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ICAM-1: Isoforms and Phenotypes
Theresa N. Ramos,*† Daniel C. Bullard,‡ and Scott R. Barnum*†

ICAM-1 plays an important role in leukocyte trafficking, immunological synapse formation, and numerous cellular immune responses. Although considered a single glycoprotein, there are multiple membrane-bound and soluble ICAM-1 isoforms that arise from alternative splicing and proteolytic cleavage during inflammatory responses. The function and expression of these isoforms on various cell types are poorly understood. In the generation of ICAM-1–deficient mice, two isoform-deficient ICAM-1 mutants were inadvertently produced as a result of alternative splicing. These mice, along with true ICAM-1–deficient mice and newly generated ICAM-1–transgenic mice, have provided the opportunity to begin examining the role of ICAM-1 isoforms (singly or in combination) in various disease settings. In this review, we highlight the sharply contrasting disease phenotypes using ICAM-1 isoform mutant mice. These studies demonstrate that ICAM-1 immunobiology is highly complex but that individual isoforms, aside from the full-length molecule, make significant contributions to disease development and pathogenesis. The Journal of Immunology, 2014, 192: 4469–4474.

The membrane-bound glycoprotein ICAM-1 (CD54) plays a central role in leukocyte trafficking, activation of lymphocytes, and numerous additional immune functions (reviewed in Refs. 1–5). Originally cloned and sequenced from the neutrophilic promyelocyte cell line, HL-60, and umbilical vein endothelial cells (6), ICAM-1 is now known to be expressed on essentially all leukocyte subsets, endothelial cells, platelets, fibroblasts, epithelial cells, glial cells, and others (7–13). On most cell types and under non-inflammatory conditions, ICAM-1 expression is constitutively low and generally detectable only on endothelial cells (6, 14, 15); however, TNF-α, IL-1β, IFN-γ, and other cytokines elicit increased expression in a cell- and cytokine-specific fashion (7, 14, 16–18). ICAM-1 upregulation is a signature event during inflammation, particularly on endothelium where expression may remain elevated for extended periods of time (11, 15).

ICAM-1 is a member of the Ig supergene family, and the full-length isoform is composed of five Ig domains, a transmembrane domain, and a short cytoplasmic tail with multiple threonine residues (Fig. 1A) (6). The cytoplasmic tail interacts with the actin cytoskeleton and the lipid raft–associated ezrin/radixin/moesin complex (19, 20). ICAM-1 is heavily glycosylated, containing multiple N-glycosylation sites (8 in human, 10 in mice), and N-glycans are required for surface expression of the protein (reviewed in Ref. 21). Recently, a functional high-mannose ICAM-1 glycoform, expressed on endothelial cells, has been characterized (20). The full-length isoform can form a homodimer in which each subunit bends at the junction between Ig domains three and four (Fig. 1B). The binding between subunits in the homodimer occurs through residues exposed on structural rearrangement of Ig domain four (22, 23). Dimerization of ICAM-1 subunits significantly increases the affinity for LFA-1, which may impact the ligand’s ability to promote intracellular signaling (24). In addition to LFA-1, ICAM-1 binds to multiple ligands, including other β2-integrins (Mac-1 and p150,95), fibrinogen, hyaluronan, a Plasmodium falciparum RBC membrane protein, and major group rhinoviruses (2, 25–30). The majority of ICAM-1 ligands bind to the first Ig domain of ICAM-1 (Fig. 1A), although Mac-1 and p150,95 were shown to bind to other regions (Ig domains 3 and 3/4, respectively) (28–33). Studies demonstrated that cross-linking ICAM-1 results in association with the actin cytoskeleton and activation of several intracellular signaling pathways that contribute to cytokine production and cellular trafficking events (reviewed in Refs. 19, 34).

