT CAG TGT TAC CAG CTT CAG AAA AAA ACC CAC TGC AAG AGG GTA TTC  1169
Asp Arg Thr Ser Pro Gly Val Leu Cys Thr Gln Glu Lys Leu Ser Gln Cys Tyr Gln Leu Gln Lys Lys Thr His Cys Lys Arg Val Phe   337
        •
ATC ACA TGC AAA GAC CCA AAC CCA GTG GGC CTG GCC CCA GGC ACT GTG GCA AGC ATC ATC CTG ACC CTG GTA CTC CTG GTG GTG CTG ATG  1259
Ile Thr Cys Lys Asp Pro Asn Pro Val Gly Leu Ala Pro Gly Thr Val Ala Ser Ile Ile Leu Thr Leu Val Leu Leu Val Val Leu Met   367

GTC ATG TGC GGT CCT CTG ATC TAC AAG AAG CTG GTG AAG AAA TTT CGT CAG AAG AAG CAG CGT CAG TGG ATT GGT CCC ACA GGA GTG AAC  1349
Val Met Cys Gly Pro Leu Ile Tyr Lys Lys Leu Val Lys Lys Phe Arg Gln Lys Lys Gln Arg Gln Trp Ile Gly Pro Thr Gly Val Asn   397

CAG AGC ATG TCT TTC CAT CGA AGC CAC ACG GCA ACT GTG CGG TCC CAG GTT GAG AAC CCG GCA GCC TCT CAC GTG GAC AAT GAG TAC AGC  1439
Gln Ser Met Ser Phe His Arg Ser His Thr Ala Thr Val Arg Ser Gln Val Glu Asn Pro Ala Ala Ser His Val Asp Asn Glu Tyr Ser   427

CAG CCT CCC AGG AAC TCT CGC CTG TCG GCT TAT CCA GCT CTG GAA GGG GCC CTA CAC CGC TCT TCC ACA CAA CCT GAC AAT TCC TCT GAC  1529
Gln Pro Pro Arg Asn Ser Arg Leu Ser Ala Tyr Pro Ala Leu Glu Gly Ala Leu His Arg Ser Ser Thr Gln Pro Asp Asn Ser Ser Asp   457

AGT GAC TAT GAC CTG CAA GTG GCT CAG AGA CTG TAA                                                                          1565
Ser Asp Tyr Asp Leu Gln Val Ala Gln Arg Leu ***                                                                           468

      AAGA ACTTAAGGCC AGCAACAAAG ACCAAGCACA TCCCTCTGTC CTGAGGAAGC ATTTACCACT CTGAGTACTG CCCACTTCCA CCTCAGCCAG        1659
AACTTGGACA GAAGCCAAGG TCCTTTGGAC AAGGTCCTGG GCCCTGCAGT GGCAGGCCAG CTCCTATATC CTGTGGCCAT GGTAGCCTGG CTGCTTCCTC        1759
CAAAAGCCAA AACTCAGCAG ACAGAGCCTT CAAGCAACCG ATCCTTGCAA GCAGAGACCC CAGTATCCAG AGGAGTCCCA ATCCTTTGGG GAGCAATGAC        1859
CAGCGAGGTT GACTTTCTCC TTGGACTCTT GACGCCATGA ACGAATGATG TGATACACCC AAGTGGGATA CGTACAAGAA CTCCTTTCTC AAGCATATTT        1959
TTAATATATC TTTGTAAATA ATGGTTTTTC TACTTTCCAA GAACATGAGT ACTCCGGGCT GCTGTGTTCA GGCCCATCCT CCCGACCTTC TAAGAGACAT        2059
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Fig. 2 (a) Nucleotide and predicted amino acid sequence of R5c64 cDNA. Numbers at right margin indicate positions of amino acids or nucleotides, respectively. The start of the mature protein sequence is denoted+1. The leader peptide and the threonine/proline rich sequence are indicated by a single underline and the predicted transmembrane domain is indicated by a bold underline. The sites of potential N-linked glycosylation are marked with a pair of asterisks and cysteine residues are marked by solid circles.

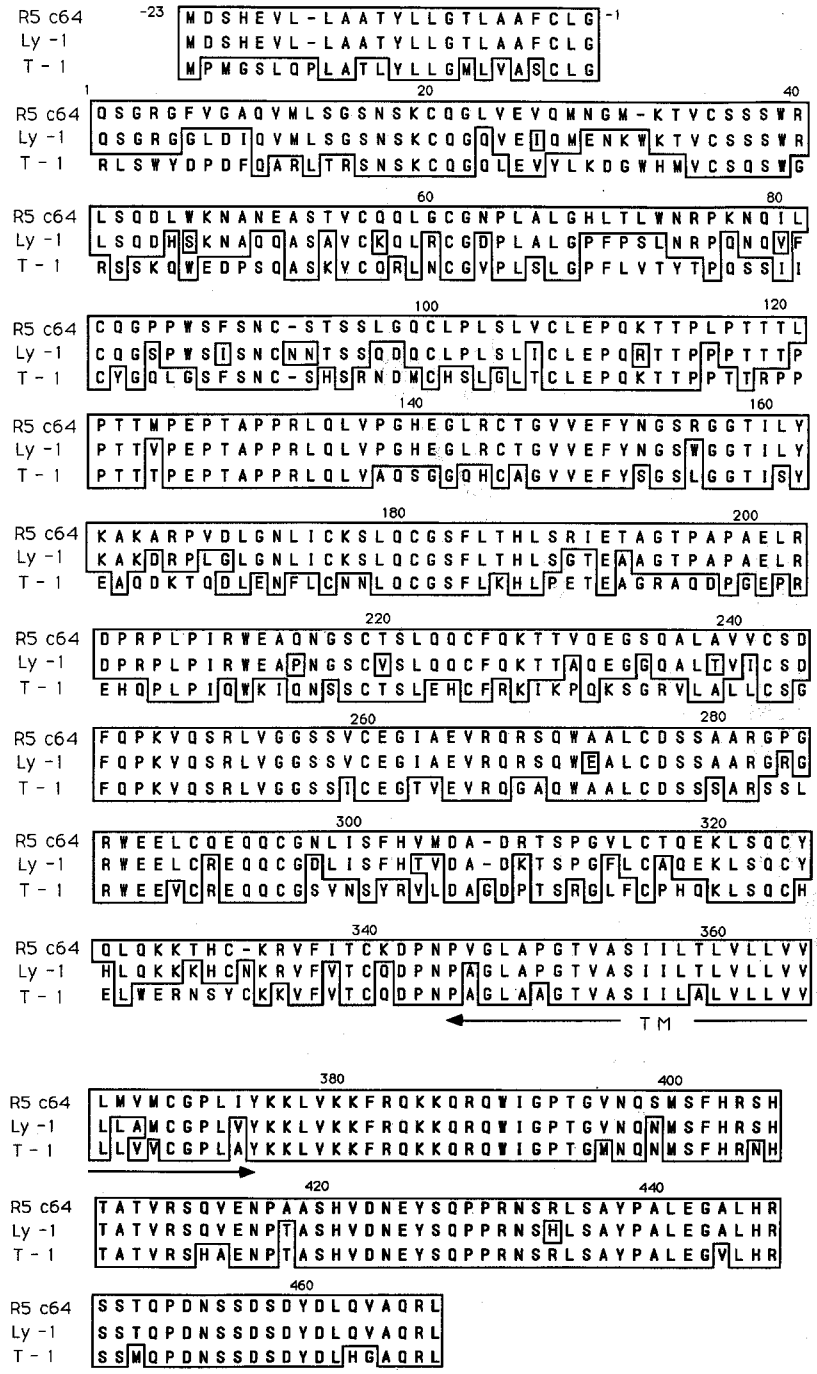

Fig. 2 (b) Amino acid sequence alignment of rat (R5c64), mouse (Ly-1), human (T-1) CD5. Standard one-letter amino acid symbols are used. Homologous residues to rat are boxed. Numbering above the sequence is that of rat. Gaps are indicated with hyphens. The transmembrane domain (TM) in the three sequences are matched and underlined.

membrane protein suitable for a receptor for peptides with a calculated molecular mass of 51,048 daltons. Analysis of the hydrophobicity plot of the predicted mature protein revealed a region (residues 345-375) of intense hydrophobicity, so we postulated this region to be a transmembrane segment (Fig. 3). Thus, the mature protein of the rat CD5 is composed of 468 amino acids including an N-terminal region of 344 residues, a transmembrane segment of 31 residues, and a cytoplasmic region of 93 residues. There are three potential N-linked glycosylation (Asn-X-Thr/Ser) sites in the extracellular domain at residues 92, 153, and 216 and two in the cytoplasmic domain at residues 397 and 454. As mentioned above, the predicted molecular weight of 51,048 is about 16 kd smaller than the value estimated by SDS-PAGE or Western blotting[10]. This difference could be accounted for by the glycosylation at three potential N-linked glycosylation sites and/or O-linked glycosylation in the extracellular domain. The extracellular domain is relatively abundant (22 residues) with cysteine but without apparent cysteine clusters. This domain seems to be divided into two homologous subregions by a 20-residue stretch at 113-132. This short sequence is rich in Threonine and Proline as was discussed by Huang *et al*[13]. A similar structure is likely to be present frequently in the hinge regions of immunoglobulin molecules[25].

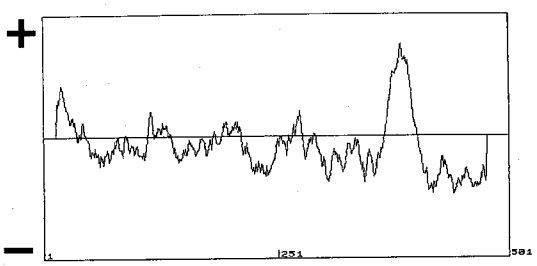

Fig. 3 Hydrophobicity plot of the predicted rat CD5. Hydrophobicity was calculated according to Kyte and Doolittle (1982) by using the plot structure program. Positive values indicate hydrophobic residues, whereas negative values indicate hydrophilic residues. Note the two hydrophobic regions corresponding to signal sequence and transmembrane domain, respectively.

3. 3 Expression of rat CD5 on the surface of COS cells

To confirm protein coding capacity of R5c64, a full-length cDNA clone, we examined the antigen expression on COS cells transfected with R5c64. The expression vector CDM8 was used to construct a recombinant R5c64/CDM8. In this vector, the cDNA is under the transcriptional control of the CMV/HIV chimeric promoter and is further followed by the SV40 termination and polyadenylation signals (Fig. 4).

COS 7 cells which constitutively expressed large T antigen were transfected with R5c64/CDM8 or Mock DNA by using DEAE-dextran method, and the surface expression was detected with mouse anti-rat (R1-3B3) or mouse anti-human (Y1) first antibody. The cells were further treated with a FITC conjugated second antibody to mouse Ig and flowcytometry analysis was done. Fig. 5 showed that transfection of the R5c64/CDM8 yielded strong expression of rat CD5 molecules on the surface of COS cells. This finding is consistent with the fact that mAb R1-3B3 recognized rat T cell antigen similar to mouse CD5[10].

3. 4 Comparison with murine and human CD5 sequences.

Comparison of rat CD5 nucleotides and the deduced amino acids sequcences with those of murine and of human CD5 was performed (Table 1). Overall the protein sequence of rat CD5 is 84.9% identical with that of murine CD5 and 64.3% with that of human CD5 after maximum matching of computer alignment. There seem to be gradients of increasing homology along corresponding domains (Fig. 6); in the comparison between rat and mouse, the first N-terminal subregion (Sb1) has 70.2% amino acid identity, the second N-terminal subregion (Sb2) has 85.7%, the transmembrane region has 87.1% and the cytoplasmic region has 96.8% identity, respectively. The similar results are shown in the comparison among rat and human. All three potential N-glycosylation sites present in rat CD5 are conserved in very similar positions in mouse, and two of them are also conserved in human. In addition, all 22 cysteines in each protein are conserved in very similar positions in the three sequences, suggesting that

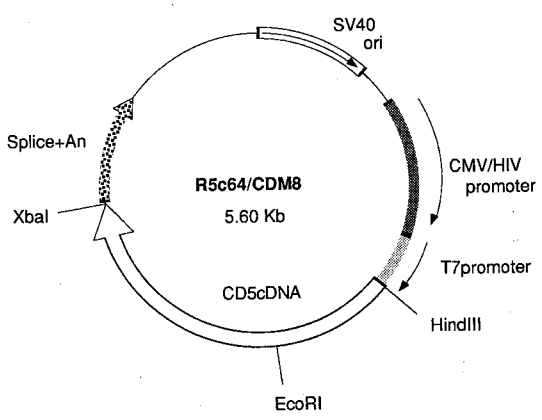

Fig. 4 The map of recombinant expression construct R5c64/CDM8.
The direction of transcription is indicated by arrows. The open white arrow indicates the insert cDNA.

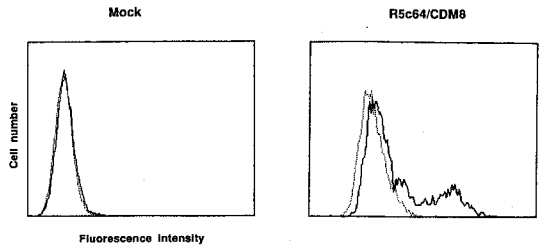

Fig. 5 FACS analysis of R5c64/CDM8 transfected COS cells.
Left panel shows a histogram from mock CDM8 transfected cells, right panel shows a histogram from R5c64/CDM8 transfected cells. The specific mAb (R1-3B3) staining profile is shown with a dark line and staining with the negative control mAb (Y1) is shown with a dashed line.

intrachain disulfide bonds formed between these residues may contribute to a certain domain structure favorable for ligand binding. Among these species, high homology values are noteworthy especially in the cytoplasmic region where the homology scores in nucleic acids and in amino acids are reversed.

In additon, a relatively high degree of conservation in 3' untranslated region of about 500 bp between rat and mouse, 77.6% homology in nucleotide alignment was observed. It seems that a very similar regulatory control might be taken in posttranscriptional modification of CD5 messages.

