Secreted and membrane attractin result from alternative splicing of the human *ATRN* gene

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Attractin, initially identified as a soluble human plasma protein with dipeptidyl peptidase IV activity that is expressed and released by activated T lymphocytes, also has been identified as the product of the murine *mahogany* gene with connections to control of pigmentation and energy metabolism. The *mahogany* product, however, is a transmembrane protein, raising the possibility of a human membrane attractin in addition to the secreted form. The genomic structure of human attractin reveals that soluble attractin arises from transcription of 25 sequential exons on human chromosome 20p13, where the 3′ terminal exon contains sequence from a long interspersed nuclear element-1 (LINE-1) retrotransposon element that includes a stop codon and a polyadenylation signal. The mRNA isoform for membrane attractin splices over the LINE-1 exon and includes five exons encoding transmembrane and cytoplasmic domains with organization and coding potential almost identical to that of the mouse gene. The relative abundance of soluble and transmembrane isoforms measured by reverse transcription–PCR is differentially regulated in lymphoid tissues. Because activation of peripheral blood leukocytes with phytohemagglutinin induces strong expression of cell surface attractin followed by release of soluble attractin, these results suggest that a genomic event unique to mammals, LINE-1 insertion, has provided an evolutionary mechanism for regulating cell interactions during an inflammatory reaction.

The regulation of the basic inflammatory response, involving recognition, recruitment of helper and effector cells, and their subsequent down-regulation, requires a complex interplay of cells, their receptors, and secreted cytokines and chemokines (1). We recently have identified the soluble human plasma protein attractin as a molecule that can facilitate initial immune cell clustering (2) and, through a putative dipeptidyl peptidase IV (DPPIV) activity similar to that of CD26 (3), has the potential to regulate chemotactic activity of chemokines (4). These activities would be required at differing stages of the inflammatory process and suggest that the functional activities of attractin may operate independent of its actual plasma levels, much as is seen for the components of complement that circulate normally at constant levels but are only active in the context of "fixed" antibody during the effector phase of a humoral response. Initial evidence for controlled expression of attractin was provided by the finding that not only was attractin a soluble protein but that its expression was induced early in T cell activation with a subsequent loss of membrane expression followed by release of soluble attractin (3).

Two recent reports describe the identification of a membrane form of attractin as the product of the murine *mahogany* gene, establishing connections to control of pigmentation and energy metabolism (5, 6). A predicted *Caenorhabditis elegans* protein, F33C8.1, is also homologous to attractin and is a transmembrane rather than a soluble protein (2). These results lead to the proposition that there exists a human membrane attractin in addition to the secreted form. Indeed, there are two prominent attractin isoforms in human lymphoid tissue, 9 kb and 4.5 kb, and a mouse cDNA probe that corresponds to the transmembrane/cytoplasmic domain hybridizes to the 9-kb isoform but not to the 4.5-kb isoform (5).

In this report, we describe the complete genomic sequence of attractin, focusing in particular on the exons coding for the 3′ region, and show how both human membrane and secreted attractin arise as a result of alternate splicing of the same gene. Remarkably, the soluble form of attractin results from an exon that appears to have been introduced by a retrotransposon insertion that generates a short carboxyl-terminal domain, stop codon, 3′ untranslated region (UTR), and polyadenylation signal. Regulation of the expression of the two isoforms may form the basis for the transient cell surface expression and subsequent release of soluble attractin observed during leukocyte activation. Thus, a genomic event unique to mammals, long interspersed nuclear element-1 (LINE-1) insertion, has provided an evolutionary mechanism for regulating the inflammatory response.

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Materials and Methods

DNA Preparation and Analysis. The bacterial artificial chromosome (BAC) clones CIT-D 3204p24 and RPCI-11 768n13 were obtained from Research Genetics (Huntsville, AL) and RPCI-5 1074c6, RPCI-4 581p3, RPCI-4 587a10, and RPCI-4 741h3 were obtained from Pieter de Jong (Roswell Park Cancer Institute, Buffalo, NY). Clones were grown in LB containing the appropriate antibiotic (12.5 μg/ml chloramphenicol or 25 μg/ml kanamycin), and DNA was purified by using the Nucleobond system (CLONTECH). Sequencing primers (all 21 bp) designed around the putative 5′ and 3′ ends of exons were used to sequence directly from the BAC template by using dye-terminator automated sequencing on ABI 373/377 machines (Applied Biosystems). Alignments were made both manually and by using the GCG package (Genetics Computer Group, Madison, WI) and compared with attractin cDNA sequence by using the BLASTN program at the National Center for Biotechnology Information (7). Analysis of the putative promoter region was performed by using MATINSPECTOR PROFESSIONAL software (Genomatix, Munich, Germany).

