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Gene structure, chromosomal location, and basis for alternative mRNA splicing of the human *VCAM1* gene

(vascular cell adhesion molecule 1/endothelial cell)

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ABSTRACT **Vascular cell adhesion molecule 1 (VCAM-1) is a cell surface glycoprotein adhesive for certain blood leukocytes and tumor cells, which is expressed by activated endothelium in a variety of pathologic conditions including atherosclerosis. Genomic clones encoding the *VCAM1* gene were isolated and the organization of the gene was determined. The gene, which is present in a single copy in the human genome, contains 9 exons spanning ≈25 kilobases of DNA. Exons 2–8 contain C2 or H-type immunoglobulin domains. At least two different VCAM-1 precursors can be generated from the human gene as a result of alternative mRNA splicing events, which include or exclude exon 5. A consensus TATAA element is located upstream of the transcriptional start site. The *VCAM1* promoter contains consensus binding sites for NF-κB, the GATA family of transcription factors, as well as an AP1 site. The *VCAM1* gene was assigned to the 1p31–32 region of chromosome 1 based on the analysis of human–mouse hybrid cell lines and *in situ* hybridization. Structural analysis of the human *VCAM1* gene provides the basis for alternative mRNA splicing and an initial approach to elucidating the regulation of VCAM-1 expression.**

A prominent feature of endothelial activation by cytokines or endotoxin is the alteration of their surface adhesive properties by induction of leukocyte adhesion molecule expression (reviewed in ref. 1). These molecules contribute to the hyperadhesive surface changes observed in activated cultured endothelium and have been identified *in vivo* in various pathologic conditions (reviewed in ref. 1). Vascular cell adhesion molecule 1 (VCAM-1) was identified in activated human umbilical vein endothelial cells by monoclonal antibody E1/6 (2) and by expression cloning (3). This inducible endothelial cell surface molecule has been shown *in vitro* to mediate intercellular adhesion via interaction with a counterreceptor, the integrin VLA4, which is expressed on monocytes, lymphocytes, basophils, eosinophils, and certain tumor cells, but not neutrophils (4–7). Expression of VCAM-1 is inducible on vascular endothelium in pathologic conditions; however, it is constitutively expressed on some nonvascular cells including dendritic cells in lymphoid tissues and skin, some monocyte-derived cells, and epithelial cells (7, 8). In inflammatory processes and cardiac allografts undergoing rejection, VCAM-1 is upregulated on endothelium of postcapillary venules (9). Arterial expression of VCAM-1 is found in experimental models of atherosclerosis in the rabbit. The rabbit homologue of VCAM-1 is induced locally on arterial endothelium overlying early foam cell lesions in dietary and heritable hypercholesterolemia (10).

VCAM-1 is a transmembrane protein and member of the immunoglobulin gene superfamily. Initially, VCAM-1 cloned from activated cultured human umbilical vein endothelial cells was reported to contain six extracellular C2 or H-type immunoglobulin-like domains (3). However, cDNAs were isolated from cytokine-activated endothelium that contains an additional immunoglobulin domain, designated AS-1 or domain 4 (the remaining domains were designated 5–7) (11, 12). This additional immunoglobulin domain is most homologous to domain 1, the existing N-terminal domain. Both transcript forms of VCAM-1 were detected in interleukin 1β (IL-1β)-stimulated human umbilical vein endothelial cells, although the seven-domain form appeared predominant (11, 12). To further define the nature of VCAM-1 mRNA processing, and as an initial approach to examining the mechanisms regulating *VCAM1* gene expression, we have cloned and characterized the human gene encoding VCAM-1.§

MATERIALS AND METHODS

Cell Culture, Cytokine Treatment, and RNA Isolation. Growth of passaged human umbilical vein and rabbit venous endothelial cells have been described (11, 13). Human endothelial cells were activated by treatment with recombinant human IL-1β (10 units/ml) (Biogen) for 6 hr. Poly(A)$^+$ mRNA was prepared as described (14).