Alternative splicing gives rise to multiple ICAM-1 isoforms

Alternative splicing is a common posttranscriptional mechanism, occurring in >90% of multiexon genes, which broadly serves to regulate gene expression (reviewed in Refs. 35–37). ICAM-1 undergoes alternative splicing, similar to many genes in the immune system, including cell surface receptors, transcriptional regulators, and intracellular signaling molecules (38). Alternative splicing generates at least six membrane-bound forms and one soluble form of ICAM-1 (Fig. 1C) (39–44). The membrane-bound isoforms contain two, three, four, or five Ig domains (see Fig. 1A for Ig domain numbering), and all isoforms contain at least Ig domains 1 and 5. The ability of these isoforms to bind LFA-1 in vitro was shown...
to be highly variable based on which Ig domains are present (40). The known soluble form of ICAM-1 is a full-length isoform and is derived from an mRNA transcript lacking a transmembrane domain. Expression of soluble ICAM-1 is, in part, cell specific (endothelial and PBMCs) and modulated by cytokines, such as IFN-\(\gamma\) (39). Soluble ICAM-1 is also generated by proteolytic cleavage of the membrane-bound form by neutrophil elastase, cathepsin G, and bacteria-derived enzymes (42, 45, 46). Interestingly, the smaller ICAM-1 isoforms are more susceptible to proteolytic cleavage than is the full-length isoform (42). In addition to the six membrane-bound isoforms, studies demonstrated a splice variant in which the fifth Ig domain is truncated by 24 aa, with a corresponding loss of Ig domain structure (Fig. 1D) (47). This variant was detected at significantly lower levels than was the full-length isoform in multiple tissues, including lung, spleen, and kidney, after pulmonary LPS challenge. Truncation of the fifth Ig domain in the remaining membrane-bound ICAM-1 isoforms appears to be minimal, suggesting that this modification is largely restricted to the full-length isoform, at least in the model system used in the study. Whether the use of this alternative splice site occurs in any of the other isoforms, as a function of infection or disease-induced inflammation, remains unexplored. The functional effects of this in-frame exon truncation event have not been examined, but they could affect ICAM-1 dimerization, inter- and intracellular signaling, and enzymatic cleavage of membrane forms of the protein (42). The result of these posttranscriptional and enzymatic modifications is at least seven membrane-bound versions of ICAM-1 arising from alternative splicing through cassette exon and alternative splice-site usage and numerous potential soluble forms of ICAM-1. Understanding how these isoforms contribute to ICAM-1-mediated immune responses has been problematic because of multiple factors, such as limited awareness of multiple ICAM-1 isoforms in mammals, limited reagents to identify the specific isoforms expressed on different cell types, changes in isoform expression during the immune response, and how these isoforms function during different disease states.

**Alternative splicing and the generation of isoform-deficient ICAM-1 mice**

Shortly after the characterization of ICAM-1, two groups independently undertook efforts to generate ICAM-1-deficient mice. Unaware that ICAM-1 was alternatively spliced, both groups used replacement constructs designed to insert a neomycin selection gene into a single exon to generate a truncated and nonfunctional molecule. Deletion of exon 4, which encodes Ig domain 3, led to the production of *Icam*\(^{tmJcgr}\) mice, whereas deletion of exon 5, which encodes Ig domain 4, led to the production of *Icam*\(^{tmBay}\) mice (48, 49). These ICAM-1 isoform-deficient mice both express two of the small isoforms, one composed of Ig domains 1, 2, and 5, and the other composed of Ig domains 1 and 5, *Icam*\(^{tmJcgr}\) mice also express the isoform composed of Ig domains 1, 4, and 5 (Fig. 2A), whereas *Icam*\(^{tmBay}\) mice lack this isoform and instead express an isoform containing Ig domains 1, 2, 3, and 5 (Fig. 2B). Neither the *Icam*\(^{tmJcgr}\) or the *Icam*\(^{tmBay}\) mutant mouse expresses the full-length isoform. At the time of their construction, these mice were considered ICAM-1-null mutants based on screening strategies that examined for alterations in genomic DNA surrounding the neomycin gene insertion site and the low expression of their isoforms under basal or inflammatory conditions, as assessed by immunohistochemistry or flow cytometry (48, 49). Although subsequent studies demonstrated that these mice expressed the unique subsets of alternatively spliced isoforms described above (40, 43), numerous investigators performed studies in various disease or injury model systems assuming that they were deficient

