Active sequences in cell adhesion molecules: targets for therapeutic intervention

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The adhesion of cells to molecules of the extracellular matrix or to other cells is basic to many biological processes, including cell growth, cell trafficking, wound healing, metastasis, formation of tissue architecture, regulation of the immune system and signal transduction. Interest in cell adhesion molecules continues to increase as new molecules are identified and their functions determined. The author discusses the binding domains of such molecules as targets for the design of therapeutic cell adhesion antagonists.

A range of therapeutic applications for cell adhesion processes and the inhibition of such pocesses has been proposed. These include cell–cell adhesion molecules involved in the blood–brain barrier, adhesion molecules in tumor metastasis, recognition sequences in cell adhesion, adhesion receptors of the immune system, binding sites involved in integrin–ligand interactions, leukocyte–endothelial adhesion molecules, adhesion molecules as therapeutic targets, regulation of cell adhesion and proliferation by carbohydrates and soluble lectins, the selectins and their ligands, and the use of inhibitors of cell adhesion as anti-inflammatory agents.

Cell adhesion can be inhibited in a variety of ways, using forms of soluble adhesion molecules or their counter-receptors, antisense oligonucleotides, transcription inhibitors, post-translational modification inhibitors (e.g. sialyltransferases), or antibodies against adhesion molecules or their counter-receptors.

This review addresses primarily the identification of sequences or motifs directly involved in the adhesion process, as targets for therapeutic intervention.

Identification of active sites or chemical targets

Ideally, the first step in the rational design of inhibitors of cell adhesion is the determination of a high-resolution three-dimensional crystal structure of the receptor–counter-receptor complex. However, relatively few structures have been determined and rational design generally begins with the identification of the binding domain of either the receptor or its counter-receptor. For a sequence to be identified as an adhesion motif, it must be shown not only to block the appropriate adhesion process but also to support adhesion and be found in either the receptor or counter-receptor. These criteria have not been demonstrated for all sequences claimed as adhesion motifs.

Adhesion molecules are responsible for both cell–cell and cell–matrix interactions. Several families of molecules have been implicated in these processes. Because of the similarity in the sequence of active sites of apparently unrelated or distantly related proteins, it has been suggested that different adhesion molecules were evolutionarily drawn from a pool of common oligopeptides. Sequences cited in support of this hypothesis are the RGD motif in matrix proteins and the EKKD motif of integrin cell surface receptors[1]. Other adhesion sites appear to be structurally unique. In several instances, multiple

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adhesion sites have been identified on one molecule that contribute to binding to either the same or different counter-receptors. The function of these multiple sites may be to increase affinity or to provide redundancy in the adhesion process.

Selectins

The selectins are a family of three glycoproteins (E-, L- and P-selectin) of the vascular system, each consisting of an N-terminal lectin domain, followed by an epidermal growth factor (EGF)-like region, a series of complement regulatory-like repeats, a transmembrane region (for membrane-bound forms) and a cytoplasmic domain (Figure 1). The selectins are involved in both leukocyte and lymphocyte adhesion and may play an important role in inflammation by mediating the initial attachment of leukocytes to endothelial cells. Although the lectin domain is generally accepted to contain the binding motif, antibodies against the EGF (Ref. 2) and complement-like repeats[3] can also affect adhesion, suggesting the presence of adhesion sequences within these domains. Several proteins have been identified as selectin ligands, including a sialoglycoprotein from leukocytes and PSGL-1 for P-selectin, ESL-1 for E-selectin, and GlyCAM-1 and MAdCAM-1 for L-selectin. Although the common motif in these selectin ligands is glycosylation, they may be too structurally dissimilar to constitute a family (Figure 2).

Antibodies and recombinant selectin constructs have been shown to block selectin-dependent cell adhesion. With certain cell types there is redundancy in selectin counter-receptor recognition. Sequences involved in selectin-dependent adhesion are given in Table 1. Numerous carbohydrate structures have been shown to inhibit selectin-dependent adhesion. Sialyl Lewis X (sLeX) has been shown to bind to all three selectins and inhibit cell adhesion[4], although some sLeX-negative cell lines also show selectin-dependent adhesion[5]. Other carbohydrate structures that bind selectins and are putative ligands include di-sLeX, sulfofuco-oligosaccharides, and sialylated and nonsialylated lacto-*N*-fucopentanoses.

Three peptides from the lectin domain of P-selectin (YTDLVAIQ, RNKKNTWTWV and TNEAENWADN) were initially identified as adhesion sequences by their ability both to block leukocyte adhesion to P-selectin and to support leukocyte adhesion[6]. Subsequent studies identified three additional adhesive sequences (STKAYSWNISRKY, DYLNKVLPYYSSYYW and CLKKKHALCY), also within the lectin domain[7]. Two similar peptides from the P-selectin lectin domain (CQNRYTDLVAIQNKNE and IGIRKNNKTWT) and one from the EGF domain (CSKQGECLETIGN) were reported to support adhesion of U937 cells[8]. An overlapping peptide, YYWIGIRK, from P-selectin was shown to inhibit HUVEC (human umbilical vein endothelial cells) binding to HL-60 cells[9]. The peptides RALTVAELRGSGDLQEYLRHVTRGWS and RALTVAELRGNAELQTYL from the lectin domain of *B. pertussis* toxin may mimic selectin sequences because they block selectin-dependent adhesion[10]. Homologous sequences from E- and P-selectin that showed inhibition of leukocyte binding were KEEIEYLNSILSYS and VAIQNKNEIDYLNKVLPYYS (Ref. 11). Other peptides from *B. pertussis* toxin and wheat germ agglutinin reported to inhibit cell adhesion have the consensus sequence SPXGXCXX and are represented by the compound SPYGRC (Ref. 12).

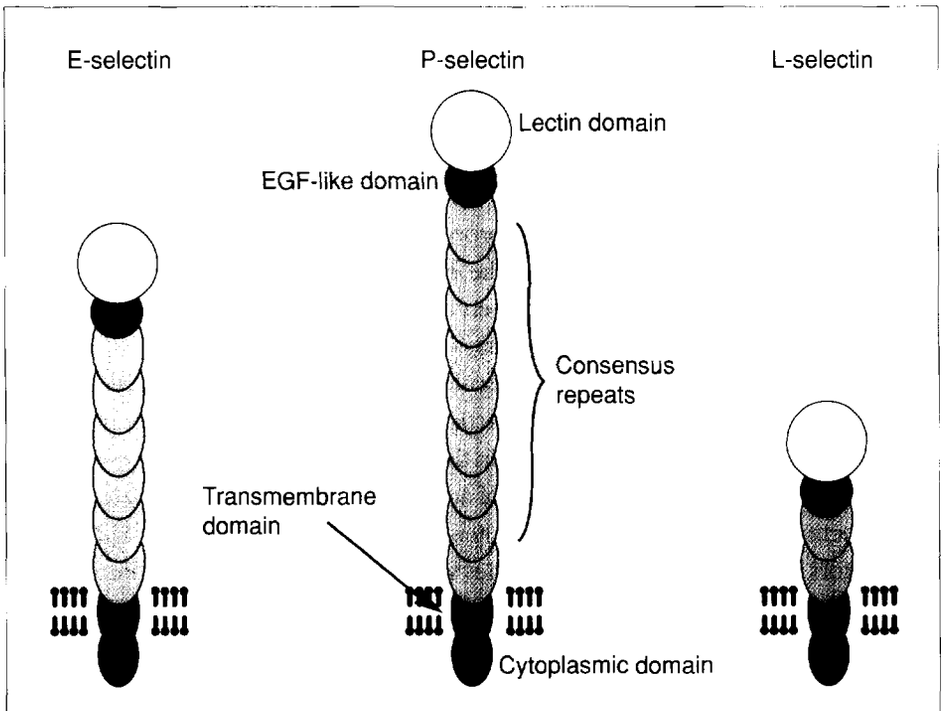

Figure 1. *Structures of the three members of the selectin family, consisting of an N-terminal lectin domain, an epidermal growth factor (EGF)-like domain, a series of consensus repeats, a transmembrane domain and a cytoplasmic C-terminal domain.*

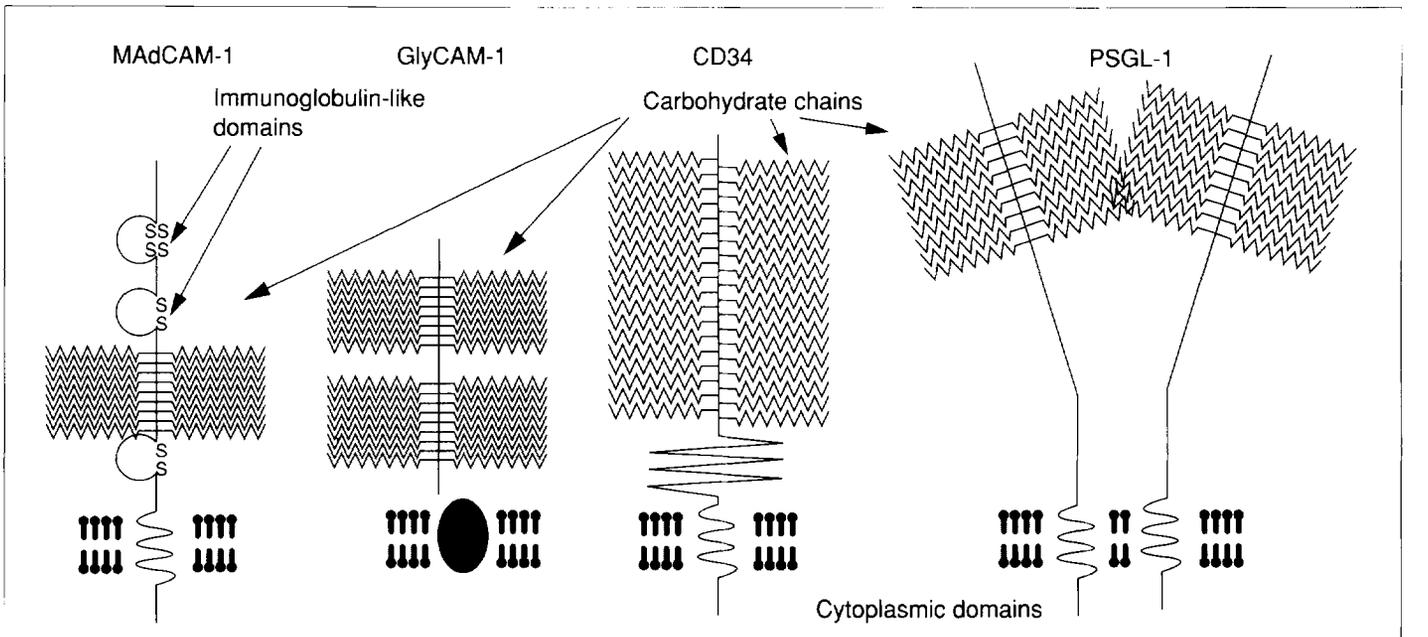

Figure 2. *The structures of several compounds that have been identified as ligands for the selectins. Glycosylation is a common structural feature in these compounds.*

Using a recombinant peptide phage library, peptides were identified that bound to E-selectin and blocked neutrophil adhesion to endothelial cells[13]. Although the adhesion mimetic HITWDQLWNVMN and related sequences inhibited adhesion, they are not found in protein sequences of known selectin ligands and did not block sLeX binding, suggesting that the sLeX motif is not the only binding domain of the selectins.

Integrins

The integrins are a family of heterodimeric, transmembrane, multifunctional cell adhesion molecules composed of unrelated α and β subunits that mediate cell adhesion (Figure 3)[14]. They are found on a multitude of membranes and can exist in different affinity or binding states[15]; several sites within one integrin can be involved in adhesion. Table 2 lists adhesion sequences from the integrins. Binding of RGD peptides to the integrin $\alpha_{IIb}\beta_3$ on platelets gives rise to high-affinity fibrinogen binding and subsequent platelet activation[16]. In addition to $\alpha_{IIb}\beta_3$, the RGD sequence has been implicated in binding to at least six other integrins: $\alpha_5\beta_1$, $\alpha_4\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_6$ and $\alpha_b\beta_8$. Sequence 656–667 of α_{IIb} (GAHYMRALSNVE) is hidden on resting platelets but becomes exposed following platelet activation with thrombin, binding to fibrinogen and inhibiting fibrinogen-mediated platelet aggregation[17]. An 11-mer from α_{IIb}, TDVNGDGRHDL (296–306), inhibited fibrinogen binding and platelet aggregation[18]. Peptides containing the β_3 sequence 211–222 (SVSRNRDAPEGG) blocked binding of $\alpha_{IIb}\beta_3$ to fibrinogen, von Willebrand factor (vWF) and vitronectin, as well as platelet aggregation[19].

Table 1. Cell adhesion sequences involved in selectin-dependent adhesion

Molecule	Sequence	Ligand
?	Sialyl Lewis X (sLeX)	Selectins
Selectin ligands	Sialyl(α2-3)Gal(β1-4)[Fuc(α1-3)]Glc	Selectins
P-selectin	YTDLVAIQ	?
P-selectin	RNKKNTWTWV	?
P-selectin	TNEAENWADN	?
P-selectin	STKAYSWNISRKY	?
P-selectin	DYLNKVLPYYSSYYW	?
P-selectin	CLKKKHALCY	?
P-selectin	CQNRYTDLVAIQNKNE	?
P-selectin	IGIRKNNKTWT	?
P-selectin	CSKQGECLETIGN	?
P-selectin	YYWIGIRK	?
B. pertussis toxin	RALTVAELRGSGDLQEYLRHVTRGWS	?
B. pertussis toxin	RALTVAELRGNAELQTYL	?
E-selectin	KEEIEYLNSILSYS	?
P-selectin	VAIQNKNEIDYLNKVLPYYS	?
B. pertussis toxin	SPYGRC	?
Phage library	HITWDQLWNVMN	E-selectin

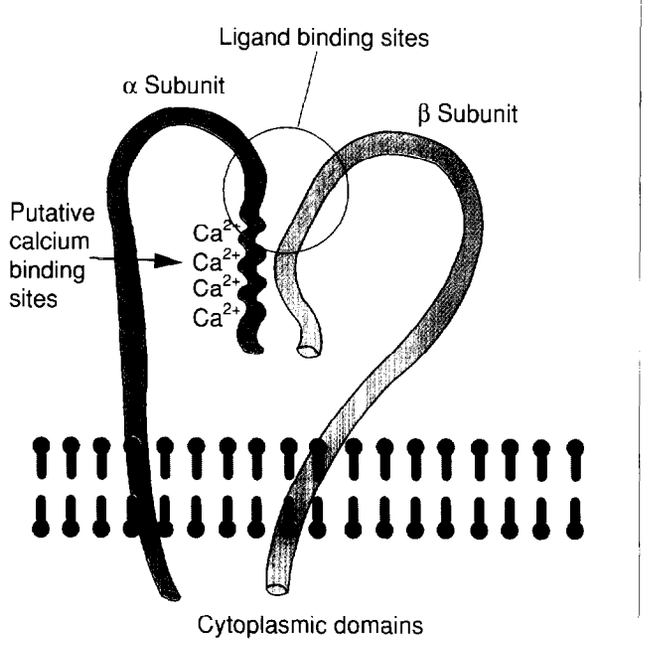

Figure 3. *Stylized structure of the calcium-dependent integrin superfamily consisting of non-equivalent membrane-bound α and β subunits.*

Table 2. Cell adhesion sequences involved in integrin- and immunoglobulin-dependent adhesion

Molecule	Sequence	Ligand
α_{IIb}	GAHYMRALSNVE	Fibrinogen
α_{IIb}	TDVNGDGRHDL	Fibrinogen
β_3	SVSRNRDAPEGG	Fibrinogen, vitronectin, vWF[a]
$\alpha_{IIb}\beta_3$	EHIPA	Fibrinogen
$\alpha_1\beta_2$	GTQIGSYFGGELCGVDVDQDGETELLLIGA-PLFYGEQRGG	ICAM-1
$\alpha_1\beta_2$	GEAITALTDINGDGLVDVAV	ICAM-1
ICAM-1	GAPL	Fibrinogen
$\alpha_{IIb}\beta_3$	DVNGDGRHDLLV	Fibrinogen
ICAM-1	VLYGPRLDERDAPGNWTWPENSQQTPMC	?
ICAM-1	GGAPRANLTVVLLRGEKELKREPAVGEP	?
ICAM-1	YELSNVQEDSQPMCYSNCPDGQSTAKTFL	?
ICAM-1	KELLLPGNNRKV	?
ICAM-1	GIET	?
ICAM-2	GSLEVNCSTTCNQPEVGGLETS	$\alpha_M\beta_2$
ICAM-2	GKSFTIECRVPTVEP	?
ICAM-2	LQCHFTCSGKQESMN	?
VCAM-1	QIDSPL	$\alpha_4\beta_1$
NCAM	KYSFNYDGSE	NCAM
PECAM	LKREKN	?

[a]vWF, von Willebrand factor

Using different adhesion sites, $\alpha_4\beta_1$ is involved in both cell–cell adhesion with VCAM-1 and cell matrix adhesion with fibronectin[20]. Identification of epitopes of blocking antibodies has also been used to localize ligand binding sites of the α_4 chain of the $\alpha_4\beta_1$ integrin that recognize both the CS-1 sequence of fibronectin and VCAM-1 (Ref. 21). Other antibodies that blocked adhesion of $\alpha_4\beta_1$ to VCAM-1 and fibronectin map to α_4(152–203)[22].

Two peptides from the β_2 subunit of LFA-1, 428–467 (GTQIGSYFGGELCGVDVDQDGETELLLIGAPLFYGEQRGG) and 497–516 (GEAITALTDINGDGLVDVAV), were shown to inhibit adhesion to ICAM-1 (Ref. 23). Based on the anti-complementarity hypothesis, the sequence GAPLRV was predicted to be a fibrinogen binding site. The peptide GAPL was shown to inhibit platelet adhesion to fibrinogen and platelet aggregation[24]. The binding site of the fibrinogen γ peptide HHLGGAKQAGDV to $\alpha_{IIb}\beta_3$ has been identified as residues 297–308 on the α_{IIb} chain (DVNGDGRHDLLV)[25].

Immunoglobulin (IgG) superfamily

Members of the immunoglobulin superfamily are defined as having at least one immunoglobulin-like domain in their sequence (Figure 4). It has been suggested that the binding domains of cell adhesion molecules of the immunoglobulin superfamily may correspond to variable regions similar to those found in antibodies[26]. In these multiple-domain molecules, the binding sites may extend over the surface of several domains or be localized to a single domain[27]. Adhesion sequences from the immunoglobulin superfamily are shown in Table 2.

A peptide analog from ICAM-1 (VLYGPRLDERDA-PGNWTWPENSQQTPMC), identified by hydropathy and homology analysis, inhibited both ICAM-1-mediated cell adhesion and ICAM-1-dependent cytotoxicity[28]. Other inhibitory sequences included GGAPRANLTVVLLRGEK-ELKREPAVGEP, YELSNVQEDSQPMCYSNCPDGQSTAKTFL and their analogs[29]. Lymphocyte–endothelial interactions were mediated with the peptide sequence KELLLPGNNRKV from ICAM-1 (Ref. 30).

A peptide from the first immunoglobulin domain of ICAM-2, GSLEVNCSTTCNQPEVGGLETS, has been shown to bind to $\alpha_1\beta_2$ (CD11a/CD18, LFA-1), activating integrin-dependent T cell adhesion[31], inhibiting endothelial cell adhesion[32] and binding to $\alpha_M\beta_2$ (CD11b/CD18, Mac-1) but not to $\alpha_X\beta_2$ (CD11c/CD18, p150, p95)[33]. Two peptides from ICAM-2, GKSFTIECRVPTVEP and LQCHFTCSGKQESMN, were identified as mediating cell adhesion[34].

Functional studies have localized a conserved integrin binding motif (IDSP) in domains 1 and 4 of the seven-domain VCAM-1 molecule that binds VLA-4 ($\alpha_4\beta_1$). The linear peptide QIDSP was found to have no effect on adhesion of Ramos cells to VCAM-1, while the cyclic variant cyclo-(CQIDSPC) inhibited adhesion in a dose-dependent manner[35]. This motif has been identified in the first domain of several molecules of the immunoglobulin superfamily and has been proposed as a primary binding site for integrins[36].

NCAM, a member of the immunoglobulin superfamily with five extracellular immunoglobulin-like domains, is involved in promotion of neurite growth, fasciculation of neurites, axonal guidance and the formation of neuromuscular synapses. The dodecapeptide sequence KYSFNYDGSE (243–252) is a homophilic binding site of NCAM (Ref. 37).

The immunoglobulin superfamily protein PECAM-1 is expressed on endothelial cells, platelets and leukocytes, and is involved in both heterotypic and homotypic aggregation. It contains a glycosaminoglycan consensus binding sequence (LKREKN) in the second immunoglobulin-like domain, similar to a sequence in NCAM. Peptides mimicking this consensus sequence inhibit PECAM-dependent aggregation[38].

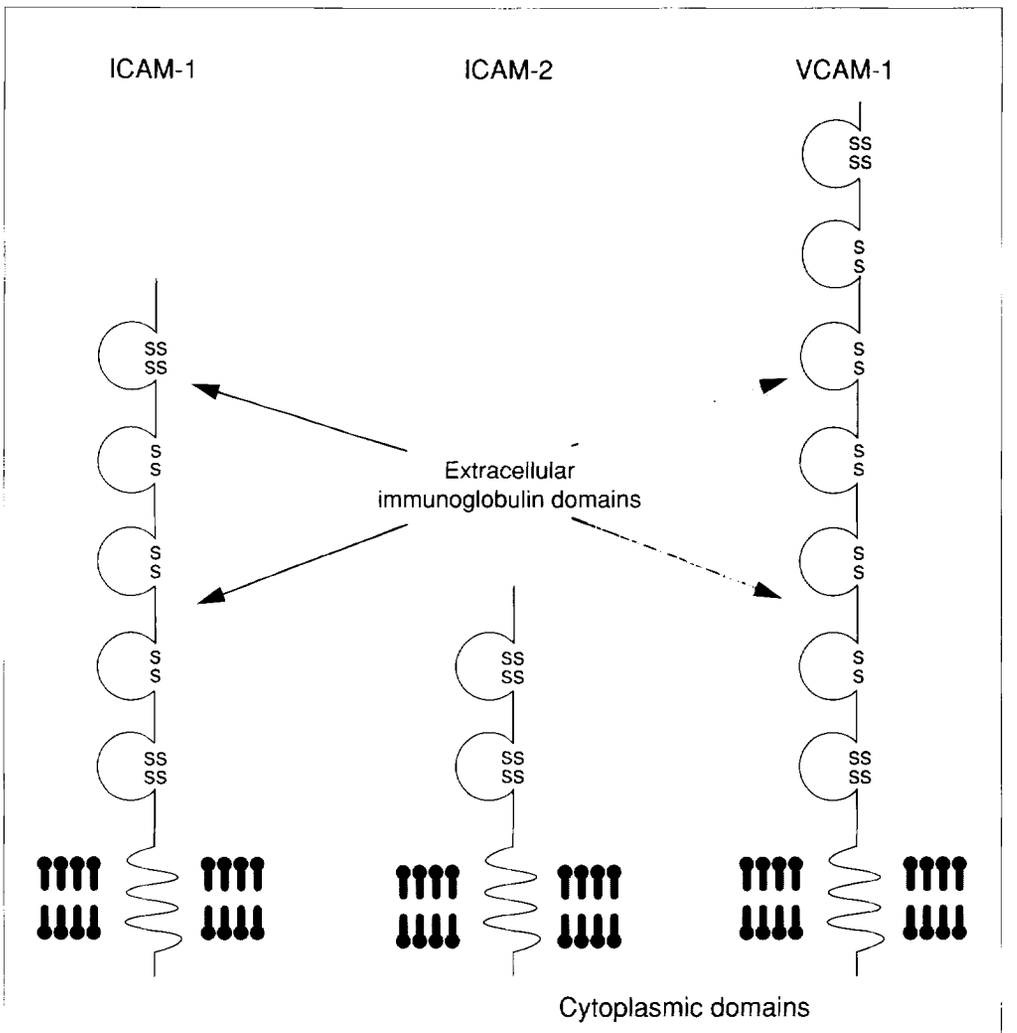

Figure 4. *Representative structures of the three members of the immunoglobulin superfamily, characterized as containing one or more immunoglobulin-like repeats.*

Fibrinogen and other RGD-containing compounds

Fibrinogen, a serum protein that is important in the blood-clotting cascade and a mediator of platelet aggregation, is a hexamer composed of two pairs of three nonidentical chains (α, β and γ). Adhesive sequences from fibrinogen are shown in Table 3. The binding of fibrinogen to the platelet integrin $\alpha_{IIb}\beta_3$ plays a key role in thrombosis and is one of the first integrin–ligand interactions to be a major target for cell adhesion intervention. The RGD sequence was initially identified in fibronectin[39] and was subsequently found in several other proteins, including the α (RGDS) and γ (RGDF) chains of fibrinogen[40], where it was shown to be an adhesive sequence. Antagonists of $\alpha_{IIb}\beta_3$, primarily involving the RGD sequence, have been reviewed[41].

Several antibodies bind to the $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) integrin receptor on platelets, including 7E3 (Ref. 42), OPG2 (Ref. 43), PAC-1 (Ref. 44) and LJ-CP3 (Ref. 45). A peptide from PAC-1 containing the RYD sequence inhibited fibrinogen binding to stimulated platelets. Mutation of RYD to RGD in OPG2 did not alter its specificity[46], suggesting that the conformation of the adhesive sequence confers specificity, an observation supported by the specificity changes observed in constrained RGD analogs. Another antibody directed against the RGD

Table 3. Cell adhesion sequences involved in fibrinogen- and fibronectin-dependent adhesion

Molecule	Sequence	Ligand
Fibrinogen	RGDS	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$
Fibrinogen	RGDF	$\alpha_{IIb}\beta_3$
Fibrinogen	HHLGGAKQAGDV	$\alpha_{IIb}\beta_3$
Fibrinogen	NNQKIVNLKEKVAQLEA	?
Fibrinogen	GWTVFQKRLDGSV	$\alpha_M\beta_2$
Fibrinogen	GPRP	?
Fibrinogen	GPRVV	?
Fibronectin	YEKPGSPPREVVPRPRPGV	?
Fibronectin	KNNQKSEPLIGRKKT	?
Fibronectin	DELPQLVTLPHPNLHGPEILDVPS (LDVPS)	?
Fibronectin	GEEIQIGHIPREDVDYHLYP (REDV)	?
Fibronectin	GPEILDVPST (LDV)	$\alpha_4\beta_1$
Fibronectin	IDAPS	$\alpha_4\beta_1$
Fibronectin	PASS	?
Fibronectin	DRVPHSRNSIT	?

binding site of $\alpha_{IIb}\beta_3$ does not contain the RYD sequence[47]. A peptide from V_H CDR_3, RQMIRGYFDV, inhibited platelet aggregation and fibrinogen binding, suggesting that the RGFY sequence may mimic RGD recognition as well.

A 12-amino acid peptide (400–411, HHLGGAKQAGDV) on the γ chain of fibrinogen is also a binding site for $\alpha_{IIb}\beta_3$ (Ref. 48). There is no apparent cooperativity of this site with the RGD sequences on the α chain[49].

A series of snake venom peptides has been characterized that inhibited binding of vWF to GPIb–IX receptors but did not inhibit the binding of fibrinogen to $\alpha_{IIb}\beta_3$ (Ref. 50), unlike other snake venom peptides[51]. A non-RGD-containing sequence (KGD) was found in the venom of the pygmy rattlesnake, *Sistrurus m. barbourin*, which did not bind to $\alpha_v\beta_3$ but was specific for $\alpha_{IIb}\beta_3$ (Ref. 52).

The interaction between ICAM-1 and fibrinogen in leukocyte transendothelial migration has been investigated using synthetic peptides from the fibrinogen γ chain[53]. The sequence 117–133 (NNQKIVNLKEKVAQLEA) was found to inhibit fibrinogen-mediated leukocyte–endothelial adhesion. Another fibrinogen γ chain peptide, GWTVFQKRLDGSV, has been shown to support $\alpha_m\beta_2$-dependent adhesion of THP-1 cells[54].

Using an anticomplementarity hypothesis to design a mimic of the vitronectin binding site of $\alpha_{IIb}\beta_3$, the peptide EHIPA was synthesized and found to bind to fibrinogen and to inhibit platelet aggregation[55].

The peptide GPRP, which is similar to the sequence GPRVV found in the N-terminal region of the Aα chain of fibrinogen, inhibited the binding of activated platelets by fibrinogen[56].

Fibronectin

Fibronectin is a dimeric glycoprotein containing three regions that are subject to alternative splicing: extra domains A and B and the type-III connecting segment (IIICS). The identification of the RGD sequence in fibronectin[57] as a cell adhesion site initiated major research efforts that led to the design of numerous analogs differing in potency and specificity. Fibronectin adhesive sequences are shown in Table 3.

An antibody that blocked melanoma adhesion to the heparin binding fragment of fibronectin recognized an epitope common to both the A and B chains of fibronectin[58]. Synthetic peptides from this region were prepared, and two peptides were identified (YEKPGSPPREVVPRPRPGV and KNNQKSEPLIGRKKT) that supported cell adhesion in a concentration-dependent manner.

A series of synthetic peptides covering the IIICS sequence of fibronectin was examined for ability to inhibit melanoma cell adhesion to fibronectin. Two nonadjacent peptides, DELPQLVTLPHPNLHGPEILDVPST (CS-1) and GEEIQIGHIPREDVDYHLYP (CS-5), were found to be inhibitory[59]. By systematic analysis using additional synthetic peptides, the active sequence of CS-5 was identified as REDV (Ref. 60). WEHI 231 lymphoid cells will adhere only to an alternatively spliced variant of fibronectin containing the IIICS region. A 10-amino acid sequence from this region, GPEILDVPST, bound to the $\alpha_4\beta_1$ integrin and inhibited the spreading of WEHI cells on the IIICS form of fibronectin, an RGD-independent process[61]. Using a series of peptides derived from CS-1, the tripeptide LDV was identified as the minimal-length sequence capable of supporting melanoma[62] or lymphocyte adhesion if the cells express an active $\alpha_4\beta_1$ complex[63]. Resting cells did not bind to LDV peptide conjugates, suggesting that cell binding can be regulated by $\alpha_4\beta_1$ expression and $\alpha_4\beta_1$ complex activation state.

The heparin binding domain of fibronectin contains a third binding site that supports $\alpha_4\beta_1$-dependent cell adhesion. The active sequence was identified as IDAPS, similar to the LDVPS site from CS-1 (Ref. 64).

The fibronectin peptide RGDSPASSKP inhibited binding of primary mesenchyme cells from the sea urchin *Clypeaster japonicus* to fibronectin. The sequence PASS was effective in inhibiting this adhesion while the peptides RGDS and GRGDSP were not[65].

An 11-amino acid peptide from the ninth type-III repeat of fibronectin (DRVPHSRNSIT) inhibited ligand binding to $\alpha_{IIb}\beta_3$ (Ref. 66). Modeling studies suggested that this site is at least 25 Å from the RGD site. Although binding to the β_3 chain of

$\alpha_{IIb}\beta_3$, the peptide failed to inhibit binding of fibronectin to $\alpha_v\beta_3$, indicating differential recognition of the β_3 integrin subunit.

Laminin

Laminin, which consists of three polypeptide chains, α1, β1 and γ1, is a self-aggregating multifunctional glycoprotein of the extracellular matrix and is implicated in cell attachment (Figure 5). Several different isoforms of laminin have been identified, and adhesive sequences found in laminins are given in Table 4. The N-linked HNK-1 carbohydrate (hexaosylceramide) is expressed by several neural recognition molecules and has been implicated in cell–cell and cell–matrix adhesion involving the G2 domain of the α1 laminin chain. A 21-mer from this domain, KGVSSRSYVGCIKNLEISRST, was found to bind to HNK-1 and mediate neural cell adhesion to laminin[67]. The sequence IKVAV within the longer peptide CSRARKQAASIKVAVSADR from the α1 chain was identified as an adhesion site[68].

Many different sequences in laminin-1 have been claimed to have cell adhesive activity. Laminin binds to $\alpha_2\beta_1$ integrin, and the RGDN sequence in laminin, found within the larger sequence CQAGTFALRGDNPQG, has been identified as a cell adhesion site[69]. In studies of the expression of fibronectin and vitronectin receptors on endothelial cells, a control peptