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Genomic Structure and Production of Variant mRNAs by Alternative Splicing in Human Lymphocyte Function-Associated Antigen-3 (LFA-3, CD58)

Toru WAKATSUKI[1)2)], **Mikio YAMAMOTO**[1)] and **Yuichi TAKAKUWA**[2)]

[1)]Department of Biochemistry II (Director: Prof. Mikio YAMAMOTO),
National Defense Medical College

[2)]Department of Biochemistry (Director: Prof. Yuichi TAKAKUWA)
Tokyo Women's Medical University, School of Medicine

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Several distinct mRNA species have been demonstrated for lymphocyte function-associated antigen-3 (LFA-3, CD58), a cell-surface glycoprotein anchored either through glycosyl phosphatidyl inositol (GPI)-linkage or by a transmembrane domain. We obtained a genomic clone for the LFA-3 gene and performed structural and functional analyses. The LFA-3 gene is at least 41.1 kb in length and consists of 6 exons. Two major mRNA species are generated by alternative splicing acceptor selection in exon 5, and two other minor ones by partial or entire elimination of exon 3 as a result of alternative splice donor selection or exon-skip. Primer extension analysis revealed that transcription may be initiated at a series of sites, probably because of the absence of TATA and CAAT box sequences. A 1.3-kb 5′-upstream flanking region was able to drive the expression of a reporter gene in an orientation-dependent manner, but only at a low level. The evolutionary relationship of LFA-3 with other two related molecules, CD2 and CD48, was also discussed on the basis of genomic structure.

Introduction

Lymphocyte function-associated antigen-3 (LFA-3, CD58), the ligand/receptor of T cell membrane protein CD2, is a highly glycosylated surface protein with a broad tissue distribution[1)2)]. It is a member of the immunoglobulin (Ig) superfamily with two extracellular Ig-like domains, mediates cell adhesion, and has a significant structural homology to two other adhesion molecules, CD2 and CD48[3)~6)]. In contrast to LFA-3, the latter two molecules are only expressed in a very limited number of cell types such as on T cells, NK cells and granulocytes[7)8)]. Interactions of LFA-3 and CD2 are important for cytolytic conjugate formation[9)10)], and for T-cell activation[11)]. Inhibition of tumor-infiltrating lymphocyte-mediated killing was observed when melanoma cells were pretreated with anti-LFA-3 monoclonal antibody (MoAb)[12)]. Two forms of mRNAs were identified for LFA-3, one encoding an ordinary intrinsic membrane protein that has transmembrane and successive cytoplasmic regions[13)], and the other encoding a protein having a stretch of hydrophobic amino acids at its C-terminus but with no cytoplasmic domain[5)]. The latter contains an extra 35-bp insertion at the last portion of the transmembrane domain, giving rise to reading frame shift and early termination. This type of protein is often seen in glycosyl phosphatidyl inositol (GPI)-linked membrane proteins. LFA-3 peptide was released from the cells expressing these GPI-linked membrane proteins following treatment with phosphatidyl inositol-dependent

phospholipase C[5]. The functional difference between the transmembrane type and GPI-link type on the cell surface *in vivo* remains unclear.

Recently another type of LFA-3 mRNA lacking almost the entire second extracellular Ig-like domain (ECD2) was found[14]. We also noticed a variant mRNA distinct from those previously reported during a study of LFA-3 gene expression. The mechanism of production of multiple diverse mRNA species from a single copy gene is not known. As a first step towards understanding LFA-3 structure and function, we isolated the LFA-3 gene from a human genomic library and studied its structure. In this report, we show that the LFA-3 gene can undertake various types of alternative splicing, and discuss differences from two other homologous genes, CD48 and CD2.

Materials and Methods

Preparation of total and poly(A)$^+$ RNA

Total RNA was prepared from cultured cells and human lymphocytes by guanidine isothiocyanate/acid phenol method[15] for reverse transcription-polymerase chian reaction (RT-PCR) and primer extension analysis. Poly(A)$^+$ RNA was prepared using oligo-dT cellulose.

RT-PCR

Reverse transcription of RNA samples was performed using SuperScript II reverse transcriptase (LIFE TECHNOLOGIES, Gaitersberg, MD) with oligo-$(dT)_{20}$ as a primer. CDNA was amplified using the Expand High Fidelity™ PCR system (Boehringer, Mannheim, Germany). PCR conditions were 35 cycles of 1 min at 95 °C, 1 min at 58 °C, and 1 min at 72 °C, followed once for 2 min at 72 °C.

The sequence of the antisense primer 608 (corresponds to nucleotides (nt) 719 to 700, according to Wallner et al's numbering[13]) and the sense primer 609 (nt 429 to 448) were 5′-ATA CCA TTC ATA TAC AGC AC-3′ and 5′-CCA ATG CAT GAT ACC AGA GC-3′, respectively. Primers 618 and 619 are described in the legend to Figure 6.

Isolation and characterization of genomic clones

Genomic DNA clones were isolated from an EMBL3 human placenta genomic library containing 15- to 20-kb inserts with EcoRI linkers (Clontech, Palo Alto, CA). Screening was performed as described previously[16] utilizing the LFA-3 cDNA[5][13], which contains almost the full coding region. Genomic clones positive after three rounds of plating and screening were grown up as described[17]. Phage DNA was characterized by restriction endonuclease mapping and Southern blot analysis using cDNA fragments. Further restriction mapping and DNA sequencing were performed following subcloning into pBluescript SK(−) or pUC118 plasmids. DNA sequencing was performed by the dideoxynucleotide chain termination method[18] using denatured double-strand templates and a commercial kit (Pharmacia, Uppsala, Sweden). If necessary, restriction fragments were further subcloned. Exon-intron boundaries were localized by comparing genomic and cDNA sequences using the GENETYX sequence analysis program (Software Development, Tokyo, Japan).

Primer extension analysis

A 40-nucleotide oligonucleotide complementary to the 5′-end region of the cDNA (5′-CCA CGC TGA GGA CCC CCA GGG CCC GCC CCG CGT CGC TCC C-3′) was synthesized (Sawady Technology, Tokyo, Japan). The oligonucleotide was purified by HPLC and 5′ end-labeled with [γ-^{32}P]ATP (NEN, Boston, MA) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA) as described[6]. The labeled oligonucleotide was separated from unincorporated ATP by a S200 spin column (Pharmacia) with a resulting specific activity of 4×10^7 cpm/pmol. Poly(A)$^+$ RNA (10 μg) was added to a 10-μl volume containing 4×10^6 cpm of end-labeled primer, 100 mM NaCl, 16.7 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, pH 7.5, and 0.03 mM EDTA, heated to 80 °C for 4 min, and then incubated at 50 °C for 3 h. The hybridized primer-RNA

complex was precipitated and resuspended in 50 μl containing 0.8 mM deoxynucleotide triphosphates, 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, and 10 mM dithiothreitol; 1,000 U of SuperScript II reverse transcriptase (LIFE TECHNOLOGIES) was added, and the mixture was incubated for 1 h at 42 °C. The reaction was terminated by the addition of 2 μl of 0.25 M EDTA, pH 8.0, followed by an additional 30-min incubation at 37 °C in the presence of 10 μg of RNase A. The digestion mixtures were phenol-chloroform extracted and ethanol precipitated in the presence of 50 μg of E. coli tRNA. The precipitated extension products were resuspended in the sample buffer and electrophoresed through a 6% polyacrylamide-7 M urea sequencing gel alongside a sequencing ladder generated with the primer used for the extension reaction and a subcloned genomic fragment containing exon 1 and the 5'-flanking region. The gel was fixed and exposed to X-ray film at −80 °C for 4 days with an intensifying screen.

Functional analysis of the promoter region

To construct plasmids containing the LFA-3 promoters connected to the CAT gene, we used the plasmid vector pUC0CAT[19]. The orientation of the insert was determined by DNA sequencing. Plasmid DNAs used for transfection were purified with CsCl.

COS-7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. A total of 3×10^5 cells per plate were transfected with LipofectAMINE reagent (LIFE TECHNOLOGIES) containing 2 μg plasmid DNA and were incubated for 40 h. Cell lysates were prepared by three cycles of freezing and thawing. The CAT activity of cell lysate was determined with the Quan-T-CAT assay system (Amersham, Tokyo, Japan). Enzymes in the extracts acetylate biotinylated chloramphenicol reagent with [^{3}H] acetyl coenzyme A. Acetylated or unacetylated biotinylated chloramphenicol binds to streptavidin-coated polystyrene beads which were centrifuged to form a pellet. The supernatant, containing unreacted acetyl coenzyme A, was removed and liquid scintillation cocktail was added. The scintillation signals generated by acetylated chloramphenicol gave a measure of CAT activity.

Cell culture

Cultured cell lines, HuH-7 (hepatoma, well differentiated), DLD-1 (colon adenocarcinoma), ECV304 (HUVEC, spontaneously transformed) and HeLa and COS-7 cells were obtained from Japanese Cancer Research Resources Bank and Riken Cell Bank and maintained in RPMI 1640 (lymphocyte, HuH-7, DLD-1), M119 (ECV304) and DMEM (HeLa, COS-7) with 10% fetal calf serum. Human peripheral blood lymphocytes were prepared by the Ficoll-Paque method from healthy volunteers.

Results

Polymorphic mRNA products from the LFA-3 gene

Two LFA-3 mRNA species have been identified, and another mRNA encoding a peptide lacking the second extracellular domain (ECD2) was also recently reported [5)13)14)]. RT-PCR using an LFA-3-specific primer pair identified another distinct mRNA species in ECV304 cells (Fig. 1, lane 1). For example, in PCR using primers, 608 and 609, a minor band appeared, in addition to a major band derived from the conventional LFA-3 mRNA (Fig. 1). Similar results were also observed in HeLa, HuH-7, and DLD-1 cells (Fig. 1, lanes 2, 3, and 4, respectively). These results indicated that the minor species is present in many diverse cell types.

DNA was recovered from these bands on the gel, cloned into pUC118 plasmid, and sequenced. The sequence of the major band was identical to a 291-bp LFA-3 cDNA clone, whereas the minor 159-bp band lacked 132-bp segment (nt 506-637 by Wallner et al's[13] numbering, data not shown). This minor product was not an artifact of the particular primer pair, since in other RT-PCR analyses using different primer pairs, a smaller minor band was also generated