Isolation of Genomic Clones. A library of human peripheral blood DNA partially digested with *Sau*3A was constructed in the vector EMBL3 (15). The library was screened with a partial rabbit VCAM-1 cDNA (M.I.C., unpublished data), as well as with 5′ and 3′ end partial human VCAM-1 cDNA probes generated by PCR (9); probes were labeled by random priming (16).

Primer Extension. Oligonucleotides AAAGTATATTTGAGGCTC and CACGACCATCTTCCCAGG complementary to the 5′ end of VCAM-1 mRNA were labeled with [γ-^{32}P]ATP and polynucleotide kinase. Primers were hybridized to 5 μg of poly(A)$^+$ RNA from human umbilical vein endothelial cells treated with IL-1β for 6 hr. Primer extension was performed as described (14, 15).

***In Situ* Hybridization.** A partial cDNA clone containing the extracellular domains of VCAM-1 (11) was biotinylated and used as a probe to localize the *VCAM1* gene by fluorescence *in situ* hybridization directly on banded metaphase chromosome preparations, as described (17).

RESULTS AND DISCUSSION

Structure of the *VCAM1* Gene. The restriction map of a 25-kilobase (kb) region containing the *VCAM1* gene was constructed by analysis of overlapping bacteriophage clones isolated from a human genomic library (Fig. 1). Restriction

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Abbreviations: IL-1, interleukin 1; VCAM-1, vascular cell adhesion molecule 1.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M73255).

maps were refined by Southern blotting with cDNA probes or specific oligonucleotides. The sizes of subcloned fragments correspond to the sizes of the hybridizing bands observed on Southern blots of digested human DNA probed with a VCAM-1 cDNA clone (data not shown); this pattern is consistent with a single copy of the gene being present in the human (haploid) genome. All of the exons, intron–exon boundaries, and portions of the introns were sequenced on both DNA strands (Fig. 2). In areas where the sequences overlap, there were no nucleotide differences between the previously published human cDNA sequences (3, 11, 12) and the genomic sequence. This suggests that polymorphism of the *VCAM1* gene is not extensive.

The *VCAM1* gene structure implies a correlation between exon/intron architecture and protein structure. Exon 1 contains the signal peptide. Each of the extracellular immunoglobulin-like domains is contained in a separate exon. In this respect, the *VCAM1* gene is similar to certain other members of the immunoglobulin gene superfamily (18). Unlike many of these genes, the transmembrane region, cytoplasmic domain, and 3′ untranslated region are contained in a single exon. The structure of the *VCAM1* gene is consistent with the proposal that exon duplication played a role in the formation of the gene. In VCAM-1, the immunoglobulin-like domains share extensive internal homology. Domains 1, 2, and 3 are highly homologous to domains 4, 5, and 6. In addition, the sizes of the introns in this region are similar. This internal homology suggests that the region of the *VCAM1* gene represented by domains 1, 2, and 3 may have been duplicated during evolution to generate domains 4, 5, and 6.

All the splice acceptor and donor sequences agree with the "GT-AG" rule (19) and conform to the consensus proposed by Mount (20). In the human open reading frame defined by existing cDNA clones, no splice junctions occur between amino acid codons (type 0), 100% occur after the first nucleotide (type 1), and none occur after the second nucleotide of a codon (type 2) (21). This can be compared to the values of 41% type 0, 36% type 1, and 23% type 2 previously reported for vertebrate genes (22). The striking conservation of phase 1 introns in the *VCAM1* gene suggests that exons could be spliced in or out of the mRNA without disturbing the reading frame.

Analysis of VCAM-1 cDNA clones has revealed several cDNA forms that differed from the originally described