3. 5 Two color staining of Wistar rat mesenteric lymph node cells

Reactivity of R1-3B3 with Wistar rat mesenteric lymph node cells was examined. As demonstrated before, mAb R1-3B3 was proved to detect rat lymphocyte antigen homologous to human CD5. The CD5 antigen was first recognized as a T cell marker, but later a certain population of normal B cells was found to express CD5 as well. Cytofluorographic analysis of rat lymphoid cells stained with R1-3B3 and RLN-9D3 antibodies is depicted in Fig. 7. The result demonstrated that a significant population of B cells (12.3%) bearing RLN-9D3 antigen also expressed R1-3B3 defined antigen, rat CD5. $CD5^+$B cells were also observed in rat thymus (data not shown).

3. 6 Southern blot analysis

To investigate whether rats have allelic forms of CD5, southern blotting analysis of several inbred rat strains were performed with the insert of R5c64 as a probe. Restriction fragment length polymorphism (RFLP) was detected in digestion of genomic DNAs by *Pst*I in certain strains. While NIG III and ACI rats deleted a 5.5 kb fragment, other strains possessed it (Fig. 8). Although further analysis is required for determining which part of gene fragment was involved in polymorphism, these results clearly demonstrated the existence of two allelic forms of CD5 which have not been clarified with R1-3B3 antibody[10].

4 Discussion

We described the cloning and expression of rat CD5 cDNA and the existence of normal $CD5^+$B cells in rat lymphoid tissues. High degree of structural homology of rat CD5 to those of other species strong-

Table 1 *Homology values of rat CD5 compared both to mouse CD5 and to human CD5 (% score).*

		ORF	EXT	TM	CYT	Sb1	Sb2	3'UT
Rat-Mouse	AA	84.9	81.6	87.1	96.8	70.2	85.7	
	NA	90.4	88.8	93.5	94.7	83.5	89.8	77.6
Rat-Human	AA	64.3	55.9	80.6	89.2	49.0	64.2	
	NA	72.8	69.2	84.9	85.8	62.1	76.7	55.0

AA; amino acid, NA; nucleic acid, ORF; Open reading frame, EXT; Extracellular domain, TM; Transmembrane domain, CYT; Cytoplasmic domain, Sb1; Subregion 1, Sb2; Subregion 2, 3'UT; 3' untranslated sequence.

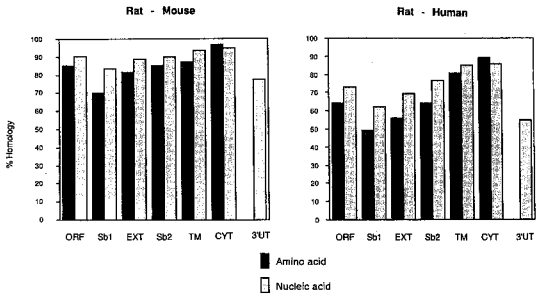

Fig. 6 Percent homology scores among rat and mouse and among rat and human are indicated both at amino acid and at nucleic acid levels. Black bars represent amino acids homology values, gray bars those of nucleic acids, respectively. Each values and the abbreviations used are the same as those of Table 1.

ly suggested that previously unidentified ligand and T cell activating property of rat CD5 may be very similar to those of mouse and human. By the computer analysis, the deduced amino acids sequence of rat CD5 reveals a structure typical of cell surface receptor for polypeptides: it contains a signal peptide and extracellular, transmembrane, and intracellular regions. The extracellular domain is relatively rich in cysteine (22 residues), whereas the cytoplasmic domain has no cysteine. Further, internal homology is found in the extracellular domain (Fig. 9). The comparison of the subregion 1 (Sb1) and the subregion 2 (Sb2) indicates 25% amino acid identity when alignment is optimized by computer, and cysteines in each domains are conserved in very similar positions. This may represent duplication events of an ancestral subdomin during the evolution of the CD5 molecule. Since the duplication of CD5 subdomain is observed in all species so far characterized, it must have been occurred before or at a very early stage of mammalian speciation. Similar phenomena was widely shown in the cases of immunoglobulin supergenefamily[25]. Moreover, two of cysteines at positions 20 and 82 are organized in a manner characteristic of immunoglobulin supergenefamily. As compared with all protein sequences in the National Biomedical Research Foundation data base, no remarkable homology was obtained except for the counterparts of murine and human CD5.