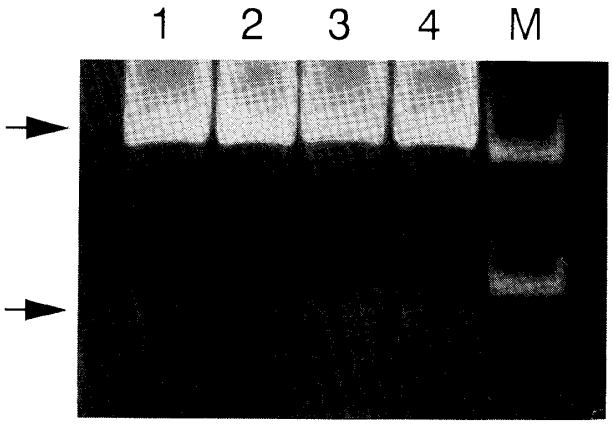

Fig. 1 Detection of a variant form of LFA-3 mRNA
RT-PCR was performed on RNA samples from HUVEC (ECV304), HeLa, HuH-7, and DLD-1 cells using the primer pair 608 and 609 (lanes 1, 2, 3, and 4, respectively). After 35 cycles of exponential amplification, samples were separated on a 6% polyacrylamide gel. One major and one minor product were detected (arrows). M indicates a 100 bp-ladder molecular weight marker (Pharmacia). Apparent mobility retardation of sample DNA bands in the marker ladder might be due to salts and/or excess DNA amounts in the sample.

(data not shown). The minor band was confirmed to be derived from the same minor mRNA species by cloning-sequencing (data not shown).

Isolation of LFA-3 genomic clones

We isolated genomic clones to elucidate the transcriptional mechanism of LFA-3 gene. Approximately 10^6 plaques of an EMBL3 human placenta genomic library were screened with the near full length LFA-3 cDNA obtained by the RT-PCR-cloning method. Three representative clones were arranged according to 5′-3′ order (Fig. 2A). Exons were mapped on these clones and depicted in the figure as solid boxes. Since these genomic clones did not overlap, the exact distances between exons 1 and 2, and exons 3 and 4 are not known. The LFA-3 gene was thus composed of 6 exons in total, and is distinct from the two other Ig-superfamily members CD2[20)21)] and CD48[6)] in number of exons.

Junctional sequences between exons and introns of the LFA-3 gene are shown in Figure 2B. Interestingly, the major transmembrane-type and GPI-linked type mRNA species are generated by alternative usage of exon 5 or exon 5′. In these cases, the 5′-half of exon 5′ is deleted in transmembrane type mRNA, or an extra 35 bp is added to form the GPI-linked type mRNA (Fig. 2B). Therefore alternative splicing acceptor selection gives rise to the two major mRNA forms. The sequences of region preceding these alternative exon fragments are very similar to the splicing acceptor consensus sequence, ...yyyyynyag.

The ECD2 deletion variant reported previously[14)] was found to be the exon-skip variant of the entire exon 3 in this experiment (Fig. 2B, gray area). The variant mRNA species described in this study had a 132-bp deletion in the 3′-half of exon 3 and is shown in Figure 2B as exon 3′. Just 3′ to exon 3′, there is a splicing donor consensus sequence, gtaaa...

Pattern of LFA-3 gene transcription

The LFA-3 gene generates multiple transcripts by alternative splicing, as shown above (Figs. 1 and 2). Splicing alternation occurs exclusively in exons 3 and 5, among the 6 exons of the LFA-3 gene. To clarify whether such events occur independently or in a coodinated manner in these exons, we devised two pairs of PCR primers as schematically presented in Figure 3A. The primer 618 amplifies mRNA lacking the entire exon 3, while the primer 619 amplifies the partially-deleted form. These primers are therefore predicted to give two distinct bands, if the splicing alteration occurs in exon 5 in the LFA-3 mRNA minor variants. Results of RT-PCR using these primers combined with the common antisense primer 32 are shown in Figure 3B, in which only a single 141-bp product was visible in ECV304 total RNA. DNA from these bands was recovered, cloned and sequenced, and represented only the transmembrane LFA-3 mRNA species (data not shown). Figure 4 schematically depicts the overall profiles of alternative splicing in the LFA-3 gene. The LFA-3 gene is thus able to generate