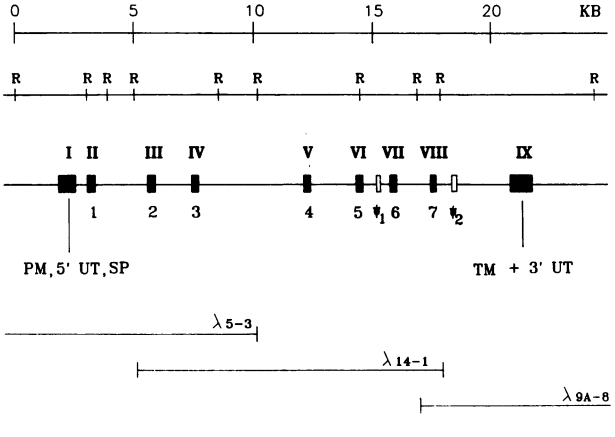

FIG. 1. Architecture of the human *VCAM1* gene. Positions of the exons are indicated by solid boxes. The scale at the top is in kb. The restriction map was derived from three overlapping bacteriophage clones (bottom), and *Eco*RI sites (R) are indicated. Roman numerals designate exons. PM, promoter; 5′ UT, 5′ untranslated region; SP, signal peptide; TM, transmembrane region; 3′ UT, 3′ untranslated region. Arabic numerals designate immunoglobulin-like domains. ψ_1 and ψ_2 designate regions in the human gene that are homologous to regions in rabbit VCAM-1 cDNAs.

VCAM-1 structure. First, human umbilical vein endothelial cells (11, 12), as well as rabbit endothelial cells (M.I.C., unpublished data), express a transcript containing a C2 or H-type immunoglobulin domain located between domains 3 and 5. This 276-base-pair (bp) domain is present in the human *VCAM1* gene (Figs. 1 and 2) and demonstrates that these forms of VCAM-1 are derived by alternative mRNA splicing.

Additional VCAM-1 transcripts have been identified, revealing new elements of the *VCAM1* gene. In a single rabbit cDNA clone, another domain is located between the fifth and sixth immunoglobulin domains. In addition, in ≈50% of the identified rabbit VCAM-1 transcripts obtained from cultured endotoxin-activated (3 hr) aortic endothelial cells, an additional domain is located between the seventh immunoglobulin domain and the transmembrane region (M.I.C., unpublished data). Regions were identified in the human *VCAM1* gene that are homologous to these domains expressed in the rabbit. Thus far we have been unable to detect expression of VCAM-1 transcripts containing these regions of the *VCAM1* gene in IL-1β-treated (6 hr) human umbilical vein RNA using the PCR (data not shown), and as such these domains have been designated ψ1 and ψ2 (Figs. 1 and 2). It is possible that other pathophysiologic stimuli, or expression of the *VCAM1* gene in other cell types, may be associated with splicing patterns that include ψ1 and ψ2. It is also possible that the ψ1 and ψ2 domains have diverged in the human and rabbit *VCAM1* genes and thus are not expressed in humans. However, the percentage identity between the established human and rabbit immunoglobulin-like domains is relatively high (80–90%) and is not consistent with a rapid gene divergence,

Table 1. Segregation of *VCAM1* with human chromosomes in *Bam*HI-digested human–mouse cell hybrid DNA

	Concordant hybrids, no.		Discordant hybrids, no.		
Chromosome	(+/+)	(−/−)	(+/−)	(−/+)	% discordancy
1	12	30	0	0	0
2	8	20	5	10	35
3	12	19	2	10	28
4	9	18	4	12	37
5	10	17	4	13	39
6	10	25	4	5	20
7	10	17	3	12	36
8	12	17	2	13	34
9	3	24	10	4	34
10	12	15	2	14	37
11	6	16	5	14	46
12	14	17	0	13	30
13	9	19	5	11	36
14	11	14	3	16	43
15	9	21	4	9	30
16	9	24	5	6	25
17	10	8	3	22	58
18	10	16	4	14	41
19	8	24	6	6	27
20	9	14	5	16	48
21	9	6	5	24	66
22	7	21	7	8	35
X	7	13	3	15	47

Data are compiled from Southern blots probed with VCAM-1 cDNA. DNA obtained from 44 human–mouse somatic hybrid cell lines involving 20 unrelated human cell lines and 4 mouse cell lines (29) was digested with *Bam*HI. The hybrids were characterized by karyotypic analysis and by mapped enzyme markers (30). Scoring was determined by the presence (+) or absence (−) of human bands in the hybrids on the blots and was compared to the presence or absence of human chromosomes in each hybrid. A 0% discordancy indicates a matched segregation of the DNA probe with a chromosome. The human *VCAM1* gene mapped to human chromosome 1.