The cytoplasmic domain is relatively large as seen in other growth factor receptors such as EGF receptor[27] and insulin receptor[28]. A notable conservation in this region is found among rat, mouse and human suggesting an essential property of this region in the physiologic role of CD5 such as signal transduction. As reported by Huang *et al*[13], the potential tyrosine phosphorylation site (position 426) surrounded by acidic amino acids [D-N-E-Y-S-Q-P] was well conserved in the three species. The similar sequence [D-N-E-Y-T-A-R] including the tyrosine autophosphorylation site was referred to be characteristic for the protooncogene c-*src* family[29] or EGF receptor and PDGF receptor. In additon, there are also many threonine and serine residues that can be phosphorylated by protein kinase. It is well konwn that CD5 stimulation induces augmentation of the CD3/antigen mediated production of IL-2 or IL-2 receptor, cell proliferation, Ca^{2+} influx and rapid hydrolysis of inositol phospholipids[30]. Protein phosphorylation is generally conceived to be the most common post-translational modification used to regu-

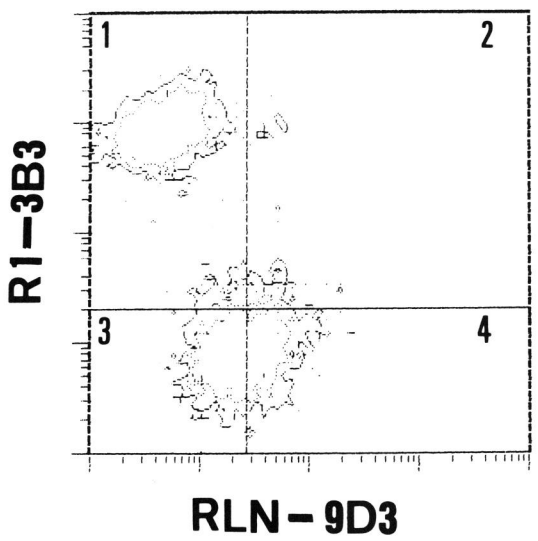

Fig. 7 Two-color analysis of Wistar/Crj rat mesenteric lymph node cells. Antibodies are labeled on the graph. Different quadrants (1, 2, 3, 4,) on the panel represent single positive red cells of 43.7% (1) or green fluorescent cells of 20.7% (4) or double positive cells of 12.3% (2) and double negative cells of 23.2% (3). Double staning cells indicated a population of B cells expressing CD5.

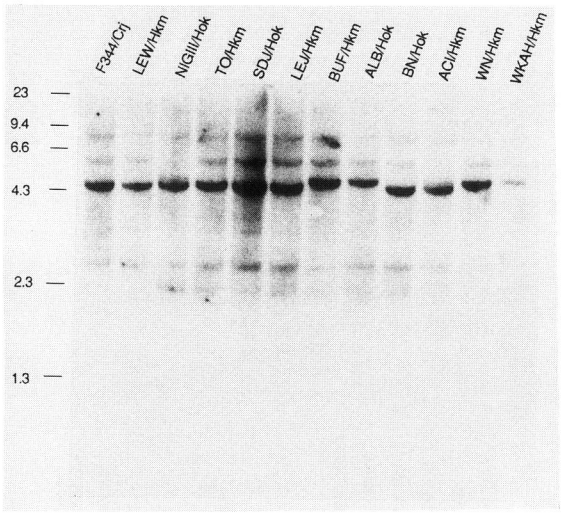

Fig. 8 Southern blot hybridization analysis. The *Pst*I-digested genomic DNAs (15 μg) were hybridized with a radiolabeled insert of R5c64. Note that 5.5 kb fragments are deleted in NIGIII/Hkm and ACI/Hkm strains indicating allelic variety of RFLP in rats. The sizes of molecular weight markers are indicated in kb.

late various cellular function[31]. Indeed, previous studies showed rapid induction of hyperphosphorylation of CD5 molecule and its higher shift of electrophoretic mobility in normal and lymphoblastoid T and B cells with stimulation of TPA which is capable of activating serine/threonine protein kinase C[32)~35)]. The phosphorylation event in cytoplasmic portions accompanied by signal transduction is frequently found in receptors for other growth and differentiation factors. Thus, the strong homology of the cytoplasmic domain of CD5 implies an important role of this molecule in signal transduction.

Recently, an attractive candidate for the ligand for CD5 have been identified to be CD72 (Lyb-2 in mice) on B cells which was first recognized as a B cell lineage specific marker[36]. CD72 is a 45-kd glycoprotein expressed at all stages of B cells except for the plasma cells. In accordance with functional studies, antibodies against CD72 could induce proliferation of resting B cells as evidenced by an increase in size, the mobilization of cytosolic Ca^{2+} and by up-regulation of surface Ia expression[37].

Structural analysis of CD72 has revealed a characteristic feature of a type II membrane protein[38] whose external domain is homologous to that of asialoglycoprotein receptor and CD23 or the low-affinity Fc receptor for IgE[23].

To date, a number of interacting pairs of molecules have been identified in relation to T-B cognate interactions. Among them are the T cell antigen receptor (TCR) and the class II MHC molecule on B cell, the adhesion molecule pairs, LFA-1 on T cell and ICAM-1 on B cell, and CD2 on T cell and LFA-3 on B cell. Although rat CD72 has hot been identified, the pair of rat CD5 which was first identified by our study[10] and putative rat CD72 could simultaneously mediate distinct activation signals through both T cells and B cells from the other ligand/receptor pair, even though the functional role of both molecules is uncertain.