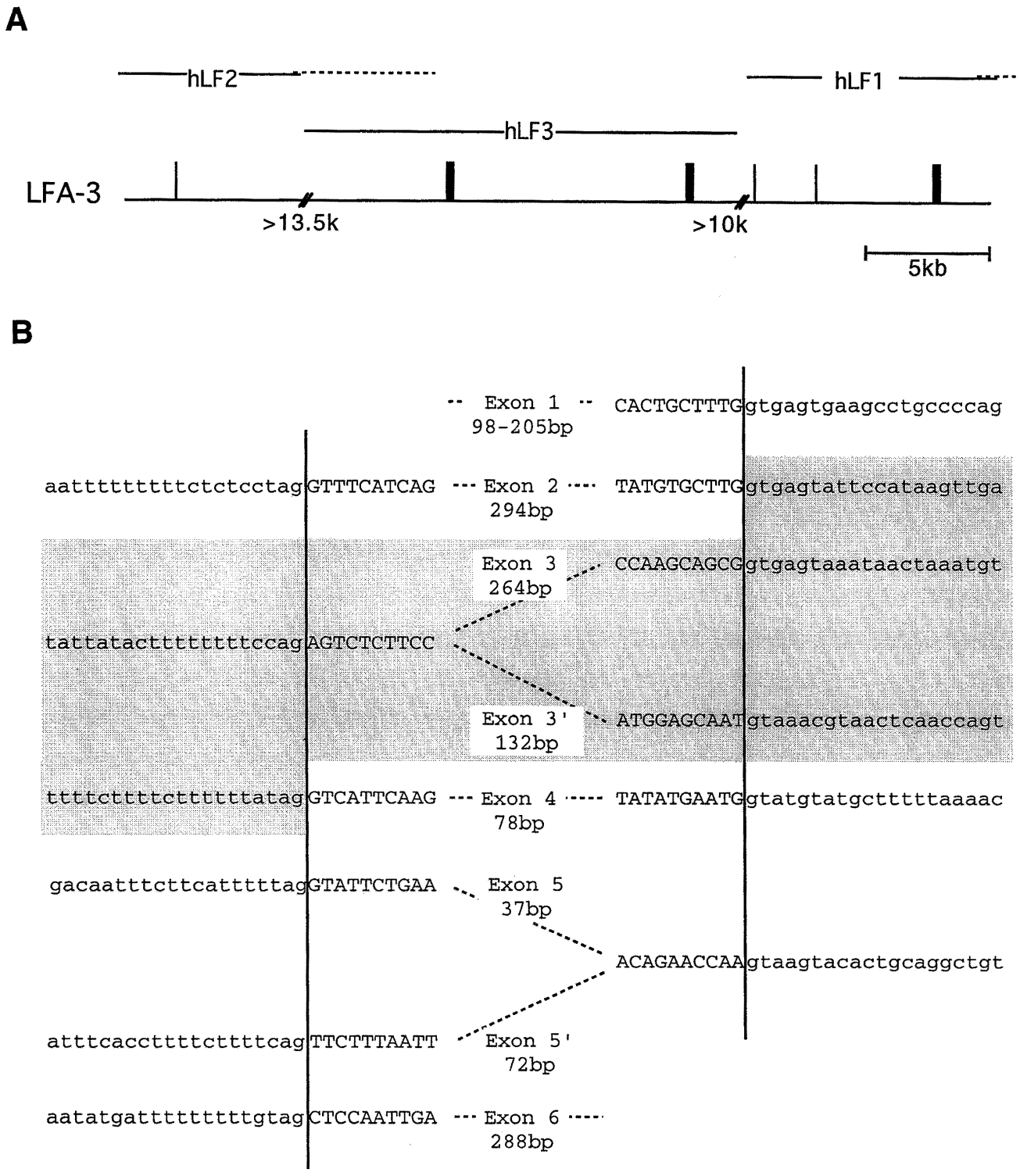

Fig. 2 Genomic structure of the LFA-3 gene
(A) Genomic clones hLF1, hLF2, and hLF3, which contain the complete coding region of the LFA-3 gene are arranged according to 5′ to 3′ orientation at the top. The undefined 3′ ends of phage clones hLF2 and hLF3 are indicated by broken lines. The location of exons are denoted by boxes. (B) Sequences of the exon-intron boundary of LFA-3. Uppercase and lowercase letters indicate exon and intron sequences, respectively. Size of the first exon is based on the results obtained from primer extension analysis (Fig. 7). Gray area indicates exon 3 and adjacent introns that is skipped in ECD2 variants.

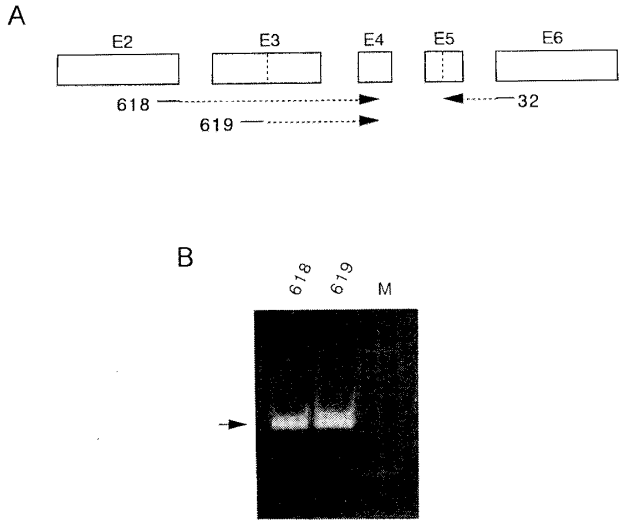

Fig. 3 Survey of alternative splicing in exon 5 in minor variant mRNAs
(A) Primer 618 represents ECD2 mRNA that skips the entire exon 3 sequence, and is composed of the 3′-most 10 bases of exon 2 and 5′-most 10 bases of exon 4 (underlined), 5′-TAT GTG CTT GGT CAT TCA AG-3′. Similarly, primer 619 represents mRNA found in this study, and is composed of the 3′-most 10 bases of alternative splicing donor site in exon 3 and 5′-most 10 bases of exon 4 (underlined), 5′-ATG GAG CAA TGT CAT TCA AG-3′. These composite primers should stably anneal only to minor variant molecules, but not to major conventional ones. RT-PCR using these and a common primer 32, 5′-TAC CAA TCA ATT GGA GTT GG-3′, is expected to generate 141-bp and 176-bp (141 bp + 35 bp) bands if there is alternative acceptor selection in exon 5. (B) The PCR products using a primer pair 618 and 32 (lane 618), and a primer pair 619 and 32 (lane 619) in ECV304 cells were separated on a 6% polyacrylamide gel. M indicates a 100 bp-ladder molecular weight marker.

four mRNA species, each one of which encodes a distinct form of LFA-3 peptide.