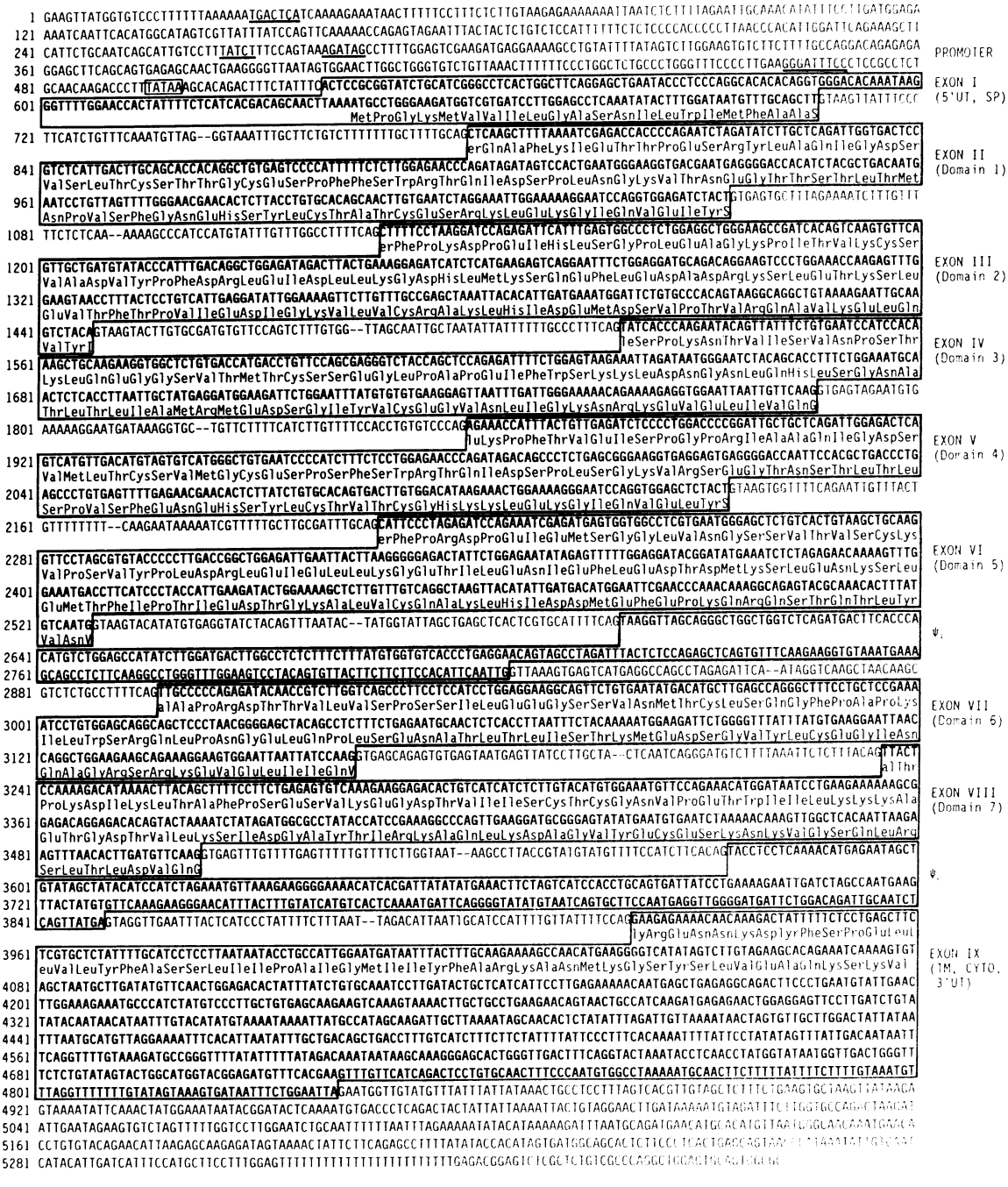

FIG. 2. Nucleotide sequence of the *VCAM1* gene. The sequences of VCAM-1 exons are enclosed in large boxes. The TATAA sequence is enclosed in a small box; the sequences corresponding to putative NF-κB, AP1, and GATA binding sites are underlined. Abbreviations are defined in Fig. 1.

as is seen in some other members of the immunoglobulin supergene family (18).