We have demonstrated the presence of normal $CD5^+$B cells in the rat peripheral lymph node. Fig. 7

shows that there are 12.3% of a small subset of CD5$^+$B cells clearly distinguishable from the so-called conventional B cells *i. e.* CD5$^-$B cells in the normal mesenteric lymph nodes. In contrast to conventional CD5$^-$B cells, a minor population CD5$^+$B cells are well known to have different biological features. They seem to be responsible for the secretion of autoantibodies mostly of the IgM isotype[39]. The number of CD5$^+$B cells is increased in the autoimmune diseases or rheumatoid arthritis[39], Sjφgren's syndrome[40], systemic lupus erythematosus[41] and Hashimoto thyroiditis[42]. Moreover, its frequent involvement in B cell leukemias and B lymphomas in both mice and human has also been described[42]. Hence CD5$^+$B cells are likely to be involved in autoimmune responses or in susceptibility to malignant transformation. Whether they contribute to the pathogenesis of these disorders is still unclear. However it is conceivable that the interaction of CD5 and CD72 might promote autostimulatory growth signals of these cells[44].

Taken together, the data described here further extend the similarity of the cell-lineage specific expression and the structure of CD5 among rats, mice and humans. It is noteworthy that only a few CD antigens such as CD4 and CD8 retained a strict tissue specificity in evolution, but others such as CD1 and CD2, despite of the extreme structural similarity, showed dramatically different patterns of tissue expression between mice and humans. Particularly high degree of conservation suggested an indispensable role of CD5 for differentiation and function of T cells and a minor subset of B cells in mammals. The availability of a cDNA clone encoding rat CD5 should help to elucidate the function and regulation of this molecules and to determine as yet unidentified ligand, rat CD72.

5 CONCLUSION

We identified the cDNA structure of rat homologue of CD5 and confirmed its expression on the transfected COS cells. The structural feature is consistent with a cell surface molecule favorable for signal transduction. Comparison of the rat CD5 cDNA sequence with those of mouse and of human showed remarkable homologies especially in cytoplasmic portion. Allelic variation of rat CD5 gene was observed with RFLP analysis.

```
  1  QSGRGFVGAQVMLSGSNSKCQGLVEV-QMNGMKTVCSSSWRLSQDLWKNANEASTVCQQL
                *  * *  * * *  ** *       * **       *         **
241  VCSDFQPKVQSRLVGGSSVCEGIAEVRQRSQWAALCDSSAARGPGRW------EELCQEQ

 60  GCGNPLALGHLTLWNRPKNQILCQGPPWSFSNC-STSSLGQCLPLSLVCLEPQK
      ***          *     **       * *        *      * *
295  QCGNLISFHVMDA-DRTSPGVLC--TQEKLSQCYQLQKKTHCKRVFITCKDPN
```

Fig. 9 Amino acid sequence alignment of two homologous subregions of rat CD5. The subregion 1 (Sb1) spans residues 1-113 and the subregion 2 (Sb2) residues 242-347. Identical residues are indicated by asterisks and gaps in the alignment are indicated by hyphens.

