Biochemical and Biophysical Research Communications **276,** 244–250 (2000)
doi:10.1006/bbrc.2000.3409, available online at http://www.idealibrary.com on IDEAL®

Molecular Cloning and Chromosomal Mapping of a Novel Five-Span Transmembrane Protein Gene, *M83*

Tsutomu Motohashi,* Sousuke Miyoshi,* Mitsujiro Osawa,* Helen J. Eyre,† Grant R. Sutherland,† Yoichi Matsuda,‡ Yukio Nakamura,* Akira Shibuya,* Atsushi Iwama,* and Hiromitsu Nakauchi*,[1]

**Department of Immunology, Institute of Basic Medical Sciences, University of Tsukuba, and CREST (JST), Tsukuba, Ibaraki, Japan; †Center for Medical Genetics, Department of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, Adelaide, South Australia, Australia; and ‡Chromosome Research Unit, Faculty of Science, and Laboratory of Cytogenetics, Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Hokkaido, Japan*

Received August 2, 2000

In an attempt to identify novel transmembrane molecules expressed on hematopoietic cells, we identified a novel transmembrane protein gene, *M83*. Cloning of the full-length cDNAs of human and mouse M83 revealed that M83 encodes a type I transmembrane protein with a region containing five hydrophobic segments within the C-terminal part of the protein, suggesting that M83 is a five-span transmembrane molecule. The M83 protein was expressed on the cell surface as a glycosylated protein with a molecular mass of 84 kDa. The *M83* gene was localized to human chromosome 16p13.3, mouse chromosome 17B1, and rat chromosome 10q12.3 distal. In human, M83 mRNA was highly expressed in placenta, pancreas, and lymphohematopoietic tissues including peripheral blood, spleen, and bone marrow. Among hematopoietic cells, it was highly expressed in resting T lymphocytes and was downregulated by cell activation, suggestive of its biological role related to the T cell resting status.

Key Words: **five-span transmembrane protein; chromosomal mapping; resting T lymphocyte.**

Cellular functions, including proliferation, differentiation, activation, and survival are precisely regulated by various signals from extracellular environments which comprise neighboring cells, extracellular matrix, and secreted factors. Secreted factors, such as cytokines and growth factors, bind to their specific receptor molecules on the cell surface, and transmit signals for cell growth and activation. On the other hand, various adhesion molecules on the cell surface interact with their counterreceptors on surrounding cells or extracellular matrix, and mediate signals generated by cell-to-cell contact. These contact-mediated regulations are also critical in maintaining cellular homeostasis (1).

Hematopoietic system is maintained by continuous proliferation and differentiation of progenitor cells generated from the pluripotent hematopoietic stem cells (2). Hematopoiesis takes place in the bone marrow (BM). Within the BM environment, hematopoietic stem cells (HSC) are compartmentalized in certain niches and maintained through interactions with stromal cells, hematopoietic cytokines, and components of the extracellular matrix. More than 30 hematopoietic growth factors and their receptors have been cloned and characterized for their ability to regulate proliferation and differentiation of hematopoietic cells. In addition, various adhesion receptors have been identified on hematopoietic cells (1). These include integrins, selectins, and other types of adhesion molecules, such as CD34 and CD44, and are selectively expressed depending on cell lineages and maturation stages. Hematopoietic progenitors express high levels of α4, α5, α6, and β1 integrins (3), among which the pivotal role of $\alpha 4\beta 1$ (VLA-4) in HSC homing to the BM has been demonstrated *in vivo* (4, 5). These adhesion receptors also play important roles in leukocyte trafficking in the circulation and functions in the periphery (1, 6). Leukocyte activation evokes coordinated changes in the expression of specific membrane molecules that allow cells to respond to environmental stimuli in an appropriate manner. All these accumulating evidences demonstrate complex regulations of hematopoietic system by extracellular signals and point out the importance of better understanding of cell membrane molecules that mediate these signals inside the cells.

In an attempt to identify novel transmembrane molecules expressed on hematopoietic cells, we identified a novel transmembrane protein gene, M83. Cloning of the full-length cDNAs of human and mouse M83 revealed that M83 encodes a novel five-span transmem-

The nucleotide sequence of human and mouse M83 have been deposited in the GenBank database under the Accession Nos. AB045292 and AB045293, respectively.

[1] To whom correspondence should be addressed. Fax: +81-298-53-6966. E-mail: nakauchi@md.tsukuba.ac.jp.

0006-291X/00 $35.00

brane protein without any significant sequence homology to known multi-span membrane proteins. Among hematopoietic cells, its expression was tightly correlated with cell resting status. These data indicate that the M83 antigen represents a new class of five-span transmembrane protein involved in the regulation of hematopoietic cell functions.