PCR. To estimate intron size within genomic sequence, forward primers based on 3′ exon sequence and reverse primers based on 5′ exon sequence (identical with those used for sequencing) were used to amplify sequence directly from BAC clone template by

Abbreviations: DPPIV, dipeptidyl peptidase IV; LINE-1, long interspersed nuclear element-1, G3PDH, glyceraldehyde-3-phosphate dehydrogenase; NF-AT, nuclear factor of activated T cells; BAC, bacterial artificial chromosome; EST, expressed sequence tag; PBL, peripheral blood leukocyte; UTR, untranslated region.

Data deposition: The exon sequences reported in this paper have been deposited in the GenBank database (accession nos. AF218889–AF218915).

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using the Advantage-GC system enzyme mix (CLONTECH) in the presence of 0.5 M GC-Melt (betaine). To differentially detect secreted and membrane attractin cDNA transcripts, primers were designed to amplify secreted attractin (forward: TTAGTCTATCCCAGGAAGATGATCG, and reverse: TAGGTATATCCCCTAATGCTATCCC; 412-bp product) or membrane attractin (forward: as above, and reverse: TCTTTATACTCCCCCCAATAAGATC; 552-bp product). Primers also were designed to amplify close to the 3′ end of the 3′UTR of membrane attractin (forward: AAGCTAATGCTAGGGTAGTGACTGA, and reverse: CTCTTGTTCCTGCTTAAATTAGCTG, yielding a product of 668 bp). Template DNA consisting of first-strand cDNA from an immune tissue panel normalized for four housekeeping genes was obtained from CLONTECH. For PCR amplification, 1 ng of template DNA was mixed with the relevant primers (0.2 μM final), Expand polymerase mix (Roche Molecular Biochemicals) and Expand PCR Buffer no. 1 and amplified by using the following cycling parameters: 30 s at 94°C (1 cycle); 30 s at 94°C, 1 min at 56°C, 2 min at 68°C (37 cycles); 7 min at 68°C (1 cycle), and held at 15°C. For amplification of glyceraldehyde-3-phosphate dehydrogenase (G3PDH), the cycle number for the amplification step was reduced from 37 to 30.

Generation of Recombinant and Membrane Attractin. All enzymes were obtained from New England Biolabs, and all expression vectors were from Invitrogen. In the initial attractin cDNA clone contig we described (AF034957), the 5′ region contains an internal deletion of 222 bp in comparison both with a cDNA isolated subsequently (AF106861) and with the sequence predicted here from the genomic structure. Two independent clones containing this deletion were identified in our initial screen, and it remains to be determined whether this is a true transcript or an artifact arising during library construction because of the GC-rich nature of the region (78% GC). Expression constructs based on the initial cDNA clones were repaired to contain the 222-bp segment by PCR amplification of the nondeleted cDNA, pCR2.1-10.43F4, that then was used as an extended forward primer together with a soluble attractin 3′ reverse primer (2) to yield a full-length soluble attractin containing the 222-bp segment that was subcloned into pcDNA3.1/B-myc-His. Sequence corresponding to the 3′ end of membrane attractin was isolated and amplified by reverse transcription–PCR from activated T cell mRNA and subcloned into pRc/CMV2, and the *Hin*dIII/*Xba*I fragment of full-length soluble attractin was ligated upstream to generate full-length membrane attractin that then was subcloned into pcDNA3.1/B-myc-His.