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**FIGURE 1.** Schematic representation of ICAM-1 isoforms. (A) Full-length ICAM-1 isoform with five Ig domains and transmembrane and intracellular domains. * denotes the binding site for LFA-1 and other ligands (see text for details) in the first Ig domain; † denotes the Mac-1 binding site in the third Ig domain; 2 denotes the p50,95 binding site in the third and fourth domains. (B) ICAM-1 full-length isoforms dimerize by interaction through the fourth Ig domains. (C) The six membrane ICAM-1 isoforms expressed on endothelial and other cell types. (D) Alternative spicing in exon 6 leads to truncation of Ig domain 5 in the full-length isoform under inflammatory conditions. In each panel, the circles represent an Ig-like domain. The numbering of each Ig domain is based on the full-length isoform containing five Ig-like domains.

**FIGURE 2.** Schematic representation of ICAM-1 isoform structure and expression in ICAM-1-mutant mice. (A) *Icam*\(^{tmBay}\) mice. (B) *Icam*\(^{tmJcgr}\) mice. (C) CD2-Icam\(^{fl}\)/ICAM-1\(^{null}\) mice. (D) CD2-Icam\(^{fl}\)/ICAM-1\(^{null}\) mice. Numbering is the same as described in Fig. 1.
in all ICAM-1 isoforms. Thus, the majority of the literature describing results using ICAM-1–deficient mice reports on findings using specific isoform-deficient mice, rather than a true ICAM-1 knockout. Mice deficient in all ICAM-1 isoforms (ICAM-1null or Icam1tm1Alb) were generated a few years later, using a cloning strategy that deleted the entire coding region (50, 51). Icam1tm1Alb mice are phenotypically normal, fertile, and have no apparent abnormalities. Recently, two transgenic ICAM-1 mice were generated to address specific functions of ICAM-1 isoforms (52–54). These transgenic mice express either the full-length isoform (CD2-Icam1fl/Icam1null, Fig. 2C) or the isoform containing Ig domains 1, 2, 3, and 5 (CD2-Icam1D4del/Icam1null, Fig. 2D) under the control of the CD2 promoter on an Icam1tm1Alb background.

Does differential ICAM-1 isoform expression alter disease phenotype?

Determining how ICAM-1 isoforms contribute to immune-mediated pathophysiology is a complex question. The ICAM-1 mutant mice described above provide a rich experimental resource to begin deciphering their functions. In the following sections, we highlight a small number of models comparing these multiple isoform–mutant mice used under the same experimental conditions or examined in the same study, allowing better insight into ICAM isoform function.

Endotoxic shock model. The first study to examine the role of ICAM-1 in LPS-induced shock used Icam1tm1Alb mice (49). Mice were treated with a lethal dose of LPS; only 20% of wild-type mice survived to 72 h, whereas 100% of Icam1tm1Alb mice survived. Interestingly, Icam1tm1Alb mice had little to no neutrophil infiltration in the liver compared with wild-type mice. These findings were confirmed and extended in a second study in which Icam1tm1Alb and Icam1tm1Bay mice were simultaneously compared with wild-type mice for LPS sensitivity (42). Using a similar dose of LPS, Icam1tm1Alb mice were again found to be resistant to endotoxemic shock compared with wild-type mice (0% versus 50% mortality, respectively). In contrast, Icam1tm1Bay mice were highly susceptible to the effects of LPS treatment, with high mortality (~80%) and significant neutrophil infiltration into the liver. Although these results demonstrate remarkably different outcomes, they provide little insight into isoform-specific mechanisms behind the phenotypes. This is due, in part, to the limited analysis of the mice with respect to inflammatory mediators, such as changes in acute-phase protein and cytokine levels and infiltration and activation of leukocytes. Studies using additional ICAM-1–mutant mice are required to determine how isoforms contribute to this acute inflammatory condition.