MATERIALS AND METHODS

Cell culture. COS7 cell were maintained in DMEM medium (Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine (Gibco BRL), and 3.32 μM 2-mercaptoethanol. Ba/F3 cells were maintained in RPMI 1640 medium (Gibco BRL) supplemented with 10% FCS, 2 mM L-glutamine, and 1/10 volume of WEHI-3 cell conditioned medium as a source of mouse interleukin-3.

cDNA cloning and sequencing. M83 cDNA fragments were isolated by an expression cloning of transmembrane proteins (see Results, Miyoshi, S., *et al.*, in preparation). To isolate full-length M83 cDNA, an UT-7 cell library in pCDM8 (Invitrogen, Carlsbad, CA) (7) was screened using an [α-^{32}P]-labeled M83 cDNA fragment according to the manufacturer's instructions. Screening of the EST database with the human M83 sequence using the BLAST 2.0 program, several ESTs were identified that represent the putative mouse homologue of M83. According to the EST sequence information, a probe was amplified by RT-PCR. A mouse thymus cDNA library in λgt10 (kindly provided by Dr. Ogata, Osaka University) was screened with an [α-^{32}P]-labeled mouse M83 cDNA. Hybridization was performed overnight at 55°C in 6× SSC, 3× Denhardt's solution, 0.5% SDS, 0.1 μg/ml salmon sperm DNA. After hybridization, the filters were washed with 2× SSC, 0.1% SDS at 55°C. The cDNA inserts of positive clones were subcloned into the Bluescript vector and sequenced using ABI 370 system (Perkin–Elmer Co., Foster City, CA).

Detection of M83 protein by FACS. M83 cDNA was tagged with a FLAG epitope at the N-terminus (FLAG-M83) or the C-terminus (M83-FLAG) in pFLAG-CMV-1 and pFLAG-CMV-5a (Sigma, St. Louis, MO), respectively and then subcloned into pcDNA3 expression vector (Invitrogen). In FLAG-M83 plasmid, its own signal sequence was replaced by preprotrypsine signal sequence followed by a FLAG epitope. These constructs were transiently expressed in COS7 cell by calcium phosphate transfection. The transfectants were stained with biotinylated mouse anti-FLAG antibody, BioM2 (Sigma), followed by avidin-APC (PharMingen, San Diego, CA). The expression of M83 protein on the cells was detected by flow cytometric analysis on a FACSVantage (Becton–Dickinson, San Jose, CA).

Cell surface biotinylation and immunodetection. COS7 cells transfected with M83 expression vector were biotinylated on the cell surface using ECL protein biotinylation module (Amersham Pharmacia Biotech, Buckinghamshire, UK). After incubation at 4°C for 30 min., the cells were washed with PBS containing 10 mM glycine three times, and were lysed in lysis buffer [50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 1% NP40, 1 mM phenylmethanesulfonyl fluoride, and 0.1 μg/ml aprotinin]. The M83 protein was immunoprecipitated with a monoclonal antibody against the FLAG epitope, M2 (Sigma). The immunoprecipitates were resolved on a 6.5% gel by SDS–polyacrylamide gel electrophoresis and transferred to a PVDF membrane (Immobilon Transfer Membranes, Millipore, Bedford, MA). For immunoblotting, membranes were incubated with 3% bovine serum albumin in TBS-T buffer [20 mM Tris–HCl (pH 8.0), 137 mM NaCl, 0.1% Tween 20] overnight at 4°C and probed with horseradish peroxidase-conjugated avidin (Amersham Pharmacia Biotech). After stripping of avidin, the membrane was then reprobed with a monoclonal antibody against the FLAG epitope, followed by horseradish peroxidase-conjugated anti-mouse antibody (Amersham Pharmacia Biotech). Blots were developed with the Supersignal system (Pierce, Rockford, IL) according to the manufacturer's instructions. Hydrolysis of N-linked oligosaccharides from the precipitated protein was performed with 0.5 unit *N*-glycosidase F (Roche Diagnostics, Tokyo, Japan) according to the manufacturer's instructions.

Northern blot analysis. Total RNA was isolated from human nonlymphoid leukemic cell line F36P and mouse myeloblastic cell line 32D by guanidium isothiocyanate extraction followed by CsCl gradient purification. RNA samples (10 μg/lane) were resolved on a 1.0% agarose/formaldehyde gel and transferred to Biodyne nylon membranes (Pall Co., Port Washington, NY). [α-^{32}P]dCTP-labeled full-length M83 cDNA was used as a probe.

RT-PCR. RT-PCR analysis of human M83 gene expression in human tissues was performed using Human MTC panel cDNAs (Clontech, Palo Alto, CA). The primer sequences were human M83 sense primer, 5′-TAC TTC GCT CAA CTG CAC CAC A-3′; M83 antisense primer, 5′-GAA GAG GTC ATC AGA GCA GCA G-3′; GAPDH sense primer, 5′-CTT CAC CAC CAT GGA GAA GGC-3′; and GAPDH antisense primer, 5′-GGC ATG GAC TGT GGT CAT GAG-3′. For human hematopoietic cells, various hematopoietic cell fractions in bone marrow and peripheral blood were collected by cell sorting on a FACSVantage (Becton–Dickinson) using monoclonal antibodies against CD3, CD4, CD8, CD13, CD14, CD19, CD34, CD56, and CD71 (PharMingen). Peripheral blood $CD4^+$ T cells were purified by MACS positive selection system using anti-CD4 antibody conjugated by magnetic beads (Miltenyi Biotec, Auburn, CA), and peripheral blood $CD8^+$ T cells were sorted on a FACSVantage using anti-CD8 antibody (PharMingen). Purified cells were activated for 12 h by 1.0 μg/ml anti-CD3 antibody (PharMingen) and 100 unit/ml interleukin-2 (kindly provided by Takeda Chemical Industry Ltd., Osaka, Japan). Total RNA was isolated by small-scaled acid guanidium thiocyanate-phenol–chloroform extraction using ISOGEN-LS solution (Nippon Gene, Tokyo, Japan) and reverse-transcribed using SuperScript II RT-PCR system (Gibco BRL) and oligo-dT primer. The amount of cDNA was normalized by the quantitative PCR using TaqMan rodent GAPDH control reagent (Perkin–Elmer Applied Biosystem, Foster City, CA). Semiquantitative RT-PCRs were then carried out for human M83. Cycling parameters were denaturation at 94°C for 20 s, annealing at 60°C for 20 s, and extension at 72°C for 30 s. PCR products were separated on an agarose gel and visualized by ethidium bromide staining.