Detection of Recombinant Proteins. 293T cells (obtained from B. Mayer, Children's Hospital, Boston, MA) were transfected transiently with the soluble or membrane attractin pcDNA3.1 constructs by using Lipofectamine Plus (Life Technologies, Grand Island, NY) as described (2), and, 48 h posttransfection, the cells were lysed directly into 2× SDS/PAGE sample buffer. Purified serum attractin (0.4 μg), soluble attractin lysate (5 μl), and membrane attractin lysate (20 μl) were separated on 7.5% SDS/PAGE gels, and the separated proteins were transferred by electroblotting onto nitrocellulose membranes (0.45 μm; Bio-Rad). The membranes were blocked in Tris-buffered saline (TBS; pH 8) containing 1% BSA followed by incubation overnight at 4°C with affinity-purified rabbit polyclonal anti-attractin (0.5 μg/ml) or with murine anti-myc (Invitrogen; 1:5,000). After washes in TBS containing 0.1% Tween 20, the anti-attractin-incubated blots were incubated with anti-rabbit-IgG-horseradish peroxidase (HRP) (Amersham Pharmacia; 1:5,000), and the anti-myc-incubated blots were incubated with anti-mouse-IgG-HRP (Amersham Pharmacia; 1:5,000) and bound antibody detected by using the Supersignal West chemiluminescent reagents (Pierce).

Results

Identification of Genomic Clones Containing Attractin Exons. To identify the relationship of secreted attractin to the putative membrane form, we investigated the genomic structure coding for attractin. Searching of GenBank revealed a 53-bp internal sequence of a 462-bp deposit (AQ244985) derived from the BAC genomic clone CIT-D 3204p24 that was identical to 3417–3469 of attractin and was flanked by appropriate splice acceptor and donor sites. A second deposit (AQ467053) sequenced from BAC genomic clone RPCI-11 768n13 contained a 234-bp sequence with 87% identity to 2951–3184 of attractin and was similarly flanked by likely splice acceptor and donor sites. Primers based on internal exon sequence were designed to amplify upstream and downstream within the BAC genomic templates. Using this technique, we were able to identify nine exons corresponding to 2765–3816 of soluble attractin and its 3′ UTR. Sequence downstream revealed five additional exons that were 100% identical at the amino acid level with the transmembrane and cytoplasmic domains of murine attractin/mahogany (corresponding to mouse 3799–4287). A human expressed sequence tag (EST) sequence (AB011120) was identified that corresponded to position 2930–3801 of attractin, diverged for the 15 bases representing the 3′ terminus of human soluble attractin, but was identical with the predicted sequence for the five downstream exons representing the putative transmembrane/cytoplasmic domain of mouse attractin. EST AB011120 had already been mapped within GenBank to chromosome 20p13 (8) and appeared to lie between the sequence tagged site (STS) markers D20S113 and D20S97. Searching of the Chromosome 20 database (Sanger Centre, Cambridge, U.K.) using the identified attractin intron and exon sequence for BAC clones mapped to the region defined by the STS markers revealed identity with the clone RPCI-4 741h3 that had been selected for shotgun sequencing and recently deposited in GenBank (AL132773). We selected three additional BAC clones that had been mapped to lie upstream of RPCI-4 741h3 but overlapped with its 5′ end to identify the 5′ genomic sequence for attractin. The complete strategy is depicted in Fig. 1*A*. This led to the identification of 30 exons coding for the complete sequence of attractin (GenBank AF218889–AF218915) of which exons 1–25 coded for the soluble secreted attractin, and exons 1–24 and 26–30 coded for a putative human membrane form with strong sequence similarity to mouse attractin (Fig. 1*B*).

To confirm that the putative genomic sequence coded for attractin, an epitope-tagged recombinant secreted attractin and membrane attractin were constructed. 293T cells were transiently transfected with the attractin constructs, and lysates were separated by SDS/PAGE. After electroblotting, the soluble and membrane form could be detected both with anti-attractin antibody and anti-myc (Fig. 2). Although the molecular weights of the recombinant protein seem smaller than that expected on comparison with native attractin, this appears to be a consequence of incomplete glycosylation in 293T cells. After deglycosylation with PNGase-F, recombinant soluble attractin has a peptide core identical in size to that of native serum attractin, and the recombinant membrane attractin is bigger than both. Furthermore, soluble recombinant attractin can be detected by Western blotting in the supernatant of transiently transfected cells, whereas none can be detected in the supernatant of recombinant membrane attractin-transfected cells (data not presented).

Analysis of Attractin Genomic Sequence. Inspection of the predicted amino acid sequence for each membrane attractin exon and comparison with *C. elegans* genomic sequences from cosmid

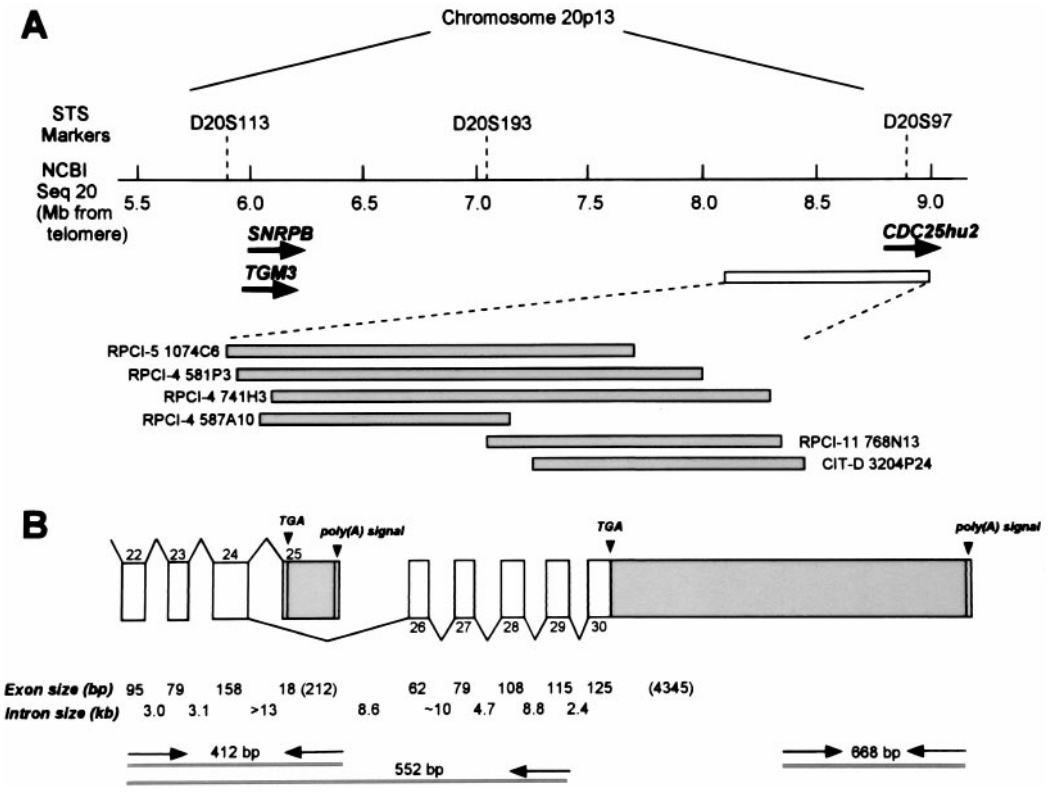

Fig. 1. (*A*) The sequence represented by AB011120 has been mapped to 20p13 (8), falling between the sequence-tagged site (STS) markers D20S113 and D20S97 that define a region of approximately 3 Mb at a distance of 6–9 Mb from the telomere. Known genes mapped to this region include small nuclear ribonucleoprotein polypeptide B/B1 (*SNRPB*), transglutaminase 3 (*TGM3*), and cell division cycle 25B phosphatase (*CDC25hu2*). The BAC clones identified as containing attractin exons map within a 1-Mb region 8–9 Mb from the telomere (white bar). (*B*) Representation of the exons coding for the 3′ ends of soluble attractin (exons 22–25) and membrane attractin (exons 22–24 and 26–30). Exon size is presented in base pairs; numbers in parentheses represent size of noncoding sequence. Intron size (kb) was determined by PCR amplification, and in some instances, confirmed by direct genomic sequencing. The thin shaded bars below represent the PCR-amplified regions used to determine relative expression of soluble and membrane mRNA, and the numbers above each bar represent the size of the amplified fragments.

F33C8 confirms a high level of sequence similarity between attractin and the F33C8.1 ORF that extends to the level of genomic structure (Fig. 3) because many, although not all, exon–intron boundaries are conserved. At the protein-coding level, it is readily apparent that the N terminal and C terminal are quite divergent, but internally there are three regions of high similarity corresponding to the CUB domain, the two laminin-like epidermal growth factor domains and a region corresponding to the transmembrane domain and the cytoplasmic domain proximal to the membrane. Attractin and F33C8.1 are clearly homologous, but the question of whether the two proteins are true orthologs or paralogs cannot be addressed, in part because there appear to be additional attractin-related genes in humans (e.g., AB011106 on chromosome 10).

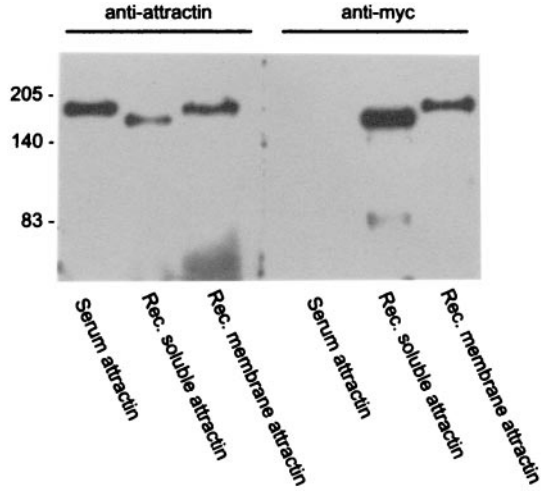

Fig. 2. Purified serum attractin, recombinant soluble attractin, and membrane attractin (lysates of transiently transfected 293T cells) were separated by 7.5% SDS/PAGE and detected with affinity-purified rabbit anti-attractin (*Left*) or anti-C-terminal myc tag (*Right*) by Western blotting and chemiluminescent detection of bound antibody.

We also examined potential transcriptional regulatory sequences in the 5′ flanking region and the likely polyadenylation signals used by the soluble and membrane isoforms. The putative promoter region is depicted in Fig. 4. Using the MATINSPECTOR package with high stringency (100% identity core binding region, 90% identity consensus region; ref. 9), the majority of potential transcription factor binding sites were associated with immunological and hematopoietic transcriptional control, including six sites for Ikaros-2 (10, 11), three sites for nuclear factor of activated T cells (NF-AT) (12), two sites for Ets-1 (13), two sites for MZF-1 (14), and a site for LyF-1 (15). Two sequences within 150 bp upstream of the putative start codon were identified as GC-boxes (16). There is no evidence of a TATA element, thus classifying this region as a TATA-less GC-rich promoter (17). There are two separate polyadenylation signals. The first is in exon 25 (soluble attractin) 196 nts downstream from the stop codon and consists of the sequence ATTAAA followed 17 nts

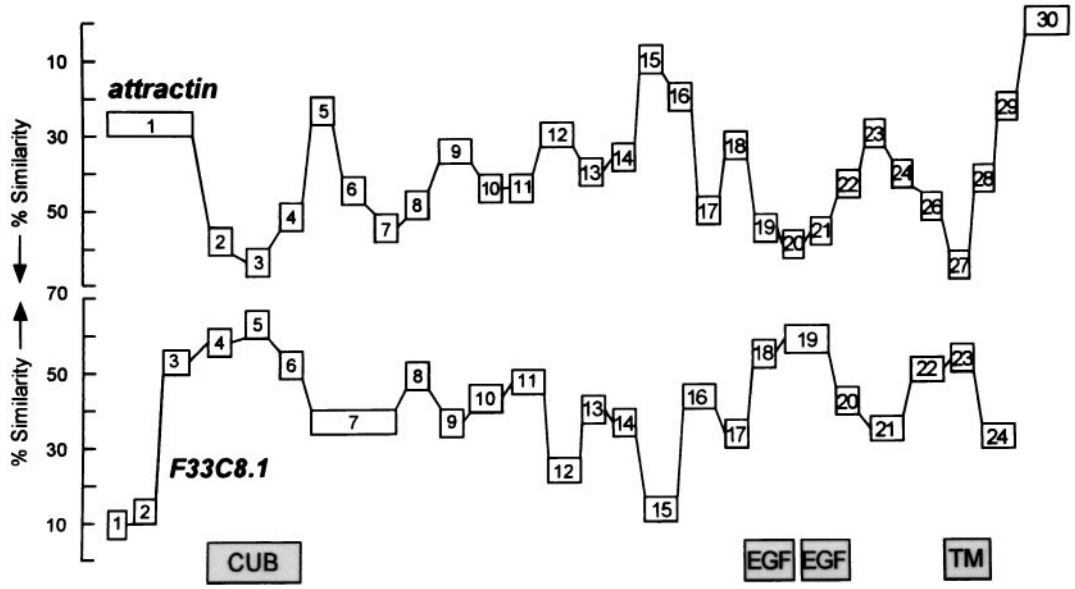

Fig. 3. Comparison of exon organization of human membrane attractin and *C. elegans* F33C8.1. Membrane attractin does not use exon 25. Similarity was determined by using the CLUSTAL-X alignment algorithm with default settings (34). Similarity (%) of each exon with the corresponding amino acid sequence in the related genomic sequence is depicted on the vertical scale, where greater similarity results in greater proximity. The shaded boxes represent identified domains of high similarity shared by attractin and F33C8.1 (TM, transmembrane domain).

further downstream by a diffusive GT-rich sequence (18). The second polyadenylation signal in exon 30 consists of the canonical sequence AATAAA followed 33 nts downstream by a diffusive GT-rich sequence. The 196 nts between the stop codon and polyadenylation signal of exon 25 correspond exactly to the 3′ end of the putative second ORF of a LINE-1 element and end in the poly(A) tract that typically accompanies insertions by non-long terminal repeat retrotransposons (19).

Differential Expression of Attractin mRNA. We had noted previously a differential expression of the two attractin mRNA species (4.5 kb and 9 kb) in different lymphoid tissues (2). In particular, the 4.5-kb isoform was almost absent from thymus, although the higher 9-kb form was present at low levels, and the low 4.5-kb form appeared to be preferentially expressed in peripheral blood leukocytes (PBLs). Based on previous results (5) and those presented here, the 4.5-kb form codes for soluble attractin with a short 3′ UTR, and the 9-kb form codes for membrane attractin and has a long 3′ UTR. PCR primers were designed to specifically amplify regions of soluble and membrane attractin cDNA, respectively. First-strand cDNA from human lymphoid organs that had been normalized for four human housekeeping genes

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-750  accttgacctttcaaaatcaaagatcctctgcctcaagctttgagtactGGGAAAaagcgggcaaccaccaagtcgggct
                                                 Ikaros-2----------
                                                  NFAT ----------
-670  aatttttgatttttagtaaagacagtttctcaccctgtTGGCcaggctgtctcggaTCCCaaagatcaagtgatccgcct
                                      NF-1----------                    ***
                                                    Ikaros-2----------
-590  gcctcagTTTCCCaaagtgctGGGAttacaggcgtaagcctccgcgcccggcctatgccaggttctacatgtgttctttc
     NFAT---------- ----------Ikaros-2
   Ikaros-2----------
     LyF-1 ---------
-510  ttgactcctgtgttacagaggctcgaatgaagcctggcttctacagtaccgcacccagcacctcgctgcgatgcagcctc
                                    ***
-430  ctgtacccttccgTCCGgcagctggagttagggcagagtgccgggtctctaggagggcaacactcaacgcagtcggctc
     c-Ets-1(p54)----------
-350  ctaggcatccagtggggctgcaaccccgccgccGGGGacgaaaggcgaagGGGGAAAactagagctttcgccggccGGGA
                                MZF-1--------   MZF-1--------
                                                Ikaros-2----------   Ikaros-2 ---------
                                                  NFAT ----------
-270  cagcgcagccgggtttcagctcgttctacggtccacgtcaaaagatcgccTCCGgcgccgcggacgccggagaccactgt
     ----                                    c-Ets-1(p54)----------
-190  gtgcgccccgcacgcacgcacccaggtactcgcgacgcgcgcgccggcccCGCCcaccgccccgctgtggcccctttgga
                                                GC box----------
-110  ccgcacgagccaccgtccgcacagcccCGCCccgcacggccaggcgaagcggagccggccgtgcggtgtgtgtgtatgtg
                          GC box----------
                                      +1
 -30  ttcgcggggcgccgtctcagccccgggaagATGGTGGCTGCAGCGGCGGCAACTGAGGCAAGGCTGAGGAGG
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Fig. 4. Promoter sites (in bold) were detected by using the MATINSPECTOR package (100% identity for core region, 90% identity with consensus flanking residues). The capitalized residues within putative sites represent the core binding motifs. * indicate in-frame stop codons; ''+1'' marks the putative start codon and numbering is relative to this. The underline indicates the longest 5′ UTR identified so far in screening cDNA libraries.

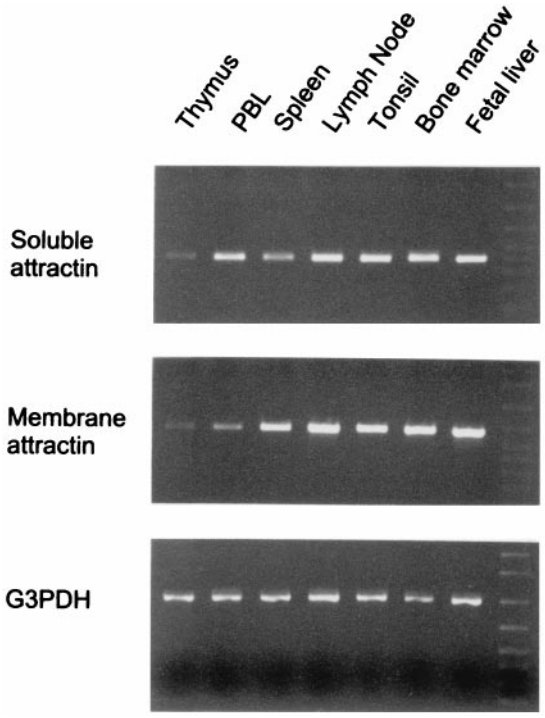

Fig. 5. First-strand cDNA immune tissue [primed from the poly(A) tail] that had been normalized for four housekeeping genes was tested for representation of soluble and membrane attractin. The lower panels depict amplification of a G3PDH (1 kb) fragment for all of the samples tested. Soluble attractin (412 bp) was amplified by using the primers depicted in Fig. 1*B*. Membrane attractin (668 bp) was amplified by using primers within the 3′ UTR. Identical results were obtained by using the primers designed from coding sequence (552 bp) but required five additional cycles to obtain a strong signal. This is a result of first-strand priming from poly(A) and the length of the membrane attractin 3′ UTR (>4 kb) leading to under-representation of the coding sequence.

was used as template (Fig. 5). We confirmed that thymic expression of attractin is very low and that the soluble form is preferentially expressed in PBL. It is important to note that detection of attractin in first-strand cDNA populations required seven additional cycles of amplification than that required for G3PDH to generate comparable signals by ethidium bromide staining, suggesting that both attractin isoforms are expressed at low to moderate levels.

Discussion

In this report, we demonstrate the genomic basis for generating both a soluble and membrane form of attractin and confirm the corroborating evidence from *C. elegans* (2) and from mouse (5, 6) that a membrane attractin exists in humans in addition to the soluble form. There is considerable evidence for the generation of secreted and membrane proteins by alternative splicing, but this is usually the result of an exon that is a composite of splice acceptor and donor sites followed closely by a poly(A) signal (20). Depending on circumstances, such exons can act as internal or terminal exons, as occurs, for example, when activation of B lymphocytes results in a switch from expression of membrane to secreted Ig (21). Attractin, however, generates alternate forms through skipping of an exon containing a termination codon and polyadenylation signal. This mechanism has been shown to operate for a relatively small number of proteins (20) of which one, rat epidermal growth factor receptor, may be particularly relevant because the soluble secreted form is capable of inhibiting/regulating the activities of the membrane form (22).

The mechanism by which exon 25 encoding the soluble attractin C-terminal has arisen is clear. The sequence between the stop codon and the polyadenylation signal is derived from the C terminal of the second ORF of a LINE-1 retrotransposon, and further evidence for non-long terminal repeat retrotransposon insertion is provided by the genomically encoded poly(A) sequence characteristic of this process (19). LINE-1 elements are a feature limited to mammalian genomes and are abundantly represented (23). Evidence is accumulating that LINE-1 insertion is an active evolutionary process that can lead to generation of gene products (24), or even disease (25). There is no evidence of any insertion in the *C. elegans* intron (only 50 nts) corresponding to the sequence between human exons 24 and 26, whereas the corresponding area in the mouse genome has not yet been fully characterized. Given that attractin in its secreted form circulates at high levels in the serum and that it appears to play a role in immune cell clustering, a major objective in the near future will be to determine whether mice also harbor such an insertion with the potential to code for a soluble molecule. The LINE-1 sequence within the 3′ UTR of exon 25 is characteristically human, raising the possibility that its presence represents a polymorphism. We consider this unlikely as we have been able to detect soluble attractin in every human serum sample we have tested.