References

1. Reinherz, E. L., Kung, P. C., Goldstein, G. and Schlossman, S. F.: A monoclonal antibody with selective reactivity with functionally mature human thymocytes and all peripheral human T cells. **J. Immunol. 123**, 1312-1317 (1979).
2. Hardy, R. R., Hayakawa, K., Herzenberg, L. A., Morse, H. C., III., Davidson, W. F. and Herzenberg, L. A.: Ly-1 as a differentiation antigen on normal and neoplastic B cells. **Curr. Top. Microbiol. Immunol. 113**, 231-236 (1984).
3. Engleman, E. G., Warnke, R., Fox, R. I. and Levy, R.: Studies of human T lymphocyte antigen recognized by a monoclonal antibody. **Proc. Natl. Acad. Sci. USA 78**, 1791-1795 (1981).
4. Thomas, Y., Glickman, E., DeMartino, J., Wang, J., Goldstein, G. and Chess, L.: Biologic functions of the OKT1 cell surface antigen. I. The T1 molecule is involved in helper function. **J. Immunol. 133**, 724-728 (1984).
5. June, C. H., Rabinovitch, P. S. and Ledbetter, J. A.: CD5 antibodies increase intracellular ionized calcium concentration in T cells. **J. Immunol. 138**, 2782-2792 (1987).
6. Williams, J. M., Derolia, D., Hansen, J. A., Dinarello, C. A., Loertscher, R., Shapiro, H. M. and Strom, T. B.: The events of primary T cell activation can be staged by use of sepharose-bound anti-T3(6401) monoclonal antibody and purified interleukin 1. **J. Immunol. 135**, 2249-2255 (1985).
7. Burastero, S. E., Casali, P., Wilder, R. L. and Notkins, A. L.: Monoreactive high affinity and polyreactive low affinity rheumatoid factors are produced by $CD5^+B$ cells from patients with rheumatoid arthritis. **J. Exp. Med. 168**, 1979-1992 (1988).
8. Casali, P., Burastero, S. E., Nakamura, M., Inghirami, G. and Notkins, A. L.: Human lymphocytes making rheumaoid factor and antibody to ssDNA belong to Leu-1 B cell subset. **Science 236**, 77-81 (1987).
9. Sato, H. and Boyse, E. A.: A new alloantigen expressed selectively on B cells: the Lyb-2 system. **Immunogenetics 3**, 565-572 (1976).
10. Matsuura, A., Ishii, Y., Yuasa, H., Narita, H., Kon, S., Takami, T. and Kikuchi, K.: Rat T lymphocyte antigens comparable with mouse Lyt-1 and Lyt-2, 3 antigenic systems: Characterization by monoclonal antibodies. **J. Immunol. 132**, 316-322 (1984)
11. Yamaguchi, A., Uede, T., Kokai, Y., Ishii, Y. and Kikuchi, K.: A novel antigenic determinant of the T200 glycoprotein expressed exclusively by Jurkat cells. **Jpn. J. Cancer Res. 78**, 1378-1389 (1987).
12. Coligan, J., Kruisbeek, A. M., Hargolies, D. H., Shevach, E. M. and Strober, W.: Current Protolols in Immunology. Vol. I. NIH. Wiley interscience.
13. Huang, H. S., Jones, N. H., Strominger, J. L. and Herzenberg, L. A.: Molecular cloning of Ly-1, a membrane glycoprotein of mouse T lymphocytes and a subset of B cells: Molecular homology to its human counterpart Leu-1/T1 (CD5). **Proc. Natl. Acad. Sci. USA 84**, 204-208 (1987).
14. Sambrook, J., Fritsch, E. F. and Maniatis, T.: Molecular Cloning: A Laboratory Manual 2nd edition. 7. 19-7. 22, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)
15. Gübler, U. and Hoffman, B. J.: A simple and very efficient method for generating cDNA libraries. **Gene 25**, 263-269 (1983)
16. Feinberg, A. P. and Vogelstein, B.: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. **Anal. Biochem. 132**, 6-13 (1983)
17. Sanger, F., Nicklen, S. and Coulson, A. R.: DNA sequencing with chain-terminating inhibitors. **Proc. Natl. Acad. Sci. USA 74**, 5463-5467 (1977)
18. Jones, N. H., Clabby, M. L., Dialynas, D. P., Huang, H. S., Herzenberg, L. A. and Strominger, J. L.: Isolation of complementary DNA clones encoding the human lymphocyte glycoprotein T1/Leu-1. **Nature 323**, 346-349 (1986).
19. Kyte, J. and Doolittle, R. F.: A simple method for displaying the hydrophobic character of a protein. **J. Mol. Biol. 157**, 105-132 (1982).
20. Sambrook, J., Fritsch, E. F. and Maniatis, T.: Molecular Cloning: A Laboratory Manual 2nd edition. 9. 14-9. 23, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)
21. Kozak, M.: An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. **Nucl. Acids Res. 15**, 8125-8148 (1987).

