Human Intercellular Adhesion Molecule-1 Gene and Its Expression in the Skin

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Cell adhesion molecules are cell-surface proteins that allow specific cell-cell interactions among leukocytes, as well as between leukocytes and other cells. Recent studies have shown that the differential expression of selected cell-adhesion molecules plays a critical role in cutaneous inflammation, immunologic responses, and wound repair. Intercellular adhesion molecule-1 (ICAM-1) is a cell-adhesion molecule that is constitutively expressed on human dermal microvascular endothelial cells (HDMEC) and is inducible on human keratinocytes (HK). Its regulated expression is vital to the initiation and evolution of localized inflammatory processes in the skin. ICAM-1 serves as a specific ligand for lymphocyte function--associated antigen-1 (LFA-1), a cell-surface protein expressed on all leukocytes. The regulated expression of ICAM-1 allows leukocytes to bind to endothelial cells at sites of inflammation and, after exiting into the tissue, to interact with specific target cells, such as HK. Furthermore, specific cytokines are capable of differentially regulating ICAM-1 expression on HDMEC, HK, and other cells. The biologic relevance of ICAM-1 expression in cutaneous inflammation is further supported by functional studies demonstrating the critical role of ICAM-1/LFA-1 interactions in mediating the binding of peripheral blood leukocytes to HDMEC and to HK—cells known to be participants and targets in specific cutaneous immunologic responses. Thus, the delineation of precise molecular mechanisms that regulate the tissue-specific and cytokine-specific expression if ICAM-1 is important to both our understanding of the biology of localized inflammation and to the development of directed anti-inflammatory therapeutic strategies. Current evidence indicates that ICAM-1 expression is regulated at the level of gene transcription. Recently our laboratory has isolated and characterized a human genomic clone that contains the 5' regulatory region of the ICAM-1 gene. In the current studies, we further describe the genomic ICAM-1 clones isolated to date and demonstrate the presence of consensus regulatory elements located within the 5' flanking region of the ICAM-1 gene that are potentially involved in regulating ICAM-1 gene transcription. J Invest Dermatol 98:618–63S, 1992

Over the past several years, numerous constitutive and inducible leukocyte adhesion molecule complexes have been described. The formation of these intercellular complexes is required for the non-antigen-dependent cell-cell interactions necessary among various types of leukocytes involved in the immune response, and for conjugate formation between leukocytes and endothelial cells and between leukocytes and target cells [1–3]. Interaction of these cell-adhesion molecules on leukocytes and endothelial cells is also pivotal in the homing of leukocytes to preferential tissue sites. Among these adhesion complexes, the most extensively characterized is that formed between lymphocyte function-associated antigen-1 (LFA-1) on leukocytes and intercellular adhesion molecule-1 (ICAM-1) expressed on both leukocytes and numerous other cell types [3,4]. LFA-1 is expressed by a variety of hematopoietic cells and its presence and interaction with ICAM-1 is critical to many adherence-dependent T-cell, B-cell, and myeloid cell functions [1,3,5–9]. ICAM-1 is constitutively expressed on the surface of numerous tissues and cell types, including leukocytes, fibroblasts, and large- as well as small-vessel endothelial cells [10–13]. The level of expression of ICAM-1 may be significantly and rapidly modulated by numerous diverse signals, including specific cytokines (IFN-γ, IL-1α, and TNF-α), bacterial products (lipopolysaccharide [LPS]), pharmacologic agents (hydrocortisone and phorbol myristate acetate [PMA]), and physical agents (UVIR) [10,14–19]. Cells and tissues that do not normally express ICAM-1 may be induced to express this surface glycoprotein by exposure to some of these agents. Indeed, numerous in vivo inflammatory processes have been shown to be accompanied by de novo expression of ICAM-1 by non-leukocyte tissues [20,21]. Moreover, in certain animal models of acute immunologic and allergic processes, anti-
body blockade of ICAM-1 surface protein or of the interaction between LFA-1 and ICAM-1 has attenuated those inflammatory processes [22,23]. In addition to its role in the proper function of lymphocytes with respect to cell–cell adherence, the ICAM-1 cell-surface protein has also been demonstrated to be in close proximity and to physically interact with the high-affinity IL-2 receptor on the surface of T cells. Thus the regulated co-expression of ICAM-1 with this critical T-cell activating receptor has been implicated in facilitating, augmenting, and focusing IL-2–mediated stimulation of T cells [24].