Within the genomic sequence, comparison with the *C. elegans* transcript F33C8.1 reveals areas of very high conservation of peptide sequence corresponding to the CUB domain and the two laminin-like epidermal growth factor domains. The role of the CUB domain is not well understood but appears to form an anti-parallel β-barrel structure that is common to a growing family of proteins involved in cell guidance, developmental decisions, and cellular communication (26). Of potentially greater importance is the complete identity of an 8-aa region of the cytoplasmic domain proximal to the membrane, as has been noted previously (6) and suggests a role in downstream signaling. The secondary structure of membrane attractin and F33C8.1 is probably very similar, given the almost complete conservation of cysteines between the two proteins (2).

Attractin expression is not restricted to the hematopoietic system. Recent reports show abundant mRNA transcripts throughout the nervous system (27); we have found strong mRNA expression in placenta and colon, and we previously have shown that attractin is constitutively expressed on a large number of cell lines derived from normal and malignant tissues with secretory capacity (3). Furthermore, the mahogany mouse deficient in attractin mRNA has clear abnormalities in control of agouti-mediated control of pigmentation and energy metabolism (5, 6). The expression of attractin on T lymphocytes is remarkable because it is activation induced, similar to that of CD26, to which we initially thought it was related (28), and, like CD26, the promoter is a TATA-less GC-rich region (29). The promoter for attractin may be of great interest because the majority of putative transcription factor binding sites in the 5′ flanking region are involved in control of lymphocyte or hematopoietic differentiation. There are six binding sites for Ikaros-2, a protein that is required for differentiation of hematopoietic stem cells to the lymphoid lineage (10, 11). Germ-line mutations in the DNA-binding domain of Ikaros lead to animals devoid of T cells, B cells, and NK cells. There are three sites for NF-AT, nevertheless there is no evidence of an up-regulation of attractin mRNA after activation (2). Additional evidence that the role of NF-AT may be limited is that stimulation with phorbol 12-myristate 13-acetate/ionomycin is known to up-regulate NF-AT activity, but such stimulation does not induce attractin expression. To date, only stimuli delivered through the TcR/CD3 pathway consistently up-regulate attractin expression. T cells from mice defective in Ets-1 (two potential binding sites in the putative promoter region) have restricted ability to proliferate in

response to multiple stimuli and undergo spontaneous apoptosis *in vitro* (13). Other potential transcription factor binding sites include two for the bifunctional MZF-1 transcription factor that represses transcription in nonhematopoietic cells and activates transcription in hematopoietic cells (14).

The possibility exists that the soluble and membrane attractin mRNA isoforms may be subject to differential posttranscriptional regulation. Within the 3′ UTR of membrane attractin, there are 10 copies of the AUUUA motif associated with binding of cytosolic proteins that target mRNA for degradation. In contrast, none are present in the short 3′ UTR of soluble attractin mRNA (30, 31). More than seven copies of an AUUUA sequence introduced into the 3′ UTR of the rabbit β-globin gene increased decay of mRNA from none at 4 h to a $t_{1/2}$ of 2 h (32). If preferential degradation of membrane attractin mRNA occurs, this may contribute to the inability of activated T cells to maintain expression of cell surface attractin for longer than 2–3 days after the initial stimulation.

It will be important to develop reagents that can distinguish soluble and membrane attractin protein and correlate protein levels at various stages of T cell activation with relative mRNA levels and their control at the transcriptional and degradation levels. Of particular importance will be to determine whether the soluble form of attractin acts as a negative regulator of the membrane form through competitive inhibition. Attractin has a DPPIV activity with specificity similar to that of CD26 that has been shown to cleave and thereby regulate the activities of a number of cytokines involved in immune and endocrine responses (4). In line with this, interaction of attractin with locally acting peptide hormones has been proposed as a common mechanism of action in the immune system and control of pigmentation and energy metabolism (33). This leads to either the more general proposal that attractin is not tissue specific, but that it is a facilitator that exerts specific functional activities dependent on the cytokines and responding cells in its environment or that there is an as yet undiscovered more specific extracellular ligand. Immune cells then may use the differential expression of soluble and membrane attractin to facilitate the change from a recirculating surveillance mode to then allow rapid localization to sites of inflammation.

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