22. Nakayama, E., von Hoegen, I. and Parnes, J. R.: Sequence of the Lyb-2 B cell differentiation antigen defines a gene superfamily of receptors with inverted membrane orientation. **Proc. Natl. Acad. Sci. USA 86**, 1352-1356 (1989).
23. Von Hoegen, I., Nakayana, E. and Parnes, J. R.: Identification of a human protein homologous to the mouse Lyb-2 B cell differentiation antigen and sequence of the corresponding cDNA. **J. Immunol. 144**, 4870-4877 (1990).
24. von Heijne, G.: A new method for predicting signal sequence cleavage sites. **Nucl. Acids Res. 14**, 4683-4690 (1986).
25. Williams, A. F. and Barclay, A. N.: The immunoglobulin superfamily-domains for cell surface recognition **Annu. Rev. Immunol. 6**, 381-405 (1988).
26. Morse, H. C., III., Shen, F.-W. and Hammerling, U.: Two allelic forms of mouse CD5, Ly-1a and Ly-1b have been defined by anti Lyt-1. and anti Lyt-1.2 antiserum and monoclonal antibodies **Immunogenetics 25**, 71-78 (1987).
27. Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. T., Gray, A., Tom, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayers, E. L. V., Whittle, N., Waterfield, M. D. and Seeburg, P. H.: Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. **Nature 309**, 418-425 (1984).
28. Ebina, Y., Ellis, L., Jarnagin, K., Edery, M., Graf, L., Clauser, E., Ou, J., Masiary, F., Kan, Y. M., Goldfine, J. D., Roth, R. A. and Ruter, W. J.: The human insulin receptor cDNA: The structural basis for hormone-activated transmembrane signalling. **Cell 40**, 747-758 (1985).
29. Hunter, T. and Cooper, J. A.: Protein-Tyrosine Kinase. **Annu. Rev. Biochem. 54**, 897-930 (1985).
30. Imboden, J. B., June, C. H., McCutcheon, M. A. and Ledbetter, J. A.: Stimulation of CD5 enhances signal transduction by the T cell antigen receptor. **J. Clin. Invest. 85**, 130-134 (1990).
31. Edelman, A. M., Blumenthal, D. K. and Krebs, E. G.: Protein serine/threonine kinases. **Annu. Rev. Biochem. 56**, 567-613 (1987).
32. Nishizuka, Y.: The role of protein kinase C in cell surface signal transduction and tumor promotion. **Nature 308**, 693-698 (1984).
33. Chatila, T. A. and Geha, R. S.: Phosphorylation of T cell membrane proteins by activators of protein kinase C. **J. Immunol. 140**, 4308-4314 (1988).
34. Griffith, L. C., Schulman, H. and Togawa, M.: In vivo and in vitro phosophorylation of murine lymphocyte differentiation antigen CD5. **Biochem. Biophys. Res. Commun. 159**, 536-541 (1989).
35. Lozano, F., Alberola-Ila, J., Places, L., Gallart, T. and Vives, J.: Phosphorylation-mediated changes in the electrophoretic mobility of CD5 molecules. **Eur. J. Biochem. 193**, 469-477 (1990).
36. Van de Velde, H., von Hoegen, I., Luo, W., Parnes, J. R. and Thielemans, K.: The B-cell surface protein CD72/Lyb-2 is ligand for CD5. **Nature 351**, 662-665 (1991).
37. Subbarao, B., Morris, J. and Baluyut, A. R.: Properties of anti-Lyb-2-mediatied B-cell activation and the relationship between Lyb-2 molecules and receptors for B-cell stimulation factor-1 on murine B lymphocytes. **Cell. Immunol. 112**, 329-342 (1988).
38. Singer, S. J., Maher, P. A. and Jaffa, M. P.: On the transfer of integral proteins into membranes **Proc. Natl. Acad. Sci. USA 84**, 1960-1964 (1987).
39. Plater-Zyberk, C., Maini, R. N., Lam, K., Kenndy, T. D. and Janossy, G.: A rheumatoid arthritis B cell subset expresses a phenotypic similar to that in chronic lumphocytic leukemia. **Arthritis Rhem. 28**, 971-976 (1986).
40. Dauphinee, M., Tovar, Z. and Talal, N.: B cells expressing CD5 are increased in Sjφgren's syndrome. **Arthritis Rheum. 31**, 642-647 (1988).
41. Sakane, T., Suzuki, N., Takada, S., Veda, Y., Murakawa, Y., Tsuchida, T., Yamauchi, Y. and Kishimoto, T.: B cell hyperactivity and its relation to distinct clinical features and the degree of disease activity in patients with systemic lupus erythematosus. **Arthritis Rheum. 31**, 80-87 (1988).
42. Casali, P. and Notkins, A. L.: Probing the B-cell repertoire with EBV: polyactive and $CD5^+$B-lymphocytes. **Annu. Rev. Immunol. 7**, 513-535 (1989).
43. Royston, I., Majda, J. A., Baird, S. M., Meserve, B. L. and Griffiths, J. C.: Human T cell antigens defined by monoclonal antibodies: the 65,000 dalton antigen of T cells(T65) is also found on chronic lymphocytic leukemia cells bearing sur-

face immunoglobulin. **J. Immunol. 125**, 725-731 (1980).
44. Kawamura, N., Muraguchi, A., Hori, A., Horii, Y., Mutsuura, S., Hardy, R. R., Kikutani, H. and Kishimoto, T.: A case of human B cell leukemia that implicates an autocrine mechanism in the abnormal growth of Leu 1B Cells. **J. Clin. Invest. 78**, 1331-1338 (1986).

ラットCD5抗原の構造と発現

村上俊也　松浦晃洋
札幌医科大学病理学第1講座（主任：菊地浩吉 教授）

リンパ球抗原CD5はT細胞や一部のB細胞に発現する糖蛋白でリンパ球の活性化や自己抗体の産生あるいは腫瘍化に関与する分子として知られている．我々はラットでのcDNA構造を明らかにすると共にCOS細胞での発現を確認した．その結果，ラットCD5 cDNAは全体で468個のアミノ酸より成り，細胞膜を貫く単一の膜貫通部分と93個のアミノ酸残基から成る細胞質ドメインを持つことがわかった．構造的にマウスやヒトのCD5と高い相同性を持ち，特にシグナル伝達に関与することが予想される細胞質ドメインはマウスとは97%，ヒトとは89%のホモロジーを示し，CD5分子の機能を考えるうえで興味深い．また，cDNAをプローブにしたラットゲノムの解析では遺伝的多型（RFLP）が認められた．