Chromosomal mapping The human M83 probe was nick-translated with biotin-14-dATP and hybridized *in situ* at a final concentration of 15 ng/μl to metaphases from two normal males. The fluorescence *in situ* hybridization (FISH) method was modified from that previously described (8) in that chromosomes were stained before analysis with both propidium iodide (as counterstain) and DAPI (for chromosome identification). Images of metaphase preparations were captured by a cooled CCD camera using the ChromoScan image collection and enhancement system (Applied Imaging Int., Ltd.). FISH signals and the DAPI banding pattern were merged for figure preparation.

The direct R-banding FISH method was used for chromosomal assignment of the *M83* gene to mouse and rat chromosomes. Preparation of R-banded chromosomes and FISH were performed as previously described (9, 10). The mouse 3.5 kb M83 cDNA fragment inserted in pBluescript KS (+) was labeled by nick translation with biotin-14-dATP (Roche Diagnostics). The hybridized biotinylated probes were reacted with goat anti-biotin antibody (Vector Laboratories) and then stained with Cy2-labeled donkey anti-goat IgG (Amersham Pharmacia Biotech). FISH images were observed under Nikon fluorescence microscope using Nikon filter sets B-2A and UV-2A. Kodak Ektachrome ASA100 films were used for microphotography.

RESULTS

Isolation and Analysis of Full-Length M83 cDNA

To isolate novel cytokine receptors, we designed an expression cloning vector that contains preprotrypsine

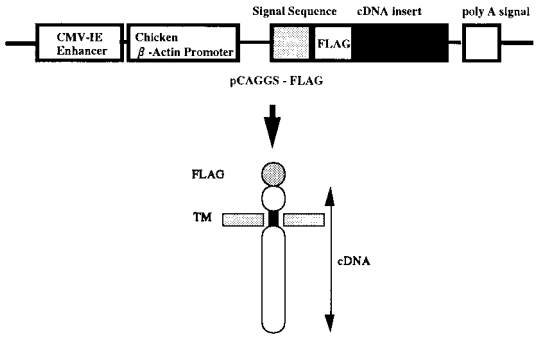

FIG. 1. Schematic representation of the pCAGGS-FLAG expression cloning vector (upper panel) and a FLAG-cDNA fusion gene product. Only the FLAG-cDNA fusion gene product that contains transmembrane domain (TM) could localize on the cell surface as a transmembrane protein (lower panel).

signal sequence followed by a FLAG epitope (pCAGGS-FLAG; Fig. 1), and a degenerate PCR primer directed to WS*x*WS motif characteristic of cytokine receptor superfamily. cDNA fragments were amplified by PCR using WS*x*WS degenerate primer and oligo-dT primer, and were incorporated downstream of the FLAG epitope so as to create FLAG-cDNA fusion libraries (Miyoshi, S., *et al.*, in preparation). If the cDNA is inserted in-frame and contains transmembrane domains, the extracellular domain is expressed on the cell surface of transfectants, and can be detected by FACS analysis using anti-FLAG antibody. An expression cloning library prepared from a human non-lymphoid leukemia cell line, F36P, was expressed transiently in COS7 cells, and cells expressing FLAG epitope were enriched by FACS cell sorting with anti-FLAG antibody. The plasmid rescued from the sorted cells was used to transfect COS7 cells for secondary screening. Positive clones were enriched by repeating this process twice, and isolated by transformation of bacteria. Screening of F36P library resulted in identification of three novel cDNA fragments in addition to several known cytokine receptors. Although these novel cDNA fragments did not have any motifs conserved among the cytokine receptor superfamily, they revealed to encode the same putative transmembrane protein, which we designated as *M83*.

Using the longest M83 cDNA fragment (nucleotides 1444 to 2578 in Fig. 2) as a probe, we screened an UT-7 cell and isolated three independent positive clones. The nucleotide sequence of the human M83 cDNA, with a length of 2578 bp, is shown in Fig. 2. The M83 cDNA contains a single open reading frame of 2316 bp encoding a polypeptide of 771 amino acids. The open reading frame is preceded by a 5′ untranslated region of 103 bp and followed by a 3′ untranslated region of 159 bp, which includes a polyadenylation signal (AATAAA) 20 bp upstream of the poly(A) tail. A hydropathy plot identified a potential hydrophobic leader sequence (residues 1–28) and a region with five hydrophobic segments within the C-terminal part of the protein (Fig. 3B). This suggests that M83 is a type I five-span transmembrane protein, with an extended extracellular region of 514 amino acids, a 198-amino-acid region that passes the membrane five times, and a short cytoplasmic tail of 31 amino acids. The mature protein supposedly consists of 743 amino acids with a predicted