Structural and functional analysis of the LFA-3 promoter region

To define the 5′ end of the LFA-3 gene, the transcriptional start site was determined by primer extension analysis using a 40-mer oligonucleotide complementary to positions 10 to 49 of the LFA-3 cDNA. Several extension products were detected with 10 μg of poly(A)$^+$ RNA from HeLa cells (Fig. 5, center lane). These extension products were not present when 50 μg of E. coli tRNA was used instead (Fig. 5, left lane). Comparing with the sequence ladder obtained with the same primer as a sequence primer, we mapped two major putative start sites on the genomic sequence upstream of the reported cDNA region (Fig. 6, asterisks). Among the region, several minor bands were also observed in this region. Appropriately located TATA and CAAT box sequences were not detected in this region. The sequence of an approximately 1.3-kb genomic 5′-flanking DNA region is shown in Figure 6. Several putative transcription factor binding sites were also identified.

To functionally analyze this putative promoter region, we generated a series of fragments, spanning nt -1147 to -18, nt -860 to -18, nt -611 to -18, nt -444 to -18, and nt -177 to -18 by PCR and cloned them into the upstream site of the CAT expression vector in both directions. Following transfection of these constructs into COS-7 cells, CAT activity was determined. The promoter fragments were only weakly active (Fig. 7). Only the shortest fragment (-177) revealed CAT activity higher than that of the pUC0CAT vector alone. The activity tended to decrease as the length of inserts became longer. All the constructs with inversely orientated inserts showed much lower activity. These results suggest that the promoter activity of LFA-3 is low. Transcriptional activation elements may reside only in the start site-proximal 180-bp region, and further upstream regions may mediate to suppression of activity. Accordingly, within the nt -125 to -86 region, four SP1 and AP2 elements are present (Fig. 6).

Discussion

The structural similarity between peptides and mRNAs of LFA-3, CD2 and CD48 suggest a common ancestral gene. Analysis of the genomic structures of CD2 and CD48 has also revealed that these two genes have similarly sized exons encoding the Ig-like domains and the introns that flank these exons[6)20)21)]. In the present study we determined the genomic

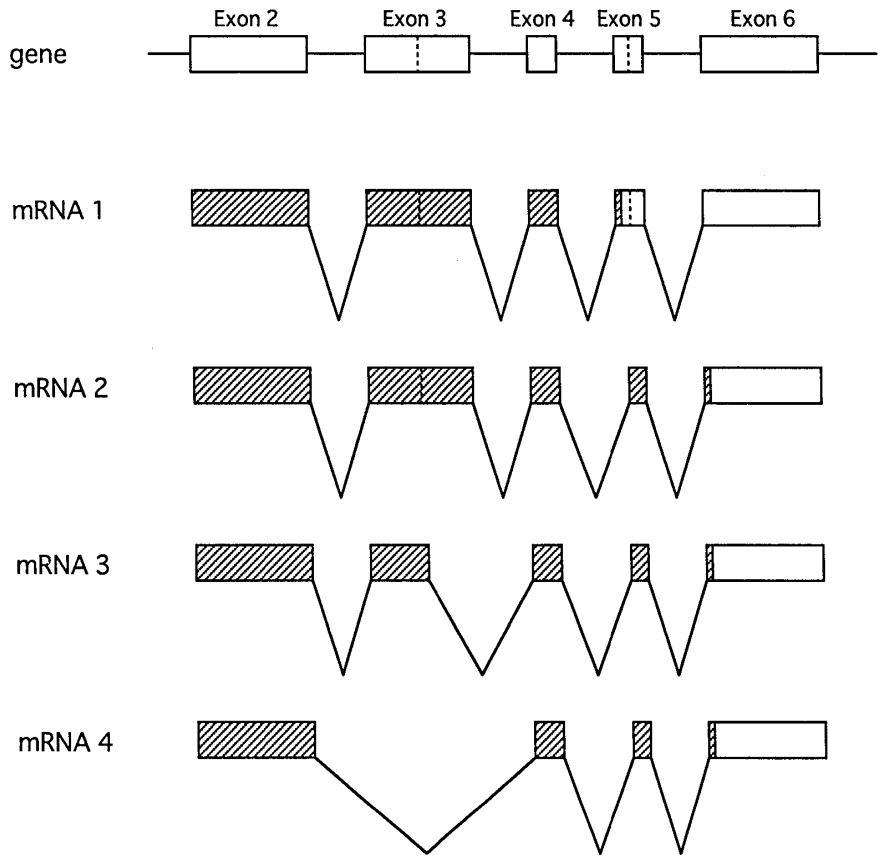

Fig. 4 Schematic representation of a part of genomic structure and multiple mRNA species of LFA-3
The open reading frame is indicated by the hatched area. The mRNA species 1, 2, and 4 corresponded to the GPI-link, transmembrane, and ECD2 forms, respectively. mRNA3 was found in this study.

arrangement of LFA-3. The most striking difference between these three genes was the number of exons they encoded, CD48, CD2, and LFA-3 encoded 4, 5, and 6 exons, respectively. Shown schematically in Figure 8, the sizes of the first three exons are similar and reminiscent of the Ig superfamily members, while the following exons are distinct in number and size, between each one of three genes. The introns in the LFA-3 gene are generally longer than the respective ones of the other two genes. The gene is therefore at least 41.1 kb in size, which is much bigger than the other two. In evolutionary terms, the sequential addition of exon sequences together with flanking introns between the D2 and the last exons may have given rise to the CD2 and then to the LFA-3 gene from the ancestral CD48 gene.

The D2 exon of the LFA-3 gene was shown to be sometimes partially or entirely omitted by exon skip or by alternative splice donor selection in both the present and a previous study[14]. The interaction of LFA-3 and CD2 involves binding of the D1 domain of both molecules[22)23)]. The LFA-3 gene may begin to evolve to eliminate D2 domain that is apparently unnecessary for the adhesion.

A 1.3-kb 5′-flanking genomic DNA fragment cloned upstream of the CAT gene was able to drive CAT expression only in the sense orientation (Fig. 7). However, the activity was very weak. There are several putative transcriptional cis-elements in this region including SP1, AP2 and AP3, but no TATA or CAAT promoter sequences. Primer extension analysis revealed several products, indicating the presence of multiple transcriptional start sites, as seen with CD2 and CD48[6)20)21)]. The expression

of a number of cellular adhesion molecules is known to be upregulated by various cytokines including interleukin (IL)-1, IL-4, tumor necrosis factor-α, and γ-interferon on both endothelial cells and leukocytes[24)~27)]. However, these cytokines had no effect on LFA-3 expression in a number of cultured cell lines as well as in freshly prepared PBL, which remaind weak but stable (unpublished results). Although the CD2/LFA-3 interaction may prime responses on both sides of cells[11)28)29)], the presence of the GPI-linked form of LFA-3 does not reconcile with the idea. Even the transmembrane type of LFA-3 has a very short cytoplasmic stretch and no cytoplasmic molecule that associates with LFA-3 has been identified to date. In addition, as mentioned before, CD2 molecules are expressed on a very limited number of cell types, while LFA-3 appears on nearly all types of cells. These observations indicate that CD2 molecules might play a regulatory role in the CD2/LFA-3 adhesion process. For example, an immunomodulatory protein, Ling Zhi-8, increased the expression of CD2, but not LFA-3 in Molt4 cells, and enhanced rosette formation between T cells and sheep red blood cells[30)]. The CD2 molecules could also act as a target for MoAb-induced mitogenesis on T lymphocytes[31)32)].

One of the features of the LFA-3 gene that is distinct from CD2 and CD48 is its diverse transcripts. Sequence analyses around the exon-intron junctions revealed that the two major forms of LFA-3 mRNA[5)13)] are generated by alternative splicing acceptor selection in exon 5. A 35-bp inserted sequence[5)] is not due to the recruitment of new exon. In addition, two

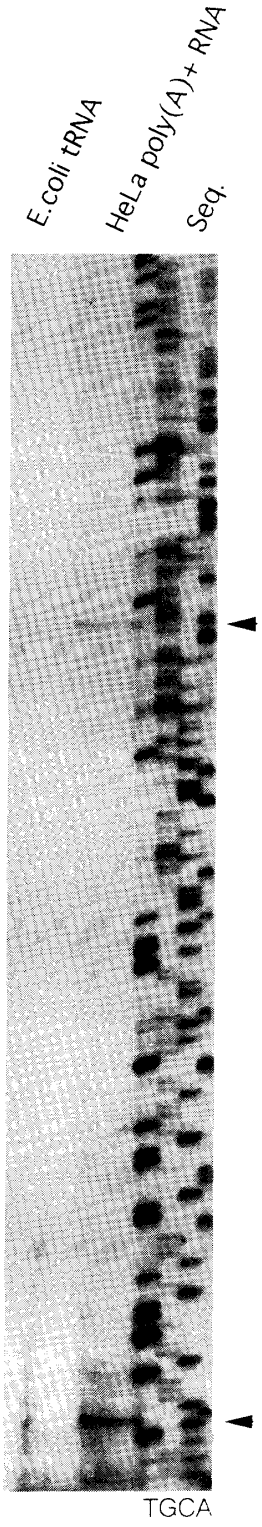

Fig. 5 Mapping of transcriptional initiation sites by primer extension analysis
Primer extension analysis was performed with 10 μg of poly(A)$^+$ RNA from HeLa cells as described in Materials and Methods (center lane). As a control, the extension reaction was done with 50 μg of E. coli tRNA (left lane). All extension reaction mixtures were run through a denaturing polyacrylamide-urea sequencing gel along with a sequencing ladder obtained with the same primer and the promoter region-containing plasmid clone (denoted as TGCA). Major transcriptional initiation sites are indicated by arrowheads.

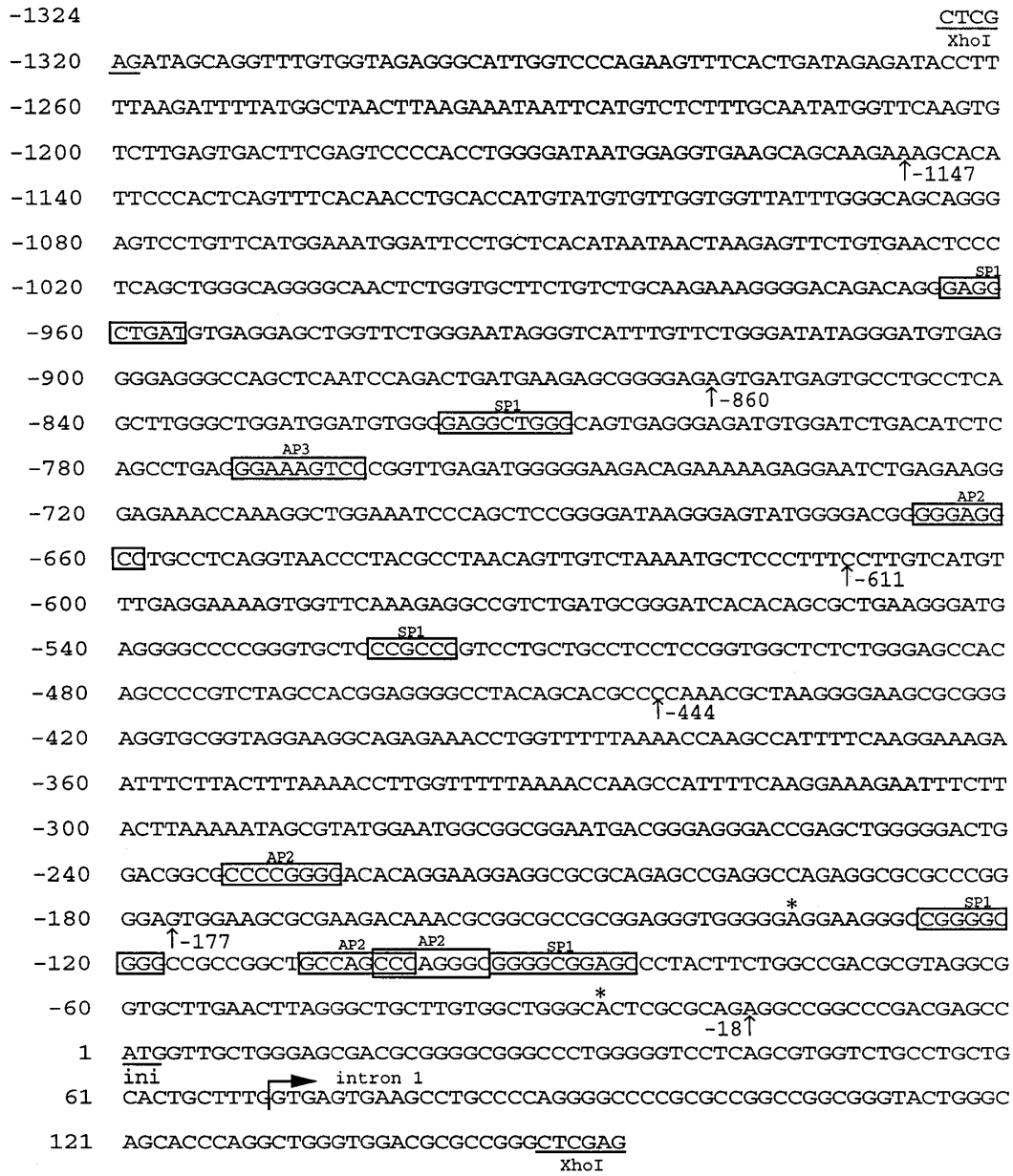

Fig. 6 Nucleotide sequence of the 1478-bp XhoI DNA fragment containing the first exon and 5'-flanking region of the LFA-3 gene
Major transcription initiation sites determined by primer extension analysis are indicated by asterisks. The numbering of the sequence is based on ATG at +1. Arrows with numbers, -1147, -860, -611, -444, -177, and -18 indicate the termini of the fragments used for the CAT constructs (Fig. 7). Putative regulatory control elements are boxed and appropriately labeled.

minor mRNA species are also produced by the entire or partial elimination of exon 3[14] (Figs. 1, 2, 3, and 4). The LFA-3 gene thus generates a diverse set of mRNA species by a variety of alternative splicing mechanisms including exon-skip and selection of donor and acceptor sites. Unexpectedly, however, the minor variants occurred only in the transmembrane type but not in the GPI-linked type mRNA (Figs. 3 and 4). This may be related to the loss of a putative GPI-linkage site that located at the 3'-most region of exon 3[33] in variant mRNAs, although it is uncertain whether the protein structure can affect the mRNA structure, from

which the protein is derived. These mRNAs encode distinct protein products, all of which are membrane proteins. Alternative splicing has not been reported in the CD2 and CD48 genes, while the mechanism generating both GPI-linked and transmembrane forms has been demonstrated in CD2-analogous NCAM gene[34].

Although the proteins encoded by two major LFA-3 mRNA species are both functional, little is known about those encoded by the minor mRNAs. Since the minor mRNA species are present at much lower levels, its physiological significance in an actual cellular adhesion processes is questionable, even if variant peptides would actually be produced.

Why then has such a mechanism to produce variant mRNAs developed in this gene? Several explanations can be raised, such as; 1) The alternative splicing sites occurred by chance. This possibility seems, however, less likely, since splicing alternations are restricted in

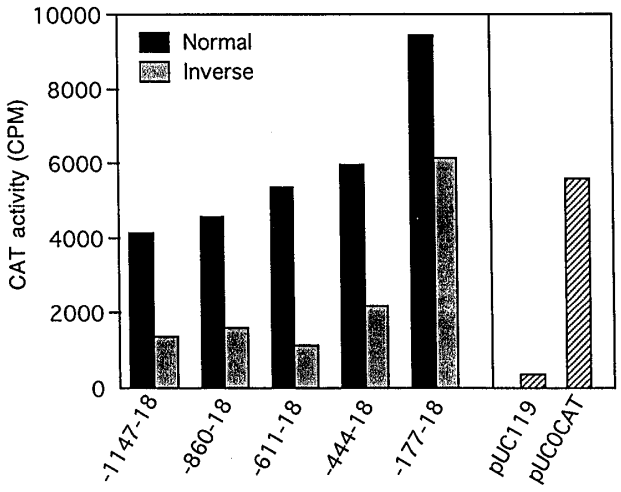

Fig. 7 Identification of the promoter region in the 5'-flanking region of the LFA-3 gene
PCR fragments (nt -1147, -860, -611, -444, and -177 to -18, Fig. 6) from the 5' end of the LFA-3 gene were ligated to the SmaI site of the pUC0-CAT plasmid in both orientations with respect to the bacterial CAT gene. COS-7 cells were transfected with 2-μg of LFA-3 CAT constructs, pUC0 CAT, or vector pUC119 plasmids. The β-gal gene was cotransfected as a reference for transfection efficiency. The normal and inverse orientation of the constructs are indicated by black and gray bars, respectively.