Experimental autoimmune encephalomyelitis. Experimental autoimmune encephalomyelitis (EAE) is the animal model for the human disease multiple sclerosis (55, 56). ICAM-1 is an iconic marker of inflammation in EAE and multiple sclerosis, with elevated expression at lesion sites on endothelial cells and on infiltrating and resident CNS cells (7, 57, 58). Although EAE can be induced in many animal species using myelin or a variety of myelin-derived proteins or peptides, the most commonly used and well-characterized model uses C57BL/6 mice and a myelin oligodendrocyte glycoprotein peptide (aa 35–55) (59, 60). This is the experimental paradigm used in the EAE studies described below. Given the multiple ICAM-1 ligands expressed by infiltrating cells, its absence would predictably result in less severe EAE. In fact, Icam1tm1Alb mice (deficient in all isoforms) failed to develop EAE, whether actively induced with myelin oligodendrocyte glycoprotein (aa 35–55) peptide or by transfer of encephalitogenic T cells (51) (Table I). Not surprisingly, leukocyte infiltration and demyelination also were significantly reduced in Icam1tm1Alb mice compared with wild-type mice. Interestingly, Ag-specific T cell proliferation assays, using various combinations of wild-type and ICAM-1–deficient T cells and APCs, demonstrated that ICAM-1 expression on T cells, but not APCs, is required for T cell proliferation (51). These studies indicated that ICAM-1 expression on multiple cell types is required for the development of EAE, but they provided little insight into isoform-specific functions.

In contrast, Icam1tm1Bay and Icam1tm1Alb mice clearly demonstrate that different combinations of isoforms (Fig. 2A, 2B) lead to different EAE phenotypes. First to be studied were Icam1tm1Bay mice, which unexpectedly developed EAE that was more severe than that observed in wild-type mice (Table I), with concomitant elevated leukocyte infiltration, demyelination, and mortality (61, 62). Transfer of Icam1tm1Bay encephalitogenic T cells to wild-type or Icam1tm1Alb mice also resulted in significantly worse disease compared with wild-type–wild-type transfers (62). However, Icam1tm1Alb mice develop a significantly milder EAE disease course compared with wild-type mice (Table I). Transfer of encephalitogenic T cells from Icam1tm1Alb mice to wild-type recipients also produces a mild disease phenotype. Collectively, the results with Icam1tm1Bay and Icam1tm1Alb mice show that alternatively spliced ICAM-1 isoforms other than the full-length isoform are functional, the full-length ICAM-1 isoform is not required for disease development, ICAM-1 expression on endothelial cells is not required for disease development based on Icam1tm1Bay to Icam1tm1Alb transfected EAE, and Icam1tm1Bay T cells are more encephalitogenic by virtue of the combination of ICAM-1 isoforms that they express. It is unclear whether Icam1tm1Bay isoforms expressed on leukocytes, APCs, and other cell types generate a highly potent T cell priming environment or whether their expression by T cells accounts for the observed enhancement in disease pathogenesis.

Table I. Comparison of EAE and ECM clinical signs between wild-type and ICAM-1 mutant mice

<table>
<thead>
<tr>
<th>ICAM-1 Genotype</th>
<th>EAE Phenotype</th>
<th>ECM Phenotype</th>
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<tbody>
<tr>
<td>Wild-type</td>
<td>Acute onset, chronic disease</td>
<td>Acute onset, uniformly fatal</td>
</tr>
<tr>
<td>Icam1tm1Alb</td>
<td>More severe than wild-type</td>
<td>Little to no disease</td>
</tr>
<tr>
<td>Icam1tm1Bay</td>
<td>Delayed onset, attenuated disease</td>
<td>Delayed onset, significant survival</td>
</tr>
<tr>
<td>CD2-Icam1fl/Icam1null</td>
<td>Delayed onset, attenuated disease</td>
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The results of EAE studies with Icam1tm1Bay and Icam1tm1Jcgr mice raised questions regarding the role of ICAM-1 isoforms on leukocytes. This led to the generation of CD2-Icam1+/Icam1null and CD2-Icam1D4del/Icam1null mice, which express a single ICAM-1 isoform under the control of the CD2 promotor on an ICAM-1 null background, thereby allowing the study of single-isofomm immunobiology (Fig. 2C, 2D) (52, 54). Expression of either isoform is comparable to wild-type levels on CD4+ and CD8+ T cells and at significantly lower levels on other leukocyte subsets (γδ T cells, B cells, and NK cells) (52, 54). Both mice develop EAE, but with unique phenotypes (Table I) (Ref. 54 and X. Hu, D.C. Bullard, and S.R. Barnum, unpublished observations). CD2-Icam1+/Icam1null mice present with significantly delayed and attenuated disease, whereas CD2-Icam1D4del/Icam1null mice present with robust disease, comparable to that seen in wild-type mice. These findings are remarkable in that they demonstrate the ability of single ICAM-1 isoforms expressed predominantly on T cells, but not endothelial cells, to drive disease development. Furthermore, these studies indicate that the isoform missing Ig domain 4 can promote severe disease pathogenesis in isolation. Future studies are necessary to determine how other ICAM-1 isoforms may contribute to disease development.

Experimental cerebral malaria. Experimental cerebral malaria (ECM) is a commonly used model for the most severe and deadly form of the human disease, cerebral malaria (63, 64). Sequestration of infected RBCs, leukocytes, and platelets on inflamed endothelium as the result of increased expression of adhesion molecules, including ICAM-1, is thought to be a major contributor to disease development (65–68). ICAM-1 binds to Plasmodium falciparum erythrocyte membrane protein 1, expressed on the surface of infected RBCs (iRBC), at a site on the N-terminal Ig domain (28–31). In rodents, inoculation of C57BL/6 mice with P. berghei ANKA iRBCs has recently become a widely used model for ECM (69–71). Additional evidence to support a role for ICAM-1 in ECM comes from studies demonstrating that anti–ICAM-1 Ab treatment abrogates iRBC adherence and rolling in both in vitro and in vivo model systems (66, 68, 72). However, direct evidence implicating an important role for ICAM-1 and its isoforms in ECM has been lacking until recently.

In studies simultaneously comparing the course of ECM in different lines of ICAM-1−mutant mice, it was shown that the ECM phenotype varies directly with the combination of isoforms expressed (53). Not surprisingly, Icam1tm1Bay mice were highly resistant to ECM, with >90% of mice surviving 10 d postinfection, a time at which all wild-type mice had succumbed to disease (Table I). Both Icam1tm1Bay and Icam1tm1Jcgr mice were more susceptible to ECM, with Icam1tm1Bay mice presenting with a more protective phenotype (60% and 40% survival, respectively; Table I). These results are an interesting reversal of the disease phenotype seen for Icam1tm1Bay and Icam1tm1Jcgr mice in EAE where Icam1tm1Bay mice developed exacerbated disease compared with wild-type mice (62). CD2-Icam1+/Icam1null and CD2-Icam1D4del/Icam1null mice presented with remarkably different ECM phenotypes. CD2-Icam1+/Icam1null mice were fully susceptible to disease similar to wild-type mice; however, the time to 50% survival was 2 d slower than for wild-type mice. In contrast, CD2-Icam1D4del/Icam1null mice were significantly more resistant to ECM, with 60% of the mice surviving 10 d postinfection (Table I). This disease course is very similar to that observed for Icam1tm1Bay mice with ECM and suggests that expression of the Icam1D4del isoform on leukocytes is sufficient to drive this disease phenotype. These studies demonstrate that ICAM-1 expression on the CNS microvascular endothelium is not required for development of ECM and suggests an important contribution for ICAM-1–mediated sequestration within the microvascular space.

Conclusions

Despite reports in the early to mid-1990s, alternatively spliced forms of different adhesion molecules, such as VCAM-1, PECAM-1, MAdCAM-1, and ICAM-1, have virtually been ignored, and the majority of the scientific field has considered these molecules to exist as single proteins. However, with expansion of genomic technologies, it is clear that the majority of genes, including those that encode for the adhesion molecules, undergo alternative splicing and have the potential to produce multiple isoforms. The expression patterns, ligand interactions, and functions of these isoforms still remain largely undefined. Future studies are needed to understand how these isoforms contribute to immune and inflammatory responses, as well as potentially modulate disease phenotypes.

Studies of the alternatively spliced forms of ICAM-1 have benefited significantly from the generation of multiple lines of ICAM-1−deficient mice. In fact, their initial discovery was facilitated by the identification of ICAM-1 isoforms expressed in Icam1tm1Bay mice. It is now evident from numerous investigations of these mice that unique combinations of ICAM-1 isoforms contribute to disease-specific outcomes. How do these ICAM-1 isoforms mediate such remarkably different pathology? In our view, there are overlapping possibilities that include differential isoform expression, structural changes of the molecule (due to the presence or absence of different Ig domains), and alterations in ICAM-1–facilitated intracellular signaling. The potential number of combinations of ICAM-1 isoforms expressed even on a single cell type is staggering. This complexity is further increased when considering the numerous cell types that express ICAM-1, thus creating a daunting task for determining isoform function. The key first step is the application of genomic and proteomic approaches to provide insight into these expression patterns.

Alternative splicing could lead to significant structural changes in the ICAM-1 molecule that may then alter ligand interactions and functional activity. In addition to potential effects on dimerization, there may be preferential interactions of ICAM-1 isoforms that serve to regulate ligand accessibility, thereby modulating adhesion strength. Such interactions may also control access to the actin cytoskeleton and adapter proteins, thereby modulating adhesion strength. Such interactions may also affect control to the actin cytoskeleton and adapter protein complexes in lipid rafts, altering intracellular signaling and disease outcome. Altered glycosylation as the result of the loss of Ig domains in alternatively spliced ICAM-1 isoforms may also contribute to the differential function of these molecules. The extent of glycosylation on both membrane-bound and soluble ICAM-1 can regulate ligand binding (21). In addition, recent studies demonstrated that the high-mannose form of ICAM-1 is functionally distinct from the complex N-glycans form of ICAM-1 with respect to monocyte rolling and adhesion and cytoskeleton interaction (20). Although these findings indicate that glycosylation plays a fundamental part
in modulating the role of ICAM-1, the effects of these modifications on isoform activity remain unexplored.

Intracellular signaling through ICAM-1 in T cells, APCs, and endothelial cells is considered costimulatory in nature, similar to CD80/86, CD28, and other costimulatory molecules. ICAM-1 dimerization is believed to be required for signaling through an ITIM-like motif that interacts with protein phosphatases containing SH2 domains. Upon ligand binding, ICAM-1 migrates to lipid rafts, leading to the activation of ERK, Akt, and JNK pathways and the subsequent production of cytokines and chemokines and changes in the expression of adhesion molecules (19, 20). These studies assumed that signaling occurs through the full-length ICAM-1 isoform; however, there is no evidence to indicate a requirement for signaling through this isoform. The distinct disease phenotypes and level of T cell activation (as determined by Ag-recall assays) in ICAM-1–mutant mice (51, 54, 62) suggest that discrete combinations of isoforms contribute to intracellular signaling events and, ultimately, to disease severity. Specifically, the severe disease phenotype observed in Icam1homo mice compared with Icam1Homo/ICAM1null mice suggests that the isoform containing Ig domains 1, 2, 3, and 5 is more critical for proinflammatory ICAM-1 signaling events than is the full-length isoform. Understanding how these isoforms modulate intracellular signaling and their downstream functions requires additional study. The currently available repertoire of ICAM-1–mutant mice and the generation of new ICAM-1–mutant mice, expressing single or unique combinations of ICAM-1 isoforms, will be useful in addressing key questions regarding ICAM-1 interactions.

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Disclosures
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BRIEF REVIEWS: ICAM-1 ISOFORMS


