Exon truncation by alternative splicing of murine ICAM-1

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Mizgerd, Joseph P., Matt R. Spieker, and Michal M. Lupa. Exon truncation by alternative splicing of murine ICAM-1. Physiol Genomics 12: 47–51, 2002.—The murine gene for intercellular adhesion molecule-1 (ICAM-1) encodes multiple products, arising from alternative splicing. Full-length ICAM-1 contains five extracellular Ig domains, each encoded by a separate exon. Alternatively spliced forms have Ig domains 2, 3, and/or 4 excised as a result of exon skipping. We report here a novel splice variant of murine ICAM-1, resulting from exon truncation rather than exon skipping and affecting Ig domain 5. A 5’ splice donor site within exon 6 generates transcripts missing 69 nucleic acids from the 3’ terminus of the exon. This in-frame exon truncation is predicted to replace 24 amino acids within Ig domain 5 with a single aspartic acid residue, yielding a structure other than an Ig domain immediately external to the membrane. Expression of this alternatively spliced form is induced in mouse lungs, spleen, and kidneys during LPS-induced pulmonary inflammation. Since the affected region is critical for ICAM-1 presentation, dimerization, and solubilization, this alternative splice variant may have unique physiological functions.

MATERIALS AND METHODS

C57BL/6 mice (Charles River Laboratories, Wilmington, MA) were anesthetized by intramuscular injection of ketamine hydrochloride (100 mg/kg) and acepromazine maleate (5 mg/kg). Escherichia coli LPS, serotype O55:B5 (Sigma Chemical, St. Louis, MO), was instilled intratracheally at 100 μg/mouse, as previously described (16). Mice were killed by inhalation of a halothane overdose. RNA was isolated from lungs, spleen, and kidneys using RNeasy kits (Qiagen, Valencia, CA). cDNA was prepared using Advantage RT-for-PCR (Clontech, Palo Alto, CA). Primer sequences for murine ICAM-1 amplification included TGTGCTTGGAGAACTGTGGC (within exon 2), TCTCTAAGTGTCTCGAGGCC (within exon 3), ACAGCAGAGACCTACAGTC (within exon 4), GGTGAAGTCTGCTCAACAGGA (within exon 5), GGGTCCAGGCAGGAGTCTCATCCAGCAGGC (within exon 6), and GGAGTAGAATGACCACTCCTC or its complement in reverse (spanning the novel splice junction between exons 6 and 7). PCR amplification products revealed by ethidium bromide staining were excised and sequenced at the Dana-Farber Cancer Institute Molecular Biology Core facility (Boston, MA). Expression levels of murine ICAM-1 with the standard or alternative splices of exons 6 and 7 were assessed using multiplex RT-PCR with 18S rRNA as the standard. Amplification of the common splice product was achieved by 19 cycles of 25 s at 95°C, 35 s at 55°C, and 50 s at 72°C with primers for exons 6 and 7 from ICAM-1 and for 18S rRNA (CTTAAAGGATTTGACCGGAAG plus TCCGAGGTTACACTACGGGA). Amplification of the alternative splice product was achieved by 24 (lung and kidney) or 27 (spleen) cycles using the novel splice junction primer, the exon 7 primer, and the pair of 18S rRNA primers. Reaction products were labeled with [α-32P]ATP for autoradiography and densitometry. ICAM-1 expression level for each sample was calculated relative to the densitometric value of 18S rRNA.

Sequences for ICAM-1 genes were collected from the National Center for Biotechnology Information (Bethesda, MD), including those for mouse (X52264), rat (D00913), hamster (AF208456), dog (L31625), pig (AF156712), sheep (AF110984), cow (U65789), chimpanzee (M86848), and hu-
Fig. 1. Unexpected product from PCR amplification of exon 6–7 junction in murine intercellular adhesion molecule-1 (ICAM-1). A: alternatively spliced forms of murine ICAM-1 generated by exon skipping. The full-length protein is schematized at top, with the corresponding full-length pre-mRNA immediately below. Exons are depicted with numbered boxes. Splicing events link the shaded exons to form mRNA, and white exons are skipped in alternatively spliced forms. As a strategy to amplify all forms of murine ICAM-1, regardless of splicing events, PCR primers were selected for sequences in exons 6 and 7, as indicated by triangles. B: amplification products from mouse lung, using primers schematized in A, without or 6 h after the intratracheal instillation of E. coli LPS. The sequence of the bottom band was identical to the top band, which corresponded to murine ICAM-1, with the exception of a missing stretch of 69 internal bases (listed in Fig. 2).

RESULTS AND DISCUSSION

ICAM-1 exon 6 encodes Ig domain 5 and the juxtamembrane region (1), essential for dimerization, surface presentation, and solubilization. Exon 7 encodes the single-spanning transmembrane domain and the cytoplasmic tail (1). Because these regions of murine ICAM-1 were structurally important and not previously noted to be alternatively spliced, primers were selected to amplify cDNA from contiguous regions of exons 6 and 7 (Fig. 1A). While optimizing PCR parameters for detecting differential levels of expression of ICAM-1, we noted that a second band of lighter intensity appeared beneath the band of expected size (Fig. 1B). The lighter band with greater electrophoretic mobility was more prominent in the lanes containing cDNA from lungs with LPS-induced inflammation, suggesting that it was induced during this inflammatory response.

The sequence of the larger band corresponded exactly to the expected product from PCR amplification of ICAM-1. The smaller band corresponded exactly to the expected ICAM-1 cDNA sequence, with the exception that a stretch of 69 internal bases was absent. The missing bases corresponded to the terminal 69 bases of exon 6. A splice donor site (AUG:GUA), with five bases including the critical “GU” consistent with the canonical $\text{AC}:\text{G}:\text{GUA}:\text{G}$ splice donor site (7, 13), was evident at the relevant position 69 bases from the common splice donor (Fig. 2). Thus this sequence functions as a novel splice donor site, resulting in an alternatively spliced mRNA which lacks 69 nucleic acids from exon 6 (Fig. 2). To our knowledge, this is the first evidence of exon truncation in murine ICAM-1.

Because this exon truncation does not cause a frame shift, the amino acid sequences of the transmembrane and cytoplasmic domains are not altered by this splicing event. However, 24 amino acids from the extracellular juxtamembrane region are no longer encoded in this alternatively spliced form, replaced by a single aspartic acid residue (Fig. 2). The missing 24 amino acids compose approximately one-third of Ig domain 5, including a cysteine required for the disulfide bond. Thus this alternatively spliced form of exon 6 encodes a transmembrane protein with a dramatically altered juxtamembrane structure.

ICAM-1 expression is induced by LPS in the lungs (2, 4) and mediates neutrophil emigration in this setting (12). To determine whether pulmonary expression of the alternate, truncated transcript was induced by LPS in the lungs, expression levels in mouse lungs were measured using a primer spanning the novel sequence arising from this splicing event. Intratracheal LPS increased levels of truncated ICAM-1 RNA in the lungs (Fig. 3A). Thus the truncated form of ICAM-1 is induced by LPS, as inferred from earlier experiments (Fig. 1B). Steady-state levels of the truncated (Fig. 3A) and full-length (Fig. 3B) forms of ICAM-1 were increased similarly over time, suggesting that the net regulation (production plus degradation) does not substantially differ for truncated and full-length transcripts.

In addition to increasing ICAM-1 message in the lungs, intrapulmonary LPS results in systemic responses including increased ICAM-1 in nonpulmonary organs. To determine whether the alternatively spliced, truncated form of ICAM-1 was specific to the lungs or the local inflammatory site, ICAM-1 message was examined in the kidneys and spleens of mice after the intratracheal instillation of LPS. The primers recognizing the alternatively spliced ICAM-1 amplified a product from the kidney and the spleen during LPS-induced pulmonary inflammation (Fig. 4). Thus LPS-induced inflammation increases expression of the alternatively spliced, truncated isoform of ICAM-1 in multiple organs of the mouse.

Transcripts containing the full-length exon 6 are far more abundant than those containing the truncated
Similarly, the full-length transcript of ICAM-1 is far more abundant than the previously identified alternative transcripts in which exons 3, 4, and/or 5 are skipped (11, 20). To determine whether ICAM-1 transcripts with truncated exon 6 contained exons 3, 4, and 5, PCR was performed using 5′/H11032 primers within exons 2, 3, and 4 and a 3′/H11032 primer spanning the alternative splice between exons 6 and 7. cDNA from lungs collected 6 h after LPS instillation was extensively amplified, through 38 cycles, to enhance detection of alternatively spliced products potentially present at low abundance. Products of the expected full-length size were amplified from exons 2, 3, and 4 to the alternative splice between exons 6 and 7 (Fig. 5 and data not shown). Products corresponding to the expected full-length size were the most abundant observed, in all cases. Additional fainter bands were occasionally observed. Amplification from exon 3 to the alternative splice site yielded a very faint band at 550 bp (Fig. 5), but sequencing demonstrated that it was not ICAM-1. These data suggest that transcripts with exon 6 (Fig. 1B).

Fig. 2. Exon 6 truncation due to an alternative 5′ splice donor site in the gene for murine ICAM-1. The pre-mRNA sequence is shown for the acceptor and donor sites relevant to joining exons 6 and 7. The two different 5′ splice donor sites are underscored. The missing sequence from the novel band resulting from PCR amplification of this region (depicted in Fig. 1B) is the 69 bases between the vertical lines. Results of splicing the alternative and common 5′ donor sites with the acceptor site in exon 7 are depicted on the bottom, with altered amino acids shaded. This alternative splicing event results in a novel juxtamembrane region, of unknown structure but no longer an Ig domain.

Fig. 3. LPS in the lungs induced similar relative increases over time in the levels of transcripts for the alternative truncated isoform of ICAM-1 (A) and for the common full-length isoform of ICAM-1 (B). E. coli LPS was instilled intratracheally to C57BL/6 mice. ICAM-1 message was assessed using multiplex RT-PCR with 18S rRNA as the standard and expressed relative to basal levels.

Fig. 4. The alternative truncated form of ICAM-1 was induced in multiple organs during LPS-induced pulmonary inflammation. Each lane shows amplification from the designated organ from a different mouse, before or 4 h after LPS was instilled intratracheally.

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the truncated form of exon 6 predominantly contain all three exons which have previously been demonstrated to be excised in a minority of ICAM-1 transcripts due to exon skipping.

To determine whether the sequence containing this alternative 5' splice donor site in the mouse genome was conserved among species, coding sequences for ICAM-1 were aligned and compared. The rat sequence is identical to mouse in this region (Fig. 6). Hamsters have a single base change (Fig. 6), corresponding to the only base in the mouse sequence that did not fit with the canonical 5’ splice donor site sequence \( \text{A/C}AG \text{GU}^{+/-} \). Thus this sequence is conserved in these rodent species, and rats and hamsters may be capable of alternatively splicing exons 6 and 7 similar to mice.

In the six non-rodent species examined, the uracil residue common to mice, rats, and hamsters was replaced by a cytosine. In all nine of these species, this codon (GGU or GGC) translates to a glycine residue. This highly conserved glycine, and 23 downstream amino acids, is lost (and predicted to be replaced by an aspartic acid residue) when the pre-mRNA is spliced at this site, as observed in the mouse (Fig. 2). This single nucleotide difference among these species could affect alternative splicing. The “GU” sequence (as observed in mice, rats, and hamsters) is canonical of recognition by U1 small nuclear ribonucleoprotein particles (7, 13). “GU” is by far the most common sequence at the 5’ end of introns, accounting for >98% of introns in the human (9). However, the second most common sequence is “GC” (9). Introns with “GC” at the 5’ terminus account for only <0.8% of human introns, but they are still far more common than any sequence other than “GU” (9). All six non-rodent species examined (dog, pig, sheep, cow, chimpanzee, and human) contain “GC” at this site. It will be essential to determine whether the frequency of exon 6 truncation due to alternative splicing is altered by this sequence difference between rodents and other species, if this alternatively spliced form of ICAM-1 proves to be functionally significant.

Splicing that alters the sequence and structure of the juxtamembrane region of ICAM-1 could have functional consequences. First, proteolytic cleavage of ICAM-1 juxtamembrane sites releases soluble ICAM-1 from cell membranes (3, 5, 6, 14). Exon 6 truncation could affect cleavage by one or more proteases, altering the release of soluble ICAM-1 during inflammatory reactions. Second, the juxtamembrane region of ICAM-1 contributes to dimerization (15, 18), which increases avidity for \( \beta_2 \)-integrins (15). In addition to facilitating adhesion, dimerized ICAM-1 functions as a multivalent ligand that cross-links \( \beta_2 \)-integrins and induces intracellular signaling into leukocytes (17). If exon 6 truncation affects ICAM-1 dimerization, then it may alter the quality and quantity of its interactions with integrins, influencing cell adhesion and signaling cascades in adherent cells. Third, the juxtamembrane region may facilitate the orientation and surface presentation of the outer extracellular domains, including those that interact with ligands and counterreceptors (21). By altering the structure of the juxtamembrane region, exon 6 truncation could influence presentation of other ICAM-1 domains on the cell surface. Finally, the juxtamembrane region could contribute to functions of ICAM-1 for which responsible domains have yet to be identified. For example, ICAM-1 cross-linking on cell surfaces induces intracellular signaling (22), although the mechanism remains unclear. If the juxtamembrane region contributes directly or indirectly to the effects of cross-linking ICAM-1, then ICAM-1 signaling also may be influenced by exon 6 truncation. The functional effects of exon 6 truncation will be foci for multiple avenues of further research.

In conclusion, multiple species have nucleotide sequences consistent with a splice donor site within exon 6 of the gene for ICAM-1. In the mouse, exon truncation due to splicing with this donor site results in an alternative ICAM-1 mRNA. Unlike previously identified isoforms of ICAM-1, ICAM-1 RNA resulting from this exon truncation encodes a juxtamembrane region.

\[
\text{mouse} \quad A\ U\ G\ U\ U\ A
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\[
\text{rat} \quad A\ U\ G\ U\ U\ A
\]
\[
\text{hamster} \quad A\ C\ G\ G\ U\ A
\]
\[
\text{dog} \quad C\ U\ G\ G\ U\ A
\]
\[
\text{pig} \quad C\ A\ G\ G\ U\ A
\]
\[
\text{sheep} \quad C\ G\ G\ G\ U\ A
\]
\[
\text{cow} \quad C\ G\ G\ G\ U\ A
\]
\[
\text{chimpanzee} \quad A\ C\ G\ G\ U\ A
\]
\[
\text{human} \quad A\ C\ G\ G\ U\ A
\]

Fig. 6. Comparative genomics of the novel 5’ splice donor site in exon 6 from murine ICAM-1. Shaded boxes depict nucleic acids that differ from the mouse sequence. The “GU” sequence typical of splice sites recognized by U1 small nuclear ribonucleoprotein particles is in bold. Although less common, “GC” sequences can also serve as splice donors (see text for discussion).
that does not form an Ig domain. Since this region is critical for ICAM-1 presentation, dimerization, and solubilization, this alternative splice variant may have unique physiological functions. Expression of this alternatively spliced form of ICAM-1 is increased in multiple organs during LPS-induced pulmonary inflammation in the mouse.

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REFERENCES