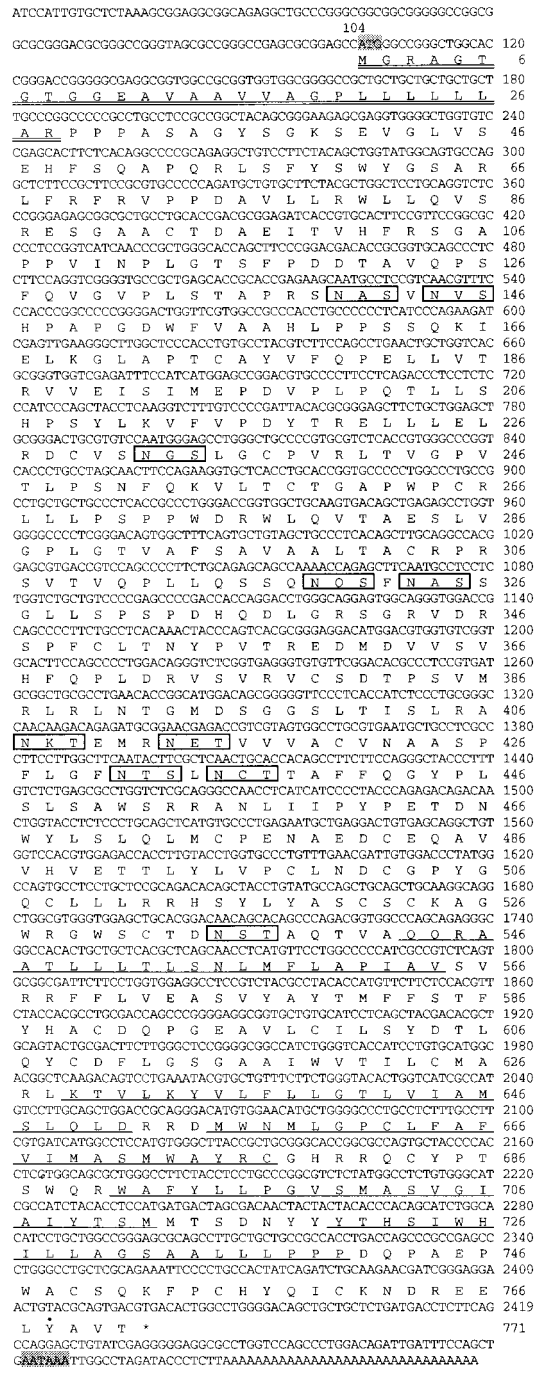

FIG. 2. Nucleotide and predicted amino acid sequence of human M83. The signal sequence is doubly underlined. Five transmembrane domains are underlined. Potential N-linked glycosylation sites are boxed. Putative tyrosine phosphorylation site is indicated by a dot.

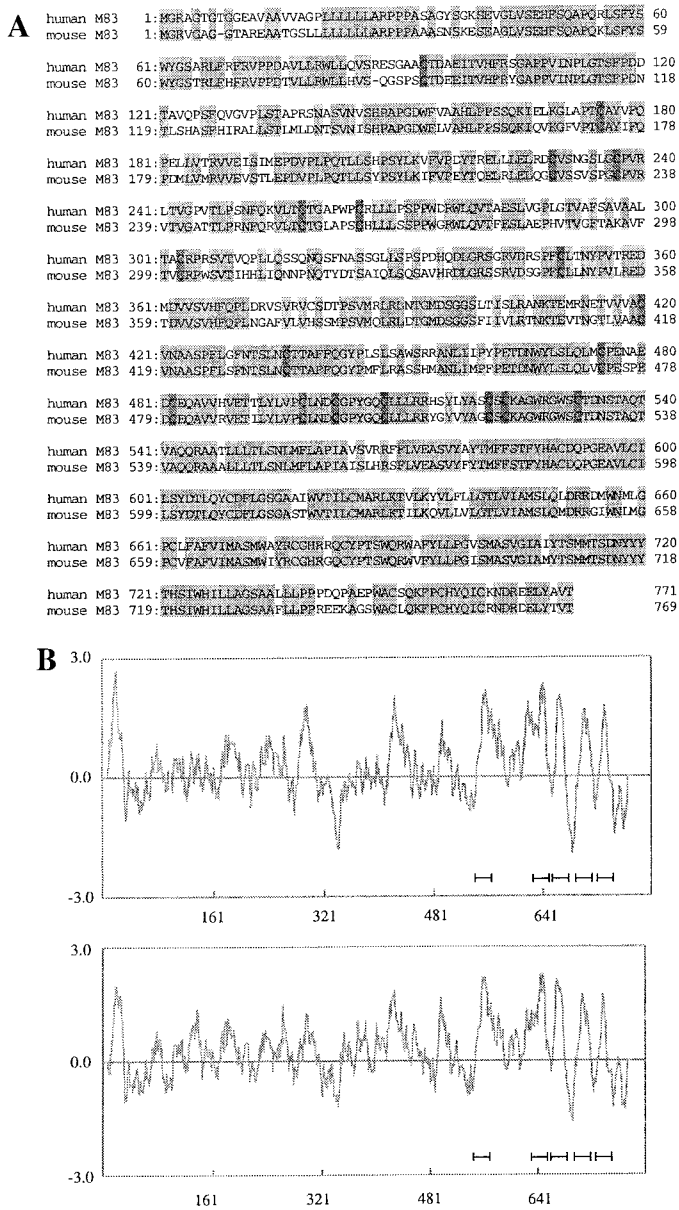

FIG. 3. (A) Alignment of human M83 and mouse M83 amino acid sequences. Conserved residues are indicated by light gray and cysteine residues in the extracellular domain are indicated by dark gray. (B) Hydropathy plots of human (upper column) and mouse M83 (lower column). The Kyte and Doolittle scale (23) was used to plot hydrophobicity and the transmembrane regions were predicted by SOSUI system (24). *X* axis indicates amino acid number. *Y* axis indicates hydrophobicity of each residue. Bars indicate putative transmembrane segments.

molecular mass of 82 kDa. The extracellular region contains ten potential N-glycosylation sites, Asn-X-Ser/Thr (Fig. 2). Membrane-proximal portion of the extracellular region is rich in cysteines and contains a sequence that matches the consensus sequence of EGF-like motif C-X-C-X (5)-G-X (2)-C (residues 521–532). The short cytoplasmic tail contains a tyrosine residue that is a potential phosphorylation site by tyrosine kinases (11). Gene database analysis identified several ESTs that represent mouse homologue of the human *M83* gene. A cDNA fragment was amplified by PCR according to EST sequence information (EST AA492649, W65862), and was used to screen a mouse thymus cDNA library. Eight independent positive clones were isolated including two clones containing mouse M83 full-length sequence. The mouse *M83* gene encodes a protein of 769 amino acid long (Fig. 3A). Identity between human and mouse was 78% in nucleotide, and 75.5% in amino acid. Database search for the M83-related protein revealed a high amino acid sequence similarity between human M83 and human NAG-5 protein (Accession No. AF149297). The NAG-5 protein shows 37% homology in total, with the highest homology to the M83 first transmembrane domain (55%), but no significant homology to the other parts of M83. Seventeen out of 18 cysteine residues in the human M83 extracellular domain are conserved between M83 and NAG-3 (data not shown).

Characterization of M83 as a Glycosylated Transmembrane Protein

To analyze cell surface expression of M83, we generated M83 expression constructs in which M83 was tagged with a FLAG epitope at the N-terminus (FLAG-M83) or at the C-terminus (M83-FLAG). In FLAG-M83, its own signal sequence was replaced by preprotrypsine signal sequence followed by a FLAG epitope. These constructs were transiently expressed in COS7 cell and analyzed by FACS. FLAG epitope was readily detected on the cell surface in FLAG-M83 transfectants, but not in M83-FLAG transfectants (Fig. 4A), suggesting that M83 is a type I transmembrane protein. The cell surface expression of M83 was also confirmed by cell surface biotinylation experiment. COS7 cells transfected with either FLAG-M83 or M83-FLAG were biotinylated on its cell surface and subjected to immunoprecipitation with anti-FLAG antibody, followed by avidin-HRP immunoblotting. Both FLAG-M83 and M83-FLAG proteins were detected as approximately 84 kDa biotinylated protein (Fig. 4B), indicating that there is no major modification of M83 protein by proteolytic cleavage. To investigate the modification of M83 protein by glycosylation, M83 protein was treated with *N*-glycanase. As shown in Fig. 4C, a clear deglycosylation was observed by *N*-glycanase treatment, resulting in the reduction of M83 molecular mass from 84 kDa into 62 kDa.

Expression of M83 Gene

Northern blot analysis detected 4.0 and 2.9 kb M83 transcripts in both human and mouse cell lines (Fig. 5A). The human cDNAs isolated in this study have short 3′ untranslated region and may correspond to the 2.9 kb transcript. On the other hand, the mouse M83

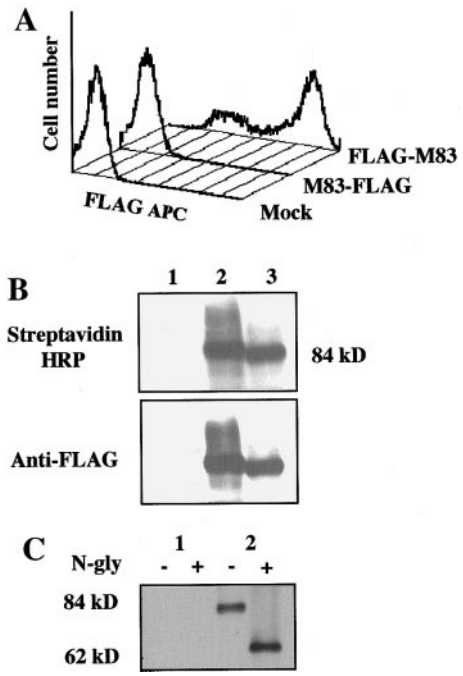

FIG. 4. Characterization of M83 protein. (A) Flow cytometric analysis of M83 expression on the cell surface. COS7 cells were transiently transfected with M83 cDNAs tagged with a FLAG epitope at the N-terminus (FLAG-M83) or C-terminus (M83-FLAG). The transfectants were stained with biotinylated anti-FLAG antibody, followed by avidin-APC. As a negative control, the flow cytometric profile of COS7 cells transfected with empty vector (Mock) is also shown. (B) Detection of M83 protein by cell surface biotinylation. COS7 cells transfected with empty vector (lane 1), FLAG-M83 cDNA (lane 2), and M83-FLAG (lane 3) were biotinylated on the cell surface. Proteins in cell lysates were then immunoprecipitated with anti-FLAG antibody. Immunoprecipitates were separated on a 6.5% SDS–PAGE gel, transferred, and probed with streptavidin–HRP (upper column). After stripping, the blot was reprobed with anti-FLAG antibody (lower column). (C) Glycosylation of M83 protein. M83-FLAG protein transiently expressed in COS7 cells (lane 2) was immunoprecipitated with anti-FLAG antibody and incubated in the presence of 0.5 unit *N*-glycosidase F (N-gly). Immunoprecipitates were then separated on a 6.5% SDS–PAGE gel, transferred, and probed with anti-FLAG antibody. Lane 1 stands for negative control COS7 cells transfected with empty vector.

clones isolated have a long 3′ untranslated region and match the 4.0 kb transcript in size. Among ESTs identical to human M83, several ESTs including EST AA368424 contain a longer 3′ untranslated region of 1,080 bp long and may represent the longer transcript. These data suggest that two different transcripts arise from differential usage of polyadenylation signals.