In addition to its physiologic role of contributing to cell-cell adhesion in immune response biology, the ICAM-1 protein has also been subverted by several pathologic processes. Most notably, ICAM-1 expressed on mucosal epithelial cells functions as the receptor for the major group of rhinoviruses [25–27] and thus plays a critical role in the pathogenesis of the common cold. Indeed, a soluble form of the ICAM-1 protein has specifically inhibited rhinovirus infection of cells expressing this receptor in vitro [28], but attempts to inhibit infectivity by selectively inhibiting ICAM-1 gene expression have not been explored. ICAM-1 has also been shown to serve as an antigen by which Plasmodium falciparum infected red blood cells attach to endothelial cells and thus escape removal from the blood stream by the reticuloendothelial system [29]. Alternatively, ICAM-1 expression is also thought to correlate positively with the metastatic potential of malignant melanoma [30]. Thus, not only is the ICAM-1 gene highly responsive to various inflammatory mediators, its expression also appears to be linked to the initiation and possible perpetuation of numerous inflammatory, infectious, and even neoplastic processes.

Immunohistochemical studies of human skin have shown that HDMEC and Langerhans cells (LC) constitutively express low levels of ICAM-1, but that HK do not [31–34]. However, in established inflammatory dermatoses [31–34], both de novo expression of ICAM-1 by HK and increased expression of ICAM-1 by HDMEC are closely linked to the appearance of infiltrating T cells. In experimental delayed-hypersensitivity reactions [35], and in normal skin injected with IFN-γ [36], a similar pattern of induced expression of ICAM-1 by HK and HDMEC is observed. Previous research efforts in our laboratory and by others [37–43] have analyzed the induction and function of ICAM-1 expression in both HK and HDMEC in vitro. In brief, whereas HK do not constitutively express ICAM-1 surface protein or mRNA, HDMEC do. Whereas IL-1α, IFN-γ, and TNF-α all increase HDMEC ICAM-1 expression, among these cytokines only IFN-γ and TNF-α are inducers of de novo ICAM-1 expression in HK (Fig 1), even though HK express functional IL-1 receptors. The combination of both IFN-γ and TNF-α appears to be synergistic in inducing ICAM-1 expression by HK [43]. Finally, in HDMEC or HK induced to express ICAM-1, T cells adhere to these cells specifically via the LFA-1/ICAM-1 conjugate formation [37–40]. With regard to the effects of UVR upon ICAM-1 expression, only HK, keratinocyte-like cell lines, and peripheral blood monocytes have been examined [17–19]. In peripheral blood monocytes, UVB exposure downregulates ICAM-1 expression. However, in cytokine-stimulated HK and keratinocyte-like cell lines, UVR exposure results in a bimodal response in ICAM-1 expression and IFN-γ inducibility, with initially an inhibition of ICAM-1 inducibility and then, in a time-dependent fashion, an enhancement of surface ICAM-1 levels after treatment with IFN-γ. More recently, studies concerning the expression and function of ICAM-1 and LFA-1 by epidermal LC have defined a clear role for these adhesion molecules in their in vitro function as either antigen-presenting or accessory cells [44,45]. Furthermore, it appears that UVB-induced inhibition of LC and peripheral blood monocyte function may be due, in part, to inhibition of ICAM-1 expression [17,44]. Clearly, there are distinct differences in the molecular mechanisms governing the expression of ICAM-1 in various cell types that merit further investigation and comparative studies. Because all current evidence indicates that ICAM-1 is differentially regulated at the level of gene transcription, elucidation of the molecular mechanisms regulating ICAM-1 gene expression in HDMEC, HK, and other cells is dependent upon the isolation, characterization, and systematic analysis of the transcriptional regulatory regions of this gene.

In order to elucidate the molecular mechanisms involved in the transcriptional regulation of ICAM-1, we have cloned and characterized both 5′ and internal portions of the human ICAM-1 gene [46]. Commercially prepared human lymphocyte and human lung fibroblast genomic libraries (Stratagene, La Jolla, CA) were screened with various portions of the characterized ICAM-1 cDNA

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Schematic representation of the possible role of induced ICAM-1 expression by keratinocytes in facilitating T-cell–mediated targeted immunologic reactions. On the left, a keratinocyte, which does not constitutively express ICAM-1, bearing antigen (Ag) associated with a major histocompatibility complex (MHC) product, and a T cell with its antigen-specific T-cell receptor (TCR) and LFA-1, an antigen-nonspecific receptor expressed by all leukocytes, which binds to ICAM-1. On the right, expression of keratinocytes to IFN-γ and/or TNF-α results in the de novo expression of ICAM-1 on the surface of keratinocytes, and the expression of ICAM-1 by these cells facilitates the binding of T cells to keratinocytes bearing targeted antigens.

![Figure 2](https://via.placeholder.com/150)

**Figure 2.** Schematic of the two ICAM-1 genomic clones, λ6 and λ8, and relationship of genomic exons to specific regions of the ICAM-1 cDNA. The 17.3 kb λ6 is shown above and the 16 kbλ8 below. Intronic and flanking regions are depicted by black horizontal lines; relative locations of EcoRI sites by R and vertical black lines, and exons by variably filled broad vertical bars. The EcoRI fragment containing the first exon and utilized to generate the subclone pG6-2.05 is indicated by the bold horizontal bar labeled λ6 2.05. The 3023 bp ICAM-1 cDNA [48] is depicted in the center (not drawn proportional to genomic clones), with the protein coding region shown as a broad horizontal bar bordered by the protein translation start codon (ATG) and the translation stop codon (TGA), and the 5′ and 3′ UTR shown as narrow horizontal bars. Regions of the ICAM-1 cDNA that correspond to specific exons are denoted by appropriately filled areas and double-headed arrows. The three exonal regions of λ6 combine for the first 5′ 633 bp of the cDNA, and the one exonal region of λ8 contains 274 bp distributed over the 3′ coding region and first part of the 3′ UTR of the cDNA. Genomic clones with exons corresponding to the intervening 979 bp of protein coding region and 1140 bp of 3′ UTR have not yet been isolated and characterized.
The genomic libraries were prepared by partial Sau IIIa digestion of either lymphocyte or lung fibroblast DNA and subsequent cloning into the Bam HI sites of the substitution vector bacteriophage aDASH or aDASH II (Stratagene). Various restriction enzyme fragments of the ICAM-1 cDNA were gel isolated and 32P-labeled by random primer extension to a specific activity of 10^8–10^9 cpm/μg and used to screen duplicate filters of 9×10^6 recombinant phage plaques, giving a 99% probability for the presence of one 15-kb insert with relevant genomic material. Hybridizing clones were plaque purified in subsequent screening rounds. Single-clone phage DNA was then prepared from lysates using phenol–chloroform extraction, SDS/EDTA phage lysis, and DNA extraction/precipitation [49,50]. The purified genomic DNA clones were then used for subsequent analytical and cloning procedures.

Using a 315 bp 5′ portion of the human ICAM-1 cDNA to screen the libraries, we isolated and subsequently characterized a genomic clone, 66, corresponding to the 5′ portion of the human ICAM-1 gene [46]. The 17.3-kb clone 66 contains portions of the first three exons covering the first 533 bases of the coding region of the previously identified ICAM-1 cDNA (Fig 2). Restriction endonuclease sites within this lambda phage clone have been mapped, plasmid subclones have been produced, and subclones containing exons, 5′ flanking, and first intron material isolated, amplified, and sequenced. Whereas six phage clones were initially isolated from the libraries using this 5′ probe, all subsequently proved to be identical by size, restriction map, and Southern blotting criteria. Using a non-overlapping, more 3′ ICAM-1 cDNA fragment as probe, a second genomic clone, 88, 16 kb in size and containing a single exon fragment corresponding to the ICAM-1 cDNA, was isolated and characterized (Fig 2). Clones containing the intervening 979 bp of corresponding cDNA sequence have not yet been isolated, nor has any clone containing the more distant 1140 bp of the 3′ untranslated region (UTR) of the ICAM-1 cDNA been identified. Subregions of these two different ICAM-1 phage clones, 66 and 88, were cloned into a pGem3Z (Promega, Madison, WI). Exon–containing subfragments, previously identified by Southern blotting, were sequenced using T7/Sp6 promoter primers or oligodeoxynucleotide primers derived either from the ICAM-1 cDNA or from sequencing data. Nine Eco RI subfragments of 66 and two Eco RI subfragments of 88 were identified (Fig 2). These studies revealed mRNA coding stretches of 124, 263, and 203 nucleotides for the three exon–containing fragments of 66 [46], respectively, and a single mRNA coding stretch within 68 of 274 bp. The first translation start codon (AUG) corresponding to the described ICAM-1 cDNA [47,48] and 67 nucleotides of ICAM-1 protein-coding region, as well as a consensus splice donor site at the beginning of the adjacent first intron, are contained within the first exon subclone of 66, designated pG6a-2.05 [46]. The next 263 nucleotides of coding region are contained in pG6a-5.4, the second exon–containing subclone, and are flanked at their 5′ and 3′ ends by consensus sequences for splice acceptor and donor sites, respectively, indicating that this region corresponds to a complete exon. The next 203 nucleotides of protein coding region are contained within the subclone pG6a-8.5, and are bordered, at their 5′ end, by a consensus splice acceptor site. There are no genomic sequences beyond this coding region and thus the 3′ end of this fragment has no flanking splice donor site. This exon subclone does contain, however, a 5′ terminal Sau IIIa site [46]. Similarly, the single exon containing subclone derived from 68, pG8b-4.1, which contains 274 bp of mRNA coding region, is bordered at its 5′ end by a consensus splice acceptor site, but contains a terminal Sau IIIa site rather than a consensus splice donor site at its 3′ end. The 274 bp of exon in pG8b-4.1 contain the final 72 bp of the ICAM-1 protein-coding region followed by a translation stop codon and an additional 205 bp of 3′ UTR (Fig 2). The 3′ ends of both the pG6a-0.85 and the pG8a-4.1 subclones correspond precisely to the 3′ ends of the entire 66 and 88 genomic fragments, respectively. As the genomic libraries from which 66 and 88 were cloned were prepared by partial Sau IIIa digests of human genomic DNA, it is most likely that these Sau IIIa sites, and apparently missing splice donor sites, are the products of preparative enzymatic restriction digests of the genomic DNA within the two affected exons [46]. Thus, these two phage clones contain 864 bp of mRNA coding sequences distributed over two complete and two partial exons. Although together they represent greater than 33 kb of genomic DNA, they contain only 52.3% of the ICAM-1 protein-coding region, as they do not encompass regions encoding the intervening 979 bp of corresponding cDNA sequence (Fig 2). Furthermore, they do not contain the most distal 1140 bp of the 3′ untranslated region of the ICAM-1 cDNA, nor any of the 5′ flanking region of the gene. This 3′ flanking region may ultimately be of considerable interest, as the corresponding region of the CD2 gene, which like ICAM-1 is a member of the immunoglobulin superfamily of genes, is responsible for tissue-specific expression in T cells [51]. These data indicate that the ICAM-1 gene is considerably larger than previously thought [46].

The 2.05 kb EcoRI subclone of 66 (pG6a-2.05), containing the 5′-most exon, an additional 1392 nucleotides upstream, and 586 nucleotides of the first intron, was selected for further study. The 5′ end of the ICAM-1 mRNA 5′ UTR and the corresponding transcription initiation site (TIS) in the ICAM-1 gene were identified by primer extension and S1 nuclease protection assays utilizing mRNA isolated from resting and IFN-α–treated HK and A431 cells and radiolabeled fragments isolated from the presumptive 5′ flanking regions encoded by pG6a-2.05 [46]. These data and sequencing information revealed a TATA box located 50 bp 5′ of the TIS, which is located 40 bp 5′ of the translation start site (AUG) of the protein coding region of the first exon. Additionally, there are appropriately located candidate CAAT boxes as well as two Sp1 binding sites within the first 200 nucleotides of the identified TIS [46]. These consensus regulatory elements are likely important in

Figure 3. Schematic of the genomic ICAM-1 2.05 kb Eco RI fragment contained within the subclone pG6a-2.05. Depicted are the 1350 bp of 5′ flanking sequence upstream of the transcription initiation site (hent arrow), designated nucleotide +1; the first exon with 40 bp 5′ UTR, translation start site (ATG), and ICAM-1 protein coding region (green stippled horizontal box); and the 3′ portion of the first intron. Consensus basal promoter and enhancer cis-elements as detected by computer analysis are depicted in their relative locations within the 5′ flanking region and first intron (GRE, glucocorticoid responsive element; IRE, interferon-responsive element).
providing basal promoter function to the ICAM-1 gene. Studies in our laboratory have demonstrated that this region just 5' of the TIS indeed contains a potent functional promoter, and that regions upstream of the potent basal promoter serve as a constitutive repressor of this promoter function [46]. Localization of the transcription initiation site at this point indicates that the genomic clone λ6 also contains 4.65 kb of 5' flanking sequences, and within these sequences can likely be found regions that are critical to the regulated expression of ICAM-1 in response to various signals.

Considerable progress has been made in recent years in defining the precise molecular components involved in the differential expression of eukaryotic genes [52]. These regulatory events occur constitutively in different tissues and in response to specific extracellular signals, such as cytokines. The effects of both intra- and extra-cellular signals that result in differential transcription of individual genes are ultimately mediated through activation or de novo expression of trans-acting proteins. These trans-acting proteins contain at least two essential domains. The first is a DNA binding domain that interacts with defined sequence cis-elements located within regulatory regions of the specific genes affected. The second is a transcription activation domain, which directly interacts with the promoter of the gene or the common protein components that regulate the activity of this promoter. Through both spatial and temporal interactions of diverse trans-acting proteins with the regulatory regions of a specific gene, transcription and expression of this gene is alternatively enhanced or repressed, depending upon the nature of the signals involved. With regard to cytokines, these agents must initially mediate changes through binding to receptors in the cell-surface membrane, which results in a variety of signal-transduction events. However, enhancement or suppression of transcriptional expression of individual genes by these agents ultimately is mediated through specific trans-acting proteins that bind to specific cis-elements as well.

In order to study the isolated regions of the ICAM-1 gene for the presence of such regulatory cis-elements, the sequence of pG6-2.05 was transferred to a computer database and analyzed for the consensus sequences that could serve as the DNA binding sites for specific trans-acting regulatory proteins using the sequence analysis program MacVector (IBI, New Haven, CT). Consensus sequences for a number of cis-elements were identified in both the 5' flanking and first intron regions (Fig 3), and a number of these have been provisionally associated with these tissue- and cytokine-specific regulation of other genes [52-56]. These include (a) two potential interferon-stimulated response elements, two potential glucocorticoid receptor elements, a potential NFKB site, two potential AP2 binding sites, and multiple potential AP1 binding sites [46]. The functional regulatory role and specific binding of trans-acting proteins associated with these and other potential cis-elements of the ICAM-1 gene are currently being determined. Identification of these potential regulatory elements is important, for they may well be involved in the basal transcription levels observed in HDMEC or the basal repression of transcription seen in HK. They may also be involved in the enhancement of ICAM-1 transcription induced by either IL-1α, IFN-γ, or TNF-α. Therefore, their relative locations will be considered in the design and construction of specific reporter gene plasmids and DNA-protein binding assays, which are currently being studied. However, it is entirely possible that through these consensus sequences are present within the 5' flanking region of the ICAM-1 gene, they are not biologically important in the regulation of ICAM-1 transcription, and that other, perhaps novel, regulatory cis-elements that are responsible for the diverse expression profiles seen in various tissues with the ICAM-1 gene can be identified.

We have presented a general review, as well as a summary of our data, concerning the role of ICAM-1 expression in inflammatory and immunologic processes, its specific expression and induction in cutaneous inflammation and in cells derived from the skin, and have summarized our findings concerning two human genomic ICAM-1 clones, λ6 and λ8. More detailed characterization of λ6, and in particular the subclone PAG6-2.05, has been previously presented [46]. These studies, as well as those of an ever-growing number of investigative laboratories, clearly demonstrate the continually emerging and diverse critical roles that the regulated expression of the human ICAM-1 gene plays in both physiologic and pathologic processes that are relevant to immunobiology in general, and to cutaneous biology in particular. It is hoped that continued characterization of the genomic structure and function of the ICAM-1 gene, as well as study of the regulatory participants governing expression of the ICAM-1 protein, will provide important insights into its role in localized inflammatory processes. Consequently, elucidation of these regulatory mechanisms not only will further our understanding of the biology of regulation of this and other genes, it may also hold important clues to the development of future therapeutic agents designed to specifically modulate the expression of ICAM-1 and other adhesion molecules, and thereby provide alternative treatment modalities for inflammatory diseases of the skin.

REFERENCES


16. Yohn JJ, Crittelli M, Lyons MB, Norris DA: Modulation of melano-


47. Simmonds D, Makgoba MW, Seed B: ICAM, an adhesion ligand for LFA-1, is homologous to the neural cell adhesion molecule NCAM. Nature 331:624–627, 1988