The 5′ and 3′ Flanking Regions. The transcription initiation site(s) of the *VCAM1* gene was defined by primer-extension analysis. The analysis was performed by using two oligonucleotide primers and IL-1-treated human umbilical vein endothelial cell poly(A)$^+$ mRNA. Marker lanes display dideoxynucleotide DNA sequencing reactions primed from the same site on a plasmid subclone of this genomic region (Fig. 3). Primer-extension analysis reveals a major transcriptional start site corresponding to an A residue 20 bp downstream of a consensus TATAA sequence. The nucleotide pair at this site in the *VCAM1* gene (A preceded by C) is the sequence most commonly found at eukaryotic transcriptional start sites (17). This result is consistent with the TATAA motif being a functional element of the *VCAM1* promoter. Located at −57 is the sequence GGGATTTCCC, which is in complete agreement with the consensus sequence [GGGR(C/A/T)TYYCC] for an NF-κB site (reviewed in ref. 23). Located at −73 is the sequence TGGGTTTCCCC, which may be a second NF-κB site. Since cytokines activate the transcription factor NF-κB, these putative promoter elements may confer IL-1 responsiveness on the *VCAM1* gene. A consensus AP1 binding site, TGACTCA (24), is located at −490. Sequences recognized by the GATA box family of zinc finger transcription factors (25), AGATAG and TATC, are located at −239 and −253. Interestingly, a member of this family is expressed by endothelial cells and is required for the restricted pattern of endothelin 1 gene expression (26). These putative promoter elements may increase the efficiency of VCAM-1 expression in cytokine-

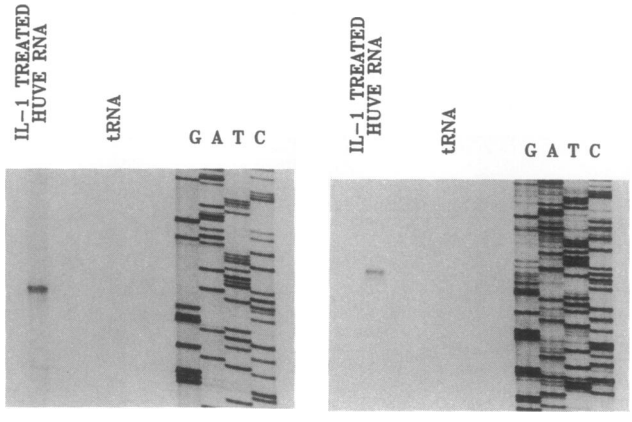

FIG. 3. Mapping of the *VCAM1* transcriptional start site. The transcriptional start site was determined by primer-extension analysis. Two oligonucleotides corresponding to the 5′ end of the VCAM-1 cDNA were labeled and hybridized to IL-1β-treated (6 hr) human umbilical vein endothelial cells (HUVE) poly(A)$^+$ RNA, or tRNA. The products of annealing served as templates for reverse transcriptase. The extension products from the first oligonucleotide (*Left*) or a second primer (*Right*) were run on a denaturing polyacrylamide gel alongside a Sanger sequence primed on a plasmid DNA template using the same primer as that used in the reverse transcription reaction.

activated endothelial cells. A consensus sterol regulatory element [CACC(C/G)(C/T)AT] (27) was not seen in the *VCAM1* promoter.