Expression of human M83 mRNA was analyzed by RT-PCR using mRNA from various tissues and highly fractionated hematopoietic cells. In human tissues, M83 mRNA was abundantly expressed in pancreas, placenta, spleen, liver, kidney, bone marrow, peripheral blood leukocytes, and tonsil (Fig. 5B). In human hematopoietic cells, the mRNA was predominantly expressed in $CD4^+$ and $CD8^+$ resting T cells in peripheral blood (Fig. 5C). To investigate the M83 gene expression during T lymphocyte activation, we isolated RNA from peripheral blood T cells either in resting or activated states. RT-PCR analysis demonstrated drastic down-regulation of M83 mRNA in $CD4^+$ and $CD8^+$ T cells activated by anti-CD3 antibody in the presence of IL-2 (Fig. 5D). These findings were also observed in peripheral blood $CD4^+$ and $CD8^+$ T cells activated by concanavalin A and phytohemagglutinin, respectively (data not shown).

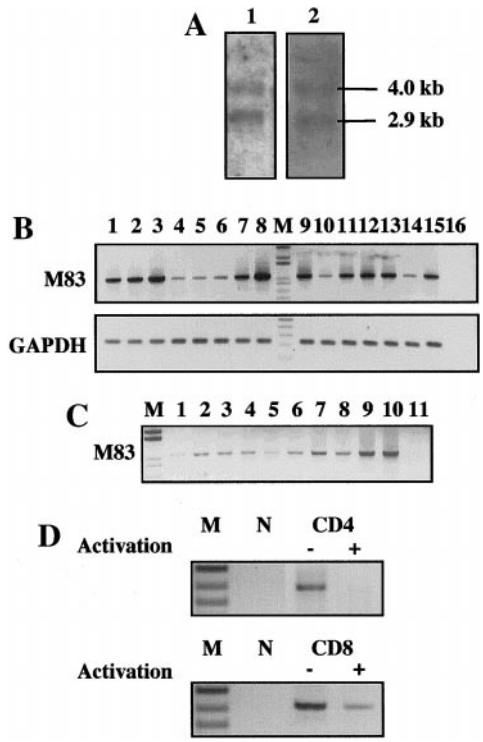

FIG. 5. Expression of M83 mRNA. (A) Northern blot analysis with M83 mRNA in human leukemic cell line F36P (lane 1) and mouse myeloblastic cell line 32D (lane 2). (B) RT-PCR analysis of M83 mRNA expression in human tissues. Semiquantitative RT-PCR was performed with primers specific for M83 (upper panel) and GAPDH (lower panel) on normalized human tissue cDNAs; kidney (lane 1), liver (lane 2), placenta (lane 3), skeletal muscles (lane 4), brain (lane 5), heart (lane 6), lung (lane 7), pancreas (lane 8), peripheral blood leukocyte (lane 9), lymph node (lane 10), bone marrow (lane 11), spleen (lane 12), fetal liver (lane 13), thymus (lane 14), tonsil (lane 15), and no cDNA (lane 16). (C) RT-PCR analysis of M83 mRNA expression in human hematopoietic cell. Semiquantitative RT-PCR was performed on normalized cDNAs for GAPDH expression using ABI 7700. Bone marrow samples: $CD34^+$ hematopoietic progenitor cells (lane 1), $CD34^-$ cells (lane 2), $CD13^+$ myeloid cells (lane 3), $CD14^+$ monocytes/macrophages (lane 4), $CD71^+$ erythroblasts (lane 5), $CD56^+$ natural killer cells (lane 6), peripheral blood samples: $CD3^+$ T cells (lane 7), $CD19^+$ B cells (lane 8), $CD4^+$ T cells (lane 9), $CD8^+$ T cells (lane 10), and no cDNA (lane 11). (D) RT-PCR analysis of M83 mRNA expression in human peripheral T cells. Purified $CD4^+$ (upper column) and $CD8^+$ T cells (lower column) were activated by anti-CD3 antibody in the presence of human IL-2. After activation for 12 h, cDNAs were prepared from each cell. Each cDNA was normalized for GAPDH expression using ABI 7700. M: Marker, N: negative control (no cDNA).

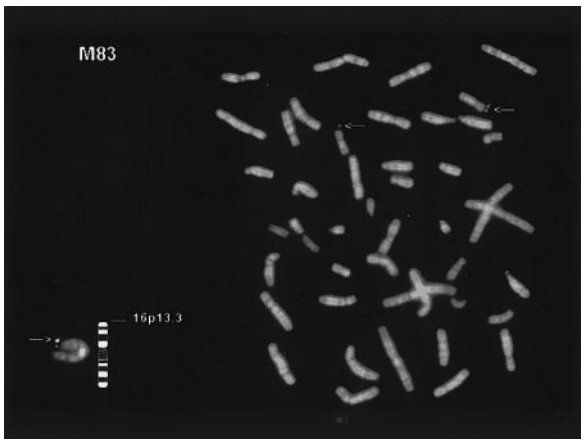

FIG. 6. Chromosomal localization of the human *M83* gene. Metaphase and partial metaphase showing FISH with the M83 probe. Normal male chromosomes stained with DAPI. Hybridization sites on chromosome 16 are indicated by arrows.

Chromosomal Localization of Human, Mouse, and Rat M83

The chromosomal localization of human *M83* gene was studied by FISH on human, mouse, and rat metaphase chromosomes. Twenty metaphases from the first normal male were examined for fluorescent signal. All of these metaphases showed signal on one or both chromatids of chromosome 16 in the region 16p13.2-16p13.3; 95% of this signal was at 16p13.3 (Fig. 6). There were a total of 28 non-specific background dots observed in these 20 metaphases. A similar result was obtained from hybridization of the probe to 10 metaphases from the normal male (data not shown). The chromosomal assignment of the *M83* gene to mouse and rat chromosomes was made by the direct R-banding FISH using a mouse cDNA fragment as a probe. The *M83* gene was localized to mouse chromosome 17B1 and rat chromosome 10q12.3 distal (Fig. 7). They were mapped in the region where the conserved linkage homology has been identified between the species analyzed (12).

DISCUSSION

A novel five-span transmembrane protein, M83, was identified in this study. Multi-span transmembrane proteins compose a growing superfamily that is divided into several subfamilies based on the sequence homology, particularly within the transmembrane domains. The majority of the multi-span cell surface proteins belong to four- or seven-span transmembrane protein family, in which members share significant homology with each other (13, 14). To our knowledge, only two proteins, CD47 and AC133, have been reported as five-span transmembrane cell surface proteins (15, 16). M83 does not share sequence homology with other multi-span transmembrane proteins, even with the known five-span transmembrane proteins. Although all of the five-span transmembrane proteins have an extracellular N-terminus and a cytoplasmic C-terminus, M83 has an extended N-terminal extracellular region, while CD47 has a shorter N-terminal extracellular region and AC133 contains two large extracellular loops. These findings suggest that M83 represents a new subfamily of five-span transmembrane proteins. Gene database search revealed that human NAG-5 protein (Accession No. AF149297) is homologous to M83, particularly to its N-terminal extracellular region and the first transmembrane domain. In addition, the spacing of cysteine residues in the extracellular region are completely conserved between the two proteins. Although the complete sequence information on NAG-5 is not yet available, M83 and NAG-5 may define a new subfamily of the multi-span transmembrane superfamily.

M83 was detected as a glycosylated cell surface protein with a molecular mass of 84 kDa. As expected from its ten potential *N*-glycosylation sites, *N*-glycanase treatment resulted in 22 kDa reduction in M83 molecular mass, indicating that 62 and 84 kDa proteins represent the core and *N*-glycosylated proteins, respectively. As shown in Fig. 4B, there was no apparent proteolytic modification of M83. Thus, there is a significant discrepancy between the predicted molecular mass of 82 kDa and the biochemical findings. However, the tendency to migrate faster than predicted on the basis of molecular mass is a well known feature of many multi-span proteins (17). This seems to be also true for M83.

Multi-span transmembrane protein superfamily involves receptors that bind to biologic active factors and adhesion molecules that mediate signals generated by cell-to-cell contact (13, 14). In addition, some of the members have been reported to associate with other cell surface molecules including integrins and modulate their function (14). For example, CD47, one member of five-span transmembrane proteins functions as a marker of self on red blood cells. CD47 on red blood cells binds to the inhibitory receptor signal regulatory protein alpha (SIRPα) on splenic red pulp macrophages and prevents red blood cells from elimination by macrophages (18). CD47 is also associated with $\alpha v\beta 3$ integrins and involved in the regulation of integrin function (19). Although no ligands or counter-receptors of M83 is known at present, its specific expression in resting T cells is suggestive of important role in the functional regulation of T cells. The traffic and tissue localization of leukocytes is regulated by a series of cell surface adhesion molecules that recognize specific ligands on endothelium and in the extracellular matrix. Modulation of the expression of these adhesion molecules results in the changes in T cell trafficking. During T cell activation and differentiation, downregula-

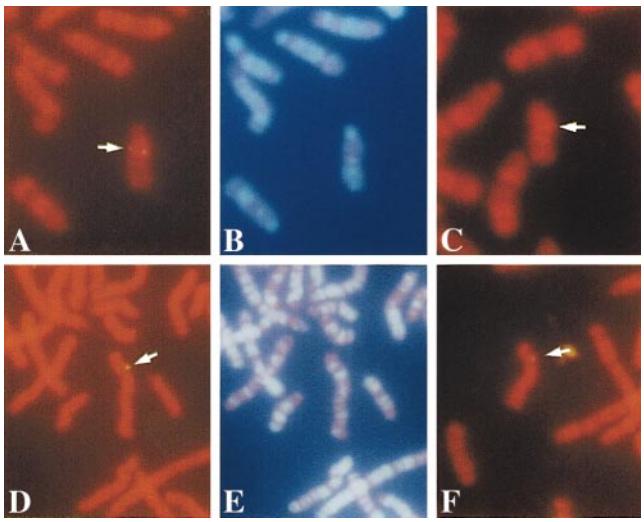

FIG. 7. Chromosomal location of the *M83* gene on mouse (A–C) and rat (D–F) R banded chromosomes. The mouse M83 cDNA fragment was used as a biotinylated probe. The hybridization signals are indicated by arrows. The *M83* gene was localized to mouse chromosome 17B1 and rat chromosome 10q12.3 distal. The metaphase spreads were photographed with Nikon B-2A (A, C, D, F) and UV-2A (B, E) filters. R-band and G-band patterns are demonstrated in (A, C, D, F) and (B, E), respectively.

tion of adhesion receptors specific for lymphoid tissue endothelium and upregulation of integrins facilitate the targeting of effector cells to the inflammation sites (6). Cytokines and the local lymphoid microenvironment are involved in these regulations of stage-specific expression of T cell adhesion molecules. One of the genes downregulated during T cell activation is L-selectin. Downregulation of L-selectin results in poor binding of lymphocytes to lymph node high endothelial venules, which causes reduced recirculation of lymphocytes through lymph nodes and facilitates lymphocyte migration to inflamed focus (20, 21). Downregulation of M83 mRNA during T cell activation, particularly by specific stimulatory pathway through the T cell receptor/CD3 complex, suggests that M83 is involved in the resting, but not activated T cell functions, such as localization and/or functional quiescence of resting T cells. Although the M83 mRNA expression is not so high in other leukocytes, M83 mRNA was also downregulated during activation of B cells and monocytes (data not show). These findings indicate universal function of M83 in resting leukocytes.

In the short C-terminal tail of M83, there is no signaling motif except for two tyrosine residues. The amino acid sequence surrounding the latter tyrosine satisfy that of potential tyrosine phosphorylation site, indicating that M83 could initiate a signal transduction cascade in response to its ligand or counter-receptor binding. Many members of the multi-span transmembrane superfamily have been characterized as a signal transducer, including seven-span family members that elicit signals through coupling with G proteins (22). Further studies are needed to address the physiologic ligands or counter-receptors of M83 as well as signaling pathways associated with M83.

ACKNOWLEDGMENTS

We thank Dr. Sibuya K. and other members in our laboratory for discussion and critical reading of the manuscript. This study was supported by Grants-in-Aid from the Ministry of Education, Science, and Culture, and the Agency for Science and Technology, Japan, and in part by the Wellcome Trust and the National Health and Medical Research Council of Australia.

REFERENCES

1. Verfaillie, C. M. (1998) *Blood* **92,** 2609–2612.
2. Metcalf, D. (1999) *Ann. NY Acad. Sci.* **872,** 289–303.
3. Voura E. B., Ballia, F., Iscove, N. N., and Hawley, R. G. (1997) *Exp. Hematol.* **25,** 1172–1179.
4. Papayannopoulou, T., and Nakamoto, B. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 9374–9378.
5. Potocnik, A. J., Brakebusch, C., and Fässler, R. (2000) *Immunity* **12,** 653–663.
6. Dailey, M. O. (1998) *Crit. Rev. Immunol.* **18,** 153–184.
7. Nakamura, Y., Komano, H., and Nakauchi, H. (1993) *Exp. Hematol.* **21,** 236–242.
8. Callen, D. F., Baker, E., Eyre, H. J., Chernos, J. E., Bell, J. A., and Sutherland, G. R. (1990) *Ann. Génét.* **33,** 219–221.
9. Matsuda, Y., Harada, Y. N., Natsuume-Sakai, S., Lee, K., Shiomi, T., and Chapman, V. M. (1992) *Cytogenet. Cell Genet.* **61,** 282–285.
10. Matsuda, Y., and Chapman, V. M. (1995) *Electrophoresis* **16,** 261–272.
11. Cooper, J. A., Esch, F. S., Taylor, S. S., and Hunter, T. (1984) *J. Biol. Chem.* **259,** 7835–7841.
12. Serikawa, T., Cui, Z., Yokoi, N., Kuramoto, T., Kondo, Y., Kitada, K., and Guenet, J. L. (1998) *Exp. Anim.* **47,** 1–9.
13. Maecker, H. T., Todd, S. C., and Levy, S. (1997) *FASEB J.* **11,** 428–442.
14. Baldwin, J. M. (1993) *EMBO J.* **12,** 1693–1703.
15. Mawby, W. J., Holmes, C. H., Anstee, D. J., Spring, F. A., and Tanner, M. J. (1994) *Biochem. J.* **304,** 525–530.
16. Miraglia, S., Godfrey, W., Yin, A. H., Atkins, K., Warnke, R., Holden, J. T., Bray, R. A., Waller, E. K., and Buck, D. W. (1997) *Blood* **90,** 5013–5021.
17. Hamann, J., Eichler, W., Hamann, D., Kerstens, H. M. J., Poddighe, P. J., Hoovers, J. M. N., Hartmann, E., Strauss, M., and van Lier, R. A. W. (1995) *J. Immunol.* **155.** 1942–1950.
18. Oldenborg, P.-A., Zheleznyak, A., Fang, Y.-F., Lagenaur, C. F., Gresham, H. D., and Lindberg, F. P. (2000) *Science* **288,** 2051–2054.
19. Brown, E., Hooper, L., Ho, T., and Gresham, H. (1990) *J. Cell. Biol.* **111,** 2785–2794.
20. Jung, T. M., Gallatin, W. M., Weissman, I. L., and Dailey, M. O. (1988) *J. Immunol.* **141,** 4110–4117.
21. Chao, C.-C., Jensen, R. J., and Dailey, M. O. (1997) *J. Immunol.* **159,** 1686–1694.
22. Watson, S., and Arkinstall, S. (1994) The G-Protein-Linked Receptor Facts Book, Academic Press, Ltd., London.
23. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 106–132.
24. Hirokawa, T., Boon-Chieng, S., and Mitaku, S. (1998) *Bioinformatics* **14,** 378–379.