Full length vascular cell adhesion molecule 1 (VCAM-1)

Tom Polte, Walter Newman and T.Venkat Gopal*
Otsuka America Pharmaceutical Inc., Maryland Research Laboratories, 9900 Medical Center Drive, Rockville, MD 20850, USA

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We have developed several monoclonal antibodies that bind specifically to IL-1 activated but not to quiescent human unbilical vein endothelial cells (HUVEC). Two antibodies, 1E7 and 2G7, were shown to react at distinct epitopes with a novel IL-1 inducible sialoglycoprotein which is implicated in the adhesion of T cells with activated endothelium (1). We used polymerase chain reaction (PCR, 2, 3) to clone VCAM-1 cDNA in order to test the relationship of VCAM-1 to 1E7/2G7 protein.

The coding region of VCAM-1 cDNA was cloned from poly A^+ RNA isolated from IL-1 activated HUVEC by PCR using the two following primers encompassing the start and stop codons respectively. Primer 1: 5'-GGGGGGCGGCCGCGCAACTT-AAAATGCCTGGGAAGATG-3' Primer 2: 5'-GGGGGCTC-GAGCATTAGCTACACTTTTGATTTCTGTG-3' The primers were designed to contain NotI and XhoI sites at the 5' and 3'ends of the cDNA, respectively. The PCR product was approximately 2.3 Kb instead of the expected 1.96 Kb band based on the published sequence (4). The 2.3 Kb band was purified and cloned into an expression vector. Four clones were sequenced completely using the primers given in Table 1. All of them contained an insert of 276 nucleotides after the nucleotide position 1034 of the published sequence (4). In addition four more clones were sequenced at the insert region. Seven out of eight clones sequenced contained identical insert sequence and one clone contained the following changes: C for T, C for A, and a deletion of C at nucleotide positions 1193, 1196, and 1198 respectively of the sequence given in Figure 1. The sequence for the 276 nucleotide insert (overlined in Figure 1) was further confirmed by direct sequencing of the PCR amplified product and found to contain the same sequence as shown in Figure 1. The four clones sequenced in entirety contained other random errors at positions corresponding to the sequence given in Figure 1. Cl #1 — G for A at positions 102 and 270 respectively; Cl #2 — C for T at positions 367, 772 and 1116 respectively; Cl #3 — T for C, G for A, C for T, and G for A at positions 953, 1150, 1403, and 1436 respectively; Cl #4 — A for G, deletion of A, and A for G at positions 305, 1711, and 1971 respectively. We belive that these differences could have arisen due to errors from PCR amplification. We have noted such random differences in the ELAM-1 cDNA cloned by PCR amplification method (5).

Table 1.

Sequencing Primers	Position on Coding Sequence (Fig. 1)
1. 5'-GCCTCGGCCTCTGAGCTATT-3'	5' Flanking Region in Vector
2. 5'-GGGGGGCGGCCGCGCAAC-TTAAAATGCCTGGGAAGATG-3'	nuc. −23−15
3. 5'-TCACAGTCAAGTGTTCAGTT-3'	nuc. 398−417
4. 5'-TCTACAGCACCTTTCTGGAA-3'	nuc. 788−807
5. 5'-GCATGTCATATTCACAGAA-CTGCCTTCCTCC-3'	nuc. 1602−1572
6. 5'-ATGGAATTCGAACCCAAACA-3'	nuc. 1474−1493
7. 5'-GTGGAAATGTTCCAGAAACA-3'	nuc. 1874−1893
8. 5'-GATCTCTAGGGAATGCTT-GAACAATTAATT-3'	nuc. 1219−1205 and 928−914
9. 5'-TGAGTCTCCAATCTGAGCAG-CAATCCGGGGTCCAGGGGAG-3'	nuc. 990−951

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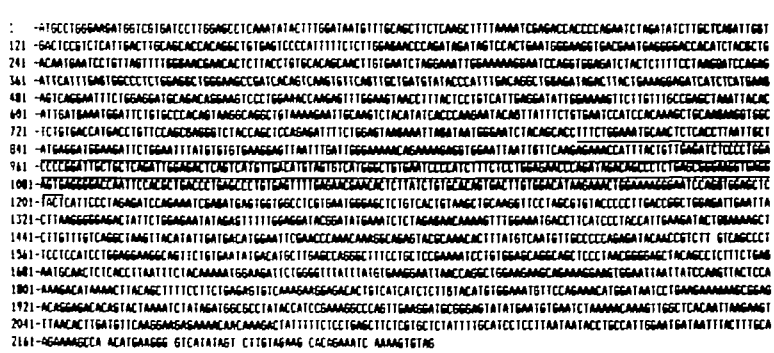

* To whom correspondence should be addressed