The 3′ end of the *VCAM1* gene does not contain a consensus polyadenylylation signal. A recently described VCAM-1 cDNA clone contains a poly(A) sequence that is 18 bp downstream of the hexamer AGTAAA. This sequence was shown to be a functional processing signal in other genes (28). Two findings support the proposal that this site is the authentic polyadenylylation signal. First, examination of the 3′ end of the *VCAM1* gene from the area of the 3′ ends of the described cDNA clones (3, 12), downstream 760 bp, failed to yield a consensus AATAAA polyadenylylation signal (Fig. 2; data not shown). Second, the position of the polyadenylylation site is consistent with the observed size of the VCAM-1 transcript. The predicted size of the VCAM-1 transcript derived from the length of the 5′ untranslated region (124 bp), the exon 4-containing open reading frame (2217 bp), as well as the size of the 3′ untranslated region obtained from the existing cDNAs (734 bp), and poly(A) tail (≈100 bp), is 3175 bp. This is consistent with the reported size of the transcript (3.2 kb) derived from the *VCAM1* gene (3).

Chromosomal Localization of the *VCAM1* Gene. DNA isolated from 44 human–mouse somatic hybrid cell lines and parental controls was examined for the presence or absence of the human *VCAM1* gene by Southern blotting techniques. On *Bam*HI-digested DNA, the VCAM-1 cDNA produced readily distinguishable patterns of restriction fragments of 23.1 and 4.8 kb for human and a 12.2-kb band for mouse (data not shown). The presence or absence of the human *VCAM1* gene sequences was tabulated against the presence or absence of human chromosomes in each hybrid (*VCAM1*/chromosome) for concordancy (+/+, −/−), and discordancy (−/+, +/−). A 0% discordancy indicates a matched segregation of the *VCAM1* gene with a chromosome and is the basis for chromosomal assignment (Table 1). *VCAM1* mapped to human chromosome 1 by analysis of the somatic cell hybrids. Two translocations, 1qter–1p34::17p13–17pter in hybrid 20L-37, and 2pter–2q37::1p21–1pter in hybrid JWR-22H, both scored positive and would localize the *VCAM1* gene to the p21–p34 region of chromosome 1.

To map the *VCAM1* gene more precisely, *in situ* hybridization to metaphase chromosomes was performed. The results of analysis of 60 metaphase spreads are shown in Fig. 4. Of the 103 fluorescent signals recorded, 57 (55.3%) were

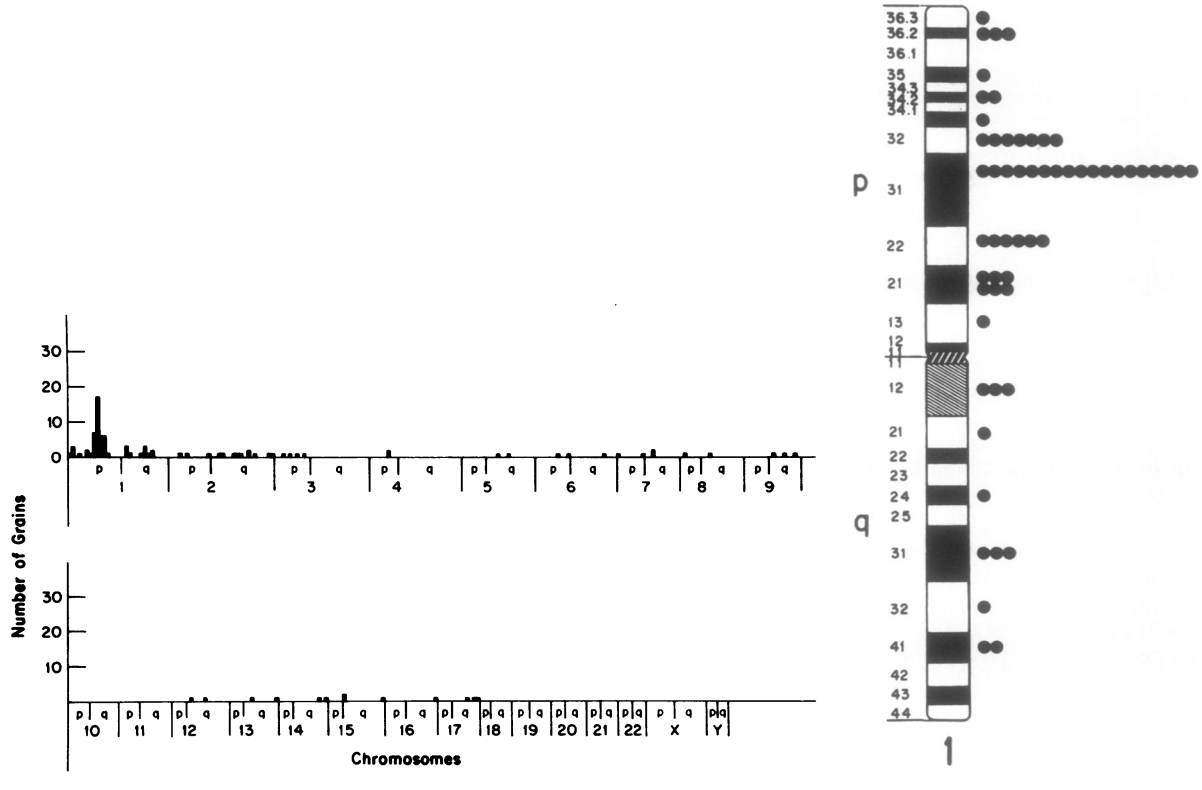

FIG. 4. *In situ* hybridization of a partial VCAM-1 cDNA to metaphase chromosomes. The histogram (*Left*) displays data for all grains counted in 60 metaphases. The ideogram of chromosome 1 (*Right*) shows the distribution of grains on that chromosome and the assignment of the *VCAM1* gene to 1p31–32.

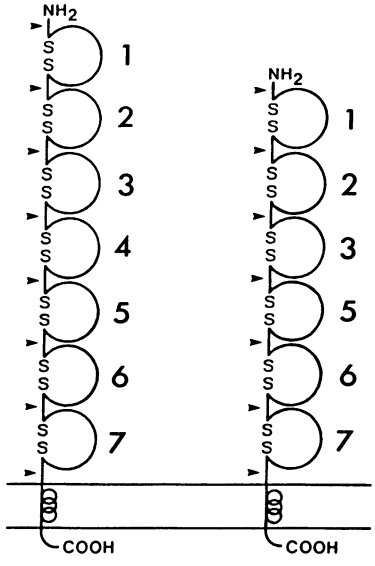

FIG. 5. Schematic illustration of the forms of VCAM-1 generated by alternative mRNA splicing. The positions of the exon/intron boundaries are designated by arrowheads. Loops represent the disulfide-linked immunoglobulin-like domains.

associated with chromosome 1 and 25 (24.3%) were located on or beside bands 1p31–32. Of the 60 metaphases, 25 (41.7%) had a signal at 1p31–32. We note that two structurally homologous members of the immunoglobulin supergene family, lymphocyte function-associated antigen (LFA-3) and CD2 are also found on the short arm of chromosome 1 (31, 32). Interestingly, a locus at 1p32, designated T-cell lymphoma 5 (TCL5), may specify a DNA binding protein with a helix–loop–helix domain (33, 34). The locus is adjacent to and rearranged by the breakpoints of several translocations t(1;14) associated with T-cell lymphoblastic leukemia. In addition, the *LCK* gene, a lymphocyte-specific tyrosine kinase, is located at 1p32–35 in a site of frequent structural abnormalities in human lymphomas (35). Human chromosome band 1p32 alterations have also been described in human cutaneous malignant melanomas (36) and human neuroblastomas (37).

VCAM-1, a cell adhesion molecule, expressed by endothelium and other cell types may play an important role in the pathogenesis of inflammatory and immune processes. Furthermore, its localized expression by arterial endothelium overlying early atherosclerotic lesions in dietary and heritable hypercholesterolemic rabbits (10), suggests a potential role in monocyte recruitment and the initiation/progression of atherosclerotic plaques. Since multiple transcripts can be derived from the *VCAM1* gene, it is possible that tissue-specific alternative splicing of *VCAM1* could generate functionally distinct cell surface molecules (Fig. 5) that may be associated with specific pathophysiologic conditions. In addition, further characterization of the structural elements of the *VCAM1* promoter may provide important insights into the induced expression of this adhesion molecule and by inference into the early events in atherogenesis.

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