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Three Soluble Form Messages of Murine CD46 Are Produced through Alternative mRNA Splicing[1]

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Murine CD46 (mCD46) is a type 1 membrane protein expressed predominantly in testicular germ cells, the distribution profile of which is in contrast to that of human CD46 showing a ubiquitous tissue distribution. We have identified an additional message of mCD46 that encodes a putative secretory form [Nomura *et al.* (1999) *Immunogenetics* 50, 245–254]. Here, we cloned three cDNAs encoding putative soluble CD46 from murine testis. These soluble form messages were yielded on insertion of unidentified nucleotide sequences, 77, 179, and 73 ntds, into the junctions between the SCR3 and SCR4 (variant 2), ST^c and UK (variant 3), and SCR4 and ST^c (variant 1) domains, respectively, the last one corresponding to the reported soluble form. The exons corresponding to these three inserts were identified in the murine CD46 genome, indicating that the alternative splicing of mRNA participates in the generation of these various CD46 messages. In normal mouse sera and cell lines, however, virtually no soluble CD46 was detected on immunoblotting. On Northern blotting analysis with specific probes, on the other hand, variant 1 was found to be predominantly expressed in the liver and heart. In addition, all variant messages were detected on PCR in all organs examined. When a rabbit cell line, RK13 cells, was transfected with cDNA of variant 1, protein synthesis was detected on immunoblotting. Although the mCD46 protein production was inefficient, this variant 1 exhibited factor I-cofactor activity as to inhibition of the complement cascade. Since the mCD46 protein was reported to be markedly up-regulated on infection of murine cells with mCMV, the soluble mCD46 proteins may act as a complement regulator that controls the systemic complement system under the conditions of a viral infection.

Key words: complement regulatory protein, mouse membrane cofactor protein (CD46), short consensus repeats (SCR), soluble isoforms, transcriptome.

Human CD46 is a complement regulatory protein that serves as a cofactor for factor I–mediated inactivation of C3b and C4b (*1*), and thus plays a major role in the protection of host cells from complement (*2*). Later, it was found to be a measles virus receptor (*3*), a human herpesvirus 6 (HHV6) receptor (*4*), and a bacterial receptor for Neisseria (*5*) and Streptococci (*6*). Stimulation of immune competent cells with these ligands may cause transmission of intracellular signaling resulting in immune responses. Recently, murine cDNA homologous to human CD46 was cloned (*7*), and the CD46 gene was mapped on the mouse RCA region of chromosome 1 (*8*). No other CD46-like genes were identified in the mouse genome on Southern and FISH analyses (*8*). Its message was predominantly expressed in the testis (*7*), although low levels of the mRNA were found in other organs. The mechanisms of the gene regulation of CD46 must be completely different between mice and humans (*9*).

It has been reported that in mice the CRRY protein serves as a potent complement regulator that functionally encompasses DAF (CD55) and CD46 (*10*). In addition, mice harbor factor H and the C4b-binding protein, both of which are functionally homologous to those in humans (*11–13*). Two mouse homologues of CD55 are also present on cells,

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Abbreviations: Ab, antibody; CHO, Chinese hamster ovary; CYT, cytoplasmic tail; DACM, a fluorescent SH reagent, dimethylamino-(4-methylcoumarinyl)maleimide; DAF, decay-accelerating factor (CD55); mCD46, mouse CD46; MCP, membrane cofactor protein (CD46); RK13, a rabbit kidney cell line for efficient transfection; RT-PCR, reverse transcription–polymerase chain reaction; SCR, short consensus repeats; ST, serine/threonine-rich domain; TM, transmembrane domain.

though their distribution is more restricted (*14*). Functional homologues (not always structural homologues) of CR1 and CR2, both of which are SCR proteins, are present in mice (*15, 16*). Mouse CD46 was not identified until recently. The main reason for this is that it is restricted to the testis (*7*). However, the properties of minute amounts of CD46 mRNA expressed in cell lines and organs have not been defined (*8*).

The mouse CD46 gene consists of 11 exons encoding 4 short consensus repeats (SCR), a serine/threonine-rich domain (ST), 13 amino acids of unknown significance as to homology (UK), a transmembrane domain (TM), and a short cytoplasmic tail (CYT), yielding a 41 kDa protein (*7, 8*). Here, we found three cDNAs encoding parts of the ectodomain of murine CD46. Since the soluble form message and protein levels were up-regulated by mCMV infection (*17*), these soluble isoforms of murine CD46 are likely to be up-regulated for host cell protection from complement during viral infection.

MATERIALS AND METHODS

Mice and Cells—Six-eight-week-old C57BL/6 and Balb/c mice were purchased from SLC, Japan. Organs including the testes and livers were surgically excised as described (*9*), and frozen at –140°C and then kept at –80°C. Rabbit kidney cell line RK13 was from the American Type Culture Collection (ATCC). RK13 cells were cultured in RPMI1640/10% FCS at 37°C in a CO_2 incubator. Other mouse cell lines, G-5 and F9, were cultured as described previously (*8*). In some experiments for the CD46 cofactor assay, GIT medium (Wako Pure Chemicals, Tokyo) was used without the addition of FCS.

cDNA Cloning—Murine CD46 cDNAs (*18*) were cloned from a cDNA library obtained from the testes of C57BL/6 mice by PCR using primers covering each junction of the domains of CD46. Oligonucleotide reverse and forward primers for PCR were synthesized according to the murine CD46 cDNA sequence (Table I) (*7, 8*). The thermocycle conditions were 35 cycles of 94°C for 30 s, 50°C for 60 s, and 72°C for 90 s for denaturing, annealing and extension, respectively. The products were run on an agarose gel containing ethydium bromide and then the major amplified DNA populations were subcloned into the TA cloning vector. Three independent cDNA clones were sequenced on both strands and were found to be identical. The new sequences were obtained between SCR3 and SCR4, SCR4 and ST, and ST and UK (accession number AB001566). Using appropriate regions of these sequences as primers (Table I), we obtained the whole sequences of these CD46 variants. The complete coding regions were predicted by computer analysis.

Genomic Analysis—λEMBL3 and λFIXII/mouse genomic DNA libraries constructed with a partial *Sau*3AI digest of the 129/SVJ mouse liver DNA (1×10^9 pfu/ml) were obtained as described previously (*8*). The probe murine CD46 cDNA (*7*) was labeled with [α-^{32}P]dCTP by random priming (Amersham). Genomic DNAs were extracted from purified phage clones and used as templates for PCR analysis for the mapping of exons. The fragments containing exons were identified by Southern blot hybridization (*19*), and subcloned into pUC19 for sequencing. DNA inserts subcloned in pUC19 vector were sequenced on both strands by the cycle sequencing method using a 373A automated DNA sequencer (Perkin Elmer, Applied Biosystems Division).

Message Analysis—Total (20 μg) RNAs were isolated from various organs of BALB/c inbred mice using an RNAzol™ (TEL-TEST). Probes were prepared by PCR as described previously (*8*). For Northern blotting, RNAs were denatured with glyoxal and DMSO at 50°C for 1 h. The RNA was electrophoresed on 1% agarose gels and transferred to Hybond N$^+$ membranes (Amersham). Tissue blotting was performed with MTN™ (Clontech). The blots were prehybridized for 30 min, hybridized for 1 h at 68°C in Clontech hybridization buffer (ExpressHyb™, Clontech) with a ^{32}P-endlabelled PCR product containing the junctional sequences unique for variant 1, 2, and 3 (Table I). The sizes of these probe were >300 bp, which recognized the variants by Southern analysis (see Fig. 3). We identified these sequences as "variant-specific probes," which were identified in Table I. The blot was washed at high stringency (50°C, 0.1 × SSC, 0.1% SDS). The blots were rehybridized with a β-actin probe to ensure equivalent loading of RNA.

For RT-PCR, RNA (0.2 μg) isolated from organs of BALB/c mice or cell lines using an ISOGEN RNA isolation kit (Nippon GENE) was reverse transcribed using random primers with RNAase H–free reverse transcriptase (Superscript, GIBCO BRL). A segment spanning the SCR3 domain and 3′-UT of murine CD46 cDNA was amplified by PCR using the primer sets indicated in Table I. In some experiments, RNA template samples were treated with DNAse to remove genomic DNA (*20*). The thermocycle program was 23–35 cycles of 94°C for 30 s, 55°C for 60 s, and 72°C for 120 s. The products were analyzed by 1.5% agarose gel electrophoresis.

Preparation of Antibodies against Murine CD46—Two methods for production of polyclonal antibodies (pAbs) against murine CD46 were described in previous reports (*7, 21*). First, the anti-peptide antibody was produced as described previously (*7*). Second, rabbits were immunized with RK13 cells expressing murine membrane CD46 (*21*). Anti-sera were collected from the rabbits and the pAb fractions were precipitated with 50% $(NH_4)_2SO_4$. The pAb fractions were dialyzed against PBS, pH 7.5.

Collection of Murine CD46 Protein—The CMV-responsive element in the promoter of mCD46 was ligated in the upstream of mammalian expression vector pME18s and the membrane form mCD46 cDNA was placed in a multicloning site of this vector (named pCMV). Since the mCD46 protein was barely detected on RK13 cells by transfection of pCMV using Lipofectamine, the Kozak sequence was inserted in front of the translation start codon Met. A minute level of mCD46 was detected on the transfectant by flow cytometry (data not shown). The variant 1 mCD46 cDNA was replaced for the membrane form mCD46 cDNA in a forward (mCD46v kozak/pCMV) or reverse orientation (mCD46v kozak/pCMVreverse). The same variant 1 mCD46 cDNA with the Kozak sequence was inserted in pEFBOS (mCD46v kozak/pEFBOS) or pME18s (mCD46v kozak/pME18s). The RK13 cell line (*21*) (~10^8 cells) were transfected with variant 1 mCD46 cDNA placed in pME18s, pEFBOS, or pCMV, and then cultured for 3 days in serum-free medium GIT (Wako Pure Chemicals), supernatants of the cells were harvested. mCD46 in the medium was checked by immunoblotting. Likewise, the NP-40

extract of the cells (*7*) was also analyzed by immunoblotting.

SDS-PAGE and Immunoblotting—The culture supernatants or NP-40 extract of cells were collected and aliquots were analyzed by SDS-PAGE by the method of Laemmli (*20*). Proteins were electrophoretically transferred to nitrocellulose membranes. The membranes were incubated with 10% of a blocking reagent (Morinaga, Tokyo) for 2 h, followed by the addition of 20 μl of anti-mCD46 antibody. One hour later, the membranes were extensively washed with PBS containing 0.02% NP-40, pH 7.4, and then incubated for 1 h with 2 μl of HRP-labeled goat anti–rabbit IgG antibody (Bio-Rad, Richmond, USA). Again after extensive washing, proteins were detected with an ECL kit (Amersham Int. Plc. Bucks, UK) (*21*).

Factor I-Cofactor Activity—Murine factor I (*11*), factor H (H.1 as referred to the Ref. *11*) and DACM (fluorescence)-labeled murine C3b (moC3b) (*7*) were prepared as described previously. The DACM-labeled substrates (5 μg) were incubated in PBS/0.02% NP-40, pH 7.5, for 2 h at 37°C with murine factor I (0.2 μg) and 0.2 ml of the culture supernatant. Samples were reduced by the addition of 5 μl of 2-mercaptoethanol. The C3b-iC3b conversion was visualized by SDS-PAGE and illumination by UV lamp (360 nm) (*7*). %Cleavage of moC3b to iC3b was calculated on F2000 Hitachi spectrofluorometer as described previously (*7*).

RESULTS

Identification of Three Additional cDNAs of Mouse CD46—PCR analyses were performed with various combinations of primer sets (Table I) and template cDNAs (Fig. 1). A two band profile was reproducibly observed for many organs when primers SCR3-5′ and 3′UT-3′ were employed (Fig. 1). The lower band corresponded to membrane mCD46 based on the size and sequencing. The upper band/lower band ratio was relatively high for the heart and skeletal muscle. In the upper band, three variant mCD46 cDNAs, out of 100 mCD46 clones, were identified with the PCR method. The nucleotide and deduced amino acid sequences are shown in Fig. 2. The insertion or retention of nucleotides at the junctions of exons 5 and 6 (variant 2), exons 6 and 7 (variant 1), and exons 7 and 8 (variant 3) resulted in a lack of the membrane anchoring facility to encode putative soluble proteins. At least variant 1 and 3 cDNA clones were not artifacts, since the primers designed in the inserted sequence (Fig. 3a) worked for PCR amplification of relevant SCR-containing regions. Variant 2 cDNA was not reproducibly identified even on repetitive trials (data not shown). Variant 1 (Fig. 3b), as well as variant 3 (not shown), was detectable with other combinations of primers. Furthermore, the message for variant 1 was detected on Northern blotting using the probe containing the inserted regions (see Fig. 5b). Variant 2 had no exon 1, which predicted a protein without the signal peptide portion, and this together with a irreproducible faint band on Southern analysis (Fig. 3a) indicated variant 2 may not be feasible. Finally, novel exons corresponding to the inserts were identified in the CD46 genome (Figs. 2a and 4). These exons were confirmed to obey the AG-GT rule. Thus, the three additional messages of murine CD46 can be produced in addition to the authentic message of membrane CD46 (*7*), and at least 2 cDNAs reflect virtual messages of alternative CD46 isoforms.

Distribution of Mouse CD46 Variants—The tissue distributions of variants 1, 2, and 3 were examined by RT-PCR with specific primer sets (Fig. 5a), and by Northern blotting analysis with specific probes (Fig. 5b). Although RT-PCR allowed the detection of all variants (Fig. 5a), a tissue distribution profile was successfully obtained on Northern analysis only with variant 1 (Fig. 5b). The conventional murine CD46 mRNA is 1.5 kb in length and is expressed predominantly in the testis (Fig. 5b), consistent with previ-

TABLE I. **Primer sequences for PCR.**

Primer/probe	Sequence/product
Primer	
SP-5′	5′-ATGACGGCGGCGCCTCTTAT-3′
SCR1	5′-CGGCCATTTGAAGCTATGGAAC-3′
SCR2-5′	5′-AGACCCTTCATTTGGCAA-3′
SCR2-3′	5′-CTTGTATGGAAGGTTATTACGTA-3′
SCR3-5′	5′-TGTGCTGGCCATAACACCTG-3′
3′UT-3′	5′-GCGTTTGCCAGACTTGAAGAAGA-3′
V1-5′	5′-GTCACTTTTTATTGACCACAAG-3′
V1-3′	5′-GTAAACCACTGGTACAGGCT-3′
V2-5′	5′-GGACTTCCCATGCAACCTTG-3′
V2-3′	5′-GACCATTTTCATTCCAGCACT-3′
V3-5′	5′-GCTGGCAGAGCCTCTCAGG-3′
V3-3′	5′-AGGAGGAAATATGGAGATAAAG-3′
Probe	
Variant 1–specific	PCR product using SCR3-5′ and V1-3′
Variant 2–specific	PCR product using SCR2-5′ and V2-3′
Variant 3–specific	PCR product using SCR3-5′ and V3-3′

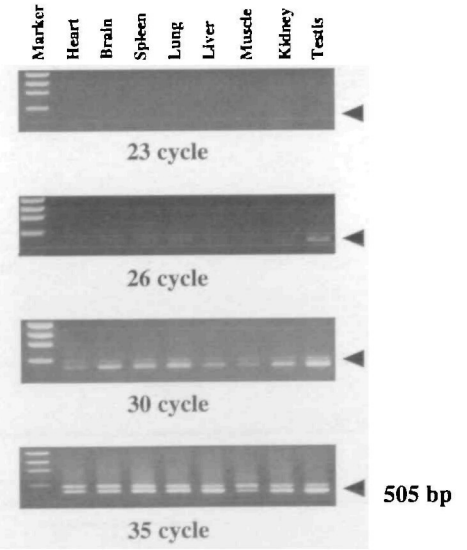

Fig. 1. **The membrane and secretory forms of mCD46.** The primer sets and conditions for PCR were described in the text and Table I. mRNAs from the indicated mouse organs were prepared as in "MATERIALS AND METHODS" and reverse-transcribed into cDNA, and then used as templates. Primer sets SCR3-5′ and 3′UT-3′ were used for PCR amplification. The amplicons were resolved on agarose gels (1%). Size variation of the products was reproducibly observed for all organs tested, and the sequences of the products were determined (see Fig. 2 for details). Arrows indicate upper (soluble form) and lower (membrane form) bands. The numbers of PCR reaction cycles are shown to the bottom. The results with other primer sets are not shown.

ous results. Unlike the membrane form, the expression of variant 1 was dominant in the liver and heart (Fig. 5b). Variants 2 and 3 were barely expressed in the organs examined (not shown). Yet the levels of the soluble form variant 1 messages were very low in all organs compared to that of the membrane form in the testis.

We next confirmed protein expression in the testis and other organs by immunoblotting. Murine CD46 protein was detected prominently in the testis, but far less or virtually not at all in other organs on immunoblotting (Inoue, Seya, and Okabe, manuscript in preparation). This CD46 should be a membrane form since a solubilizer was required for protein detection. The liver produced CD46 mRNA, but the serum CD46 protein level was less than the detection limit

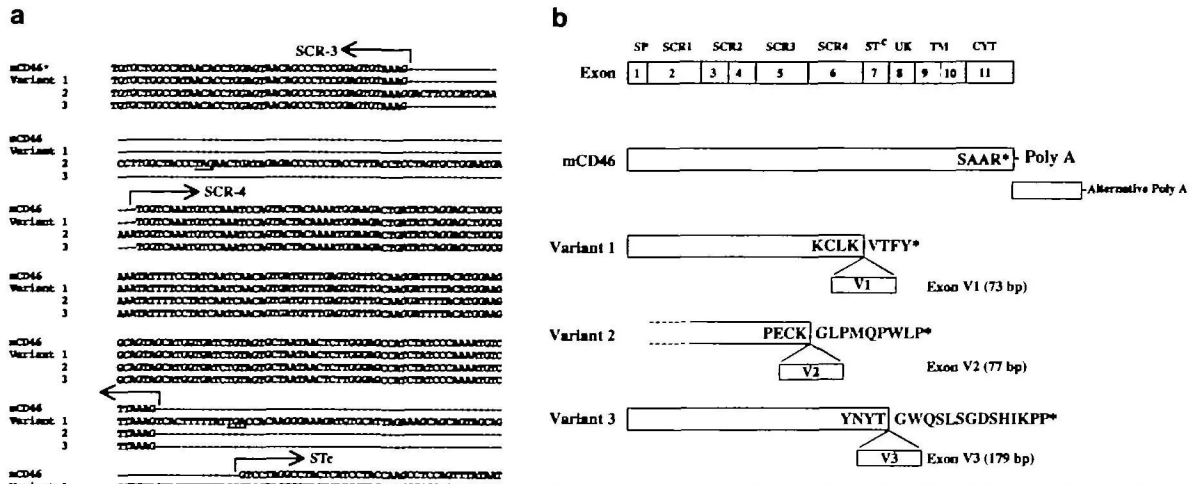

Fig. 2. **The three variants of murine CD46.** Panel a: Comparison of the nucleotide sequences of the three variants of murine CD46 cDNA. The nucleotide sequences of the three variants of murine CD46 were obtained as in the text. The three inserted sequences are encoded by independent exons (see Fig. 4). The stop codon is underlined. The nucleotide sequence data are available from EMBL Data Library/GenBank/DDBJ under accession number AB001566. Panel b: The predicted amino acid sequences of the murine variant CD46. The reported domains and corresponding exons of the reported membrane mCD46 are outlined at the top. The sequence of the conventional membrane mCD46 is shown in Ref. *8*. The unique portions of each soluble form are depicted in this figure. * means a stop codon.

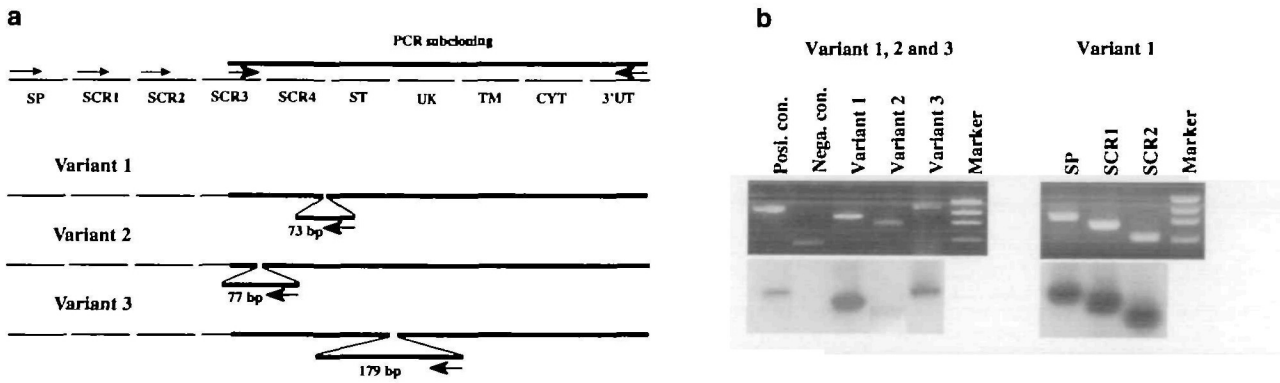

Fig. 3. **RT-PCR profiles suggestive of the presence of variant mRNAs.** Panel a: The primers were set for evaluation of the presence of variant messages. An oligonucleotide primer for PCR is indicated by underscoring. The primer sequences are shown in Table I. Variants 1 and 3 were detected with all primer combinations examined, while variant 2 was only detectable with SCR3-5′ and variant 2-specific primer (V2-3′). The amplification conditions for PCR were given in "MATERIALS AND METHODS." Panel b: PCR Southern analysis was performed on the three variants. Left panel: The testis mRNA was used throughout this analysis. The mRNA was reverse-transcribed into cDNA as described in "MATERIALS AND METHODS." The SP-5′ primer and specific primers for variant 1 (V1-3′), variant 2 (V2-3′), and variant 3 (V3-3′) were used for PCR amplification. As controls, irrelevant primers (negative control), and the primer set of SP-5′ and 3′UT-3′ (positive control) were used instead of SP-5′ and the variant-specific primers (Table I). The samples were analyzed on agarose gel (upper panel). The bands were transblotted onto nitrocellulose sheets and then probed with end-labeled variant-specific probes (lower panel), which are identified in Table I. Markers (No4; Toyobo, Tokyo) are shown to the right. Right panel: One of the three 5′-primers, SP-5′, SCR1, or SCR2-5′, and a 3′-primer, V3′, were used as the primer set for amplification. The amplicons were analyzed on agarose gel (upper panel) and by PCR Southern blotting (lower panel). Marker No4 (Toyobo) is shown alongside.

on immunoblotting, probably <5 ng (not shown). In mouse cell lines, the soluble proteins were not detectable in the supernatants of the cell lines (G-5, F9, and 3T3).

Functional Analysis of Soluble Murine CD46—Since RK13 cells produced the membrane form of mCD46 when transfected with the vectors containing the Kozak sequence and the membrane form cDNA, RK13 cells were transfected with the cDNA of variant 1 as described in "MATERIALS AND METHODS." The soluble form of variant 1 mCD46 protein was identified in the conditioned media as well as the NP-40 cell extract of cDNA-transfected RK13 cells. The proteins accumulated in the culture supernatant, which were detectable on SDS-PAGE/immunoblotting with polyclonal antibodies (Fig. 6). The molecular mass of the vari-

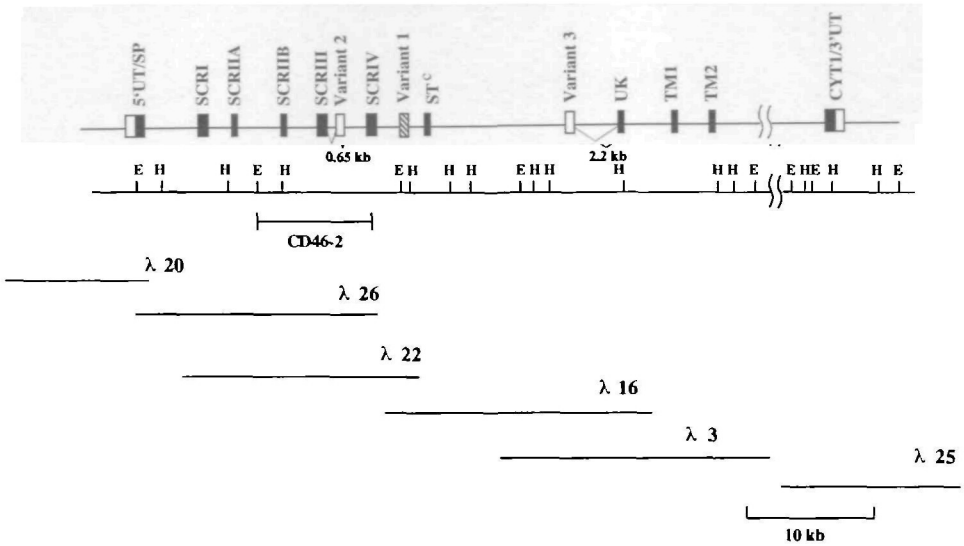

Fig. 4. **Location of the novel exons in the mouse CD46 genome.** Restriction maps and exon-intron structures of the murine *Mcp* gene including the three additional exons are presented. Boxed regions represent exons and closed boxes correspond to the open reading frame. The names of domains are shown above the map. The names, sizes and locations of representative phage clones are shown below the map. V1, V2, and V3 are alternative exons that encode putative soluble CD46. Symbols: E, *Eco*RI; H, *Hin*dIII.

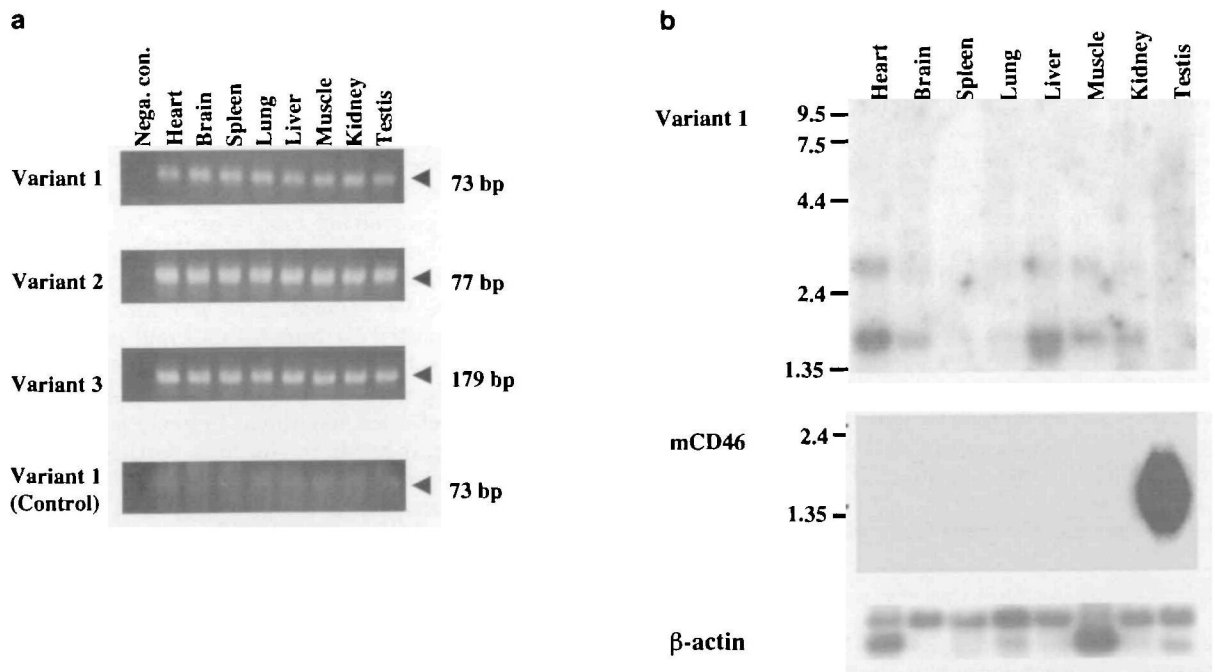

Fig. 5. **Expression of variant messages in murine tissues.** Panel a: RT-PCR analyses. The pair primers (the 5′ and 3′ primers for V1, V2, and V3 in Table I) were set within the insert sequences of variants 1, 2, and 3, and RT-PCR was performed using mRNA from each organ. The sources of the templates are indicated at the top. The negative control (Nega. Con.) had no template. The primer pairs used for analyses are shown to the left. The sizes of the amplicons are shown to the right. In the bottom panel, the same RNA samples without reverse-transcriptase treatment were used as templates to exclude the presence of amplifiable genomic DNA in the samples. Panel b: Northern blotting analysis of murine variant CD46. The membrane (Clontech MTN 7762-1, about 2 μg of poly(A)$^+$ RNA in each lane) was hybridized with the variant 1–specific probe (upper panel) (Table I). The same membrane was also rehybridized with a 0.3 kb fragment of murine CD46 cDNA (center panel), and then rehybridized with human β-actin cDNA (lower panel). The hybridized membranes were exposed for 7 days (upper panel), 36 h (center panel), and 2 h (lower panel). In the lower panel, a single 2.0 kb band was observed in all lanes, except for the lanes of heart and skeletal muscle, which contained two forms of β-actin of 2.0 and 1.8 kb. Standard markers are indicated at the left side.

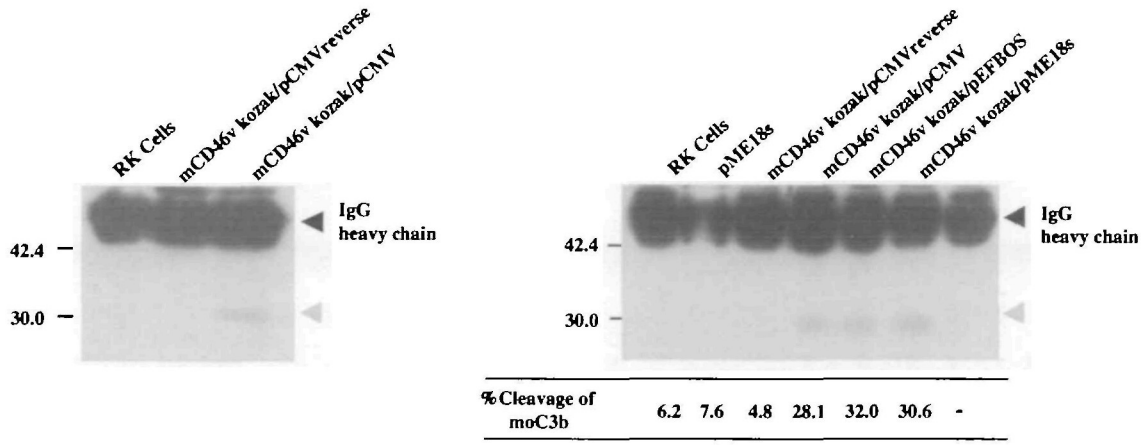

Fig. 6. **Factor I-cofactor activity of variant 1 soluble mouse CD46.** Left panel: Solubilized RK13 cells transfected with variant 1 mCD46 cDNA in the reverse or forward orientation (lanes 2 and 3, respectively) or with no vector (lane 1) were analyzed by SDS-PAGE followed by immunoblotting using the first Ab (a rabbit Ab specific for mCD46) and second Ab (Ab recognizing rabbit IgG). Open arrowhead, the soluble mCD46 band; closed arrowhead, rabbit IgG heavy chain of the first Ab. Molecular weight markers are shown on the left. Right panel: The supernatants of RK13 cells bearing no vector (the left lane) or those indicated in the panel were analyzed as in the left panel (upper panel). Supernatant samples (0.2 ml, containing no FCS) were then incubated with murine factor I (0.2 μg) and fluorescence-labeled murine C3b (5 μg), %cleavage of C3b being calculated as in Ref. 7 with a spectrofluorometer (indicated beneath the lanes). Without factor I, these preparations exhibited virtually no C3b-cleaving activity under the conditions described in "MATERIALS AND METHODS" (data not shown). The three lanes with samples containing soluble CD46 exhibited similar degrees of factor I-cofactor activity (~30% C3b cleavage). The far right lane is a medium control (serum-free medium used for cell culture). Murine factor I and H.1 (0.1 μg) cleaved mouse C3b to similar extents, ~30% (data not shown). Open arrowhead, the soluble mCD46 band; closed arrowhead, rabbit IgG heavy chain of the first Ab. Molecular weight markers are shown on the left. A representative of the three experiments is shown.

ant 1 protein was 32 kDa, *i.e.* about 10 kDa smaller than that of the membrane form of murine testis CD46 and 4 kDa larger than the predicted molecular mass of variant 1 (data not shown). These differences could be in part due to post-translational sugar modification, as for human CD46. The variant 2 and 3 proteins could not be detected with the same expression procedure (data not shown).

Factor I-cofactor activity in the variant 1 preparations was assessed using a murine substrate and protease factor I according to the established method (*7*). The cofactor activity of mCD46 was exerted for murine factor I-mediated cleavage of murine C3b (Fig. 6). Cofactor activity was assessed after 2 h incubation, since prolonged incubation disclosed cofactor activity of parent cells, presumably due to liberated membrane complement regulatory proteins (data not shown). Virtually no C3b cleavage was observed if the conditioned medium was substituted with that containing no soluble mCD46. Thus, variant 1 of soluble mCD46 exhibits factor I-cofactor activity to inhibit complement activation as in the case of murine factor H (*11*).

DISCUSSION

Human SCR-containing complement regulatory proteins often serve as virus receptors (*22*). Viral infection often regulates the levels of human complement regulatory proteins (*23*). Here, we demonstrated that there are additional mRNAs of mCD46, one of which encodes a functionally active soluble protein.

One alternative exon corresponding to a 73 bp insertion in the secretory form was found between the SCRIV and ST^C exons; alternative splicing involving this novel exon predicted the generation of a secretory form of murine CD46, variant 1, consistent with our previous report (*8*). The point of this study is that the cDNA encoding 4 SCRs without any ST or UK corresponds to the predicted genomic organization of the secretory mCD46 and the expression of a functionally-active protein product by transfectants. Thus, generation of the transcripts of secretory variant 1 and membrane mCD46 can be fully explained by alternative splicing. Likewise, other murine CD46 mRNAs, variants 2 and 3, were found to be produced through alternative splicing involving unique exons. Yet, the factors for induction of the splicing of the mCD46 message have not been determined.

Mechanisms for generation of secretory CD46 proteins have been reported for humans (*24*) and guinea pigs (*25*), respectively. In humans, neither possible messages satisfying the protein data nor possible exons encoding secretory forms have yet been identified. Proteolytic clipping-out of membrane forms has been shown to participate in the production of secretory forms in human CD46 (*24*). In guinea pigs, intron retention followed by the exon of SCRIII may facilitate generation of a secretory form message (*25*). In humans, intron retention downstream of the exon encoding SCRIV allows insertion of 39 unique amino acids, resulting in an unusual membrane (but not secretory) isoform (*26*). These mechanisms are not consistent with those for the production of secretory CD46 in mice, herein first reported. In this study, the secretory form messages including multiple splice products and relevant exons were first identified in the CD46 cDNA and genome, respectively. The mechanism for production of the secretory murine CD46 is not one of as yet known mechanisms of intron retention.

Another important finding is that the tissue distribution of the CD46 secretory form messages completely differs from that of the membrane form. A high steady state level of the membrane form of mRNA (1.5 kb) was predomi-

nantly found in the testis, especially the testicular germ cells (*7*). In contrast, the variant 1 message was predominantly distributed in the liver and heart, and showed a little slower mobility (1.6 kb) than that of the membrane mCD46 message on Northern blotting analysis (Fig. 5). The levels of the variant mRNAs were relatively low compared to that of the membrane form mRNA, as judged on RT-PCR (Fig. 1) and Northern analyses (Fig. 5). These tendencies were confirmed with another inbred mouse strain, C57BL/6. Why the soluble and membrane CD46 mRNAs were differentially regulated in each organ remains to be examined (Fig. 5).

A aim of this study was to determine what the role of soluble mCD46 is. Although we identified the three messages of soluble mCD46, only one soluble isoform was successfully expressed to analyze the function. The variant 1 soluble isoform exhibits sufficient factor I-cofactor activity to regulate complement activation in the fluid phase. Thus, the soluble mCD46 is a complement-regulatory protein of which expression is usually suppressed under gene regulation: only the membrane CD46 is constitutively expressed on spermatozoa. Since soluble mCD46 has now been found to act as a complement regulator, we favor the following interpretation in terms of soluble *vs.* membrane CD46.

According to previous reports (*17, 23*), mCD46 is up-regulated in some cell lines in conjunction with viral infection. Infection may switch from a membrane form- to a soluble form-dominant state in murine cells leading to regulation of infection-induced complement activation. In fact, the mCD46 protein is markedly up-regulated by mCMV infection in affected NIH 3T3 cells (*17*). If this is the case, cells and body fluids may provide a complement-resistant environment, which facilitates viral amplification in infected cells. A number of survival strategies of viruses have been reported. Vaccinia virus encodes a soluble complement regulatory protein and liberates it for self-protection against complement (*27*), which protect viral replication from host complement as in soluble CD46. HCMV, HTLV-1, HIV, and vaccinia virus also upregulate human CD46 and CD55 to circumvent complement attack (*28–31*). Thus, virus species have their own unique ways of evading host complement attack, and mCMV may employ soluble mCD46 to survive, circumventing host complement activation.

The role of mCD46 in the complement system has not yet been clarified since the current concept is that in intact mice membrane mCD46 is exclusively expressed in the testicular germ cells and mCD46 should play some roles in fertilization. Our present findings further extend the role of murine CD46. In virus-infected mice, the secretory mCD46 may be up-regulated to participate in complement regulation, which is crucial because the activation potential of complement can be increased in response to pathogenic stimuli. Our hypothesis is that under environmental control soluble and membrane forms of mCD46 are differentially yielded to execute their functions and roles (*17*). Further studies including promoter analysis and gene targeting of CD46 in mice are currently in progress to substantiate these hypotheses.

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