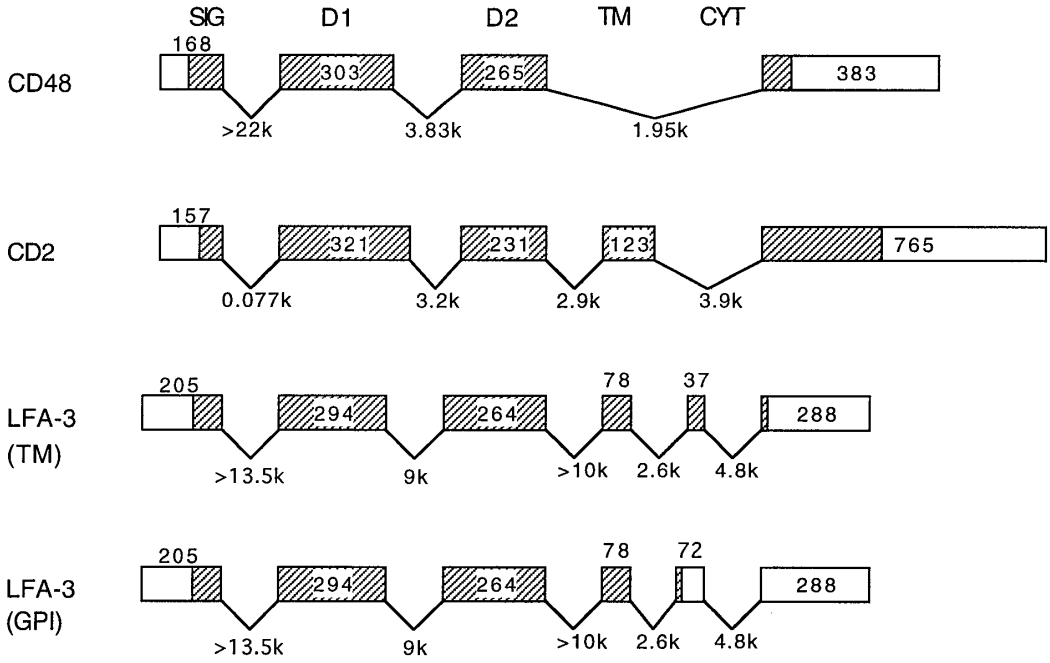

Fig. 8 Schematic presentation and comparison of CD48, CD2, and LFA-3 genes
The size of exons and introns are shown. The signal sequence, D1, D2, transmembrane, and cytoplasmic domains are aligned and represented by SIG, D1, D2, TM, and CYT, respectively. The coding region is indicated by hatched area.

exons 3 and 5, and generate no frame shift. 2) The LFA-3 gene is rapidly evolving. As mentioned above, this gene acquired extra exons that are absent in two other highly-homologous genes (Fig. 8), and can generate two functional types of membrane proteins[5)13)]. By eliminating partially or entirely the D2 domain, closer cell-to-cell contact may be expected in adhesion processes. 3) The production of variant molecules is a fail-safe device. The LFA-3 molecules are expressed in virtually all types of cells, suggesting an essential function. If mutations causing frame-shift, premature termination or fatal missense can occur in exon 3, mutant peptides might lose their functions. Skipping of exon 3 or alternative donor selection may circumvent these mutations and thereby rescue the molecule. Further studies of variant LFA-3 function, by analysis of cellular adhesion after overexpression of variant peptides are required to clarify the functions of these alternate transcripts.

Acknowledgments

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ヒト LFA-3 (CD58)におけるゲノム構造と選択的スプライシングによるバリアント mRNA の産生

[1]防衛医科大学校　生化学第二講座（指導：山本三毅夫教授）
[2]東京女子医科大学　医学部　生化学（主任：高桑　雄一教授）
若月（ワカツキ）　徹（トオル）[1)2)]・山本三毅夫（ヤマモトミキオ）[1)]・高桑　雄一（タカクワユウイチ）[2)]

細胞表面 glycoprotein である LFA-3には，glycosyl phosphatidyl inositol (GPI) 結合型および膜貫通型など，いくつかの固有の mRNA 分子種の存在が証明されている．この LFA-3の多様な転写特性と mRNA 産生のメカニズムを明らかにするために，我々はゲノムクローニングに引き続き構造および機能の解析を行なった．LFA-3 gene は少なくとも41.1 kb の大きさを持ち，6 つのエキソンにより構成されていた．2 つの主な mRNA 種は第 5 エキソンにおける選択的スプライシング acceptor selection により産生されており，2 つのマイナーなものは，第 3 エキソンの選択的スプライシング donor selection およびエキソンスキップによる部分的および完全な欠失により産生されていた．プライマー伸長法による解析では，一連の転写開始部位が観察され，それは TATA および CAT box の欠如によるものであると考えられた．1.3 kb の5′上流の隣接配列は配向依存性に CAT reporter 遺伝子の発現を促進できたが，その転写活性は低かった．LFA-3と他の 2 つの関連分子 CD2および CD48 の遺伝子進化がそれらのゲノム構造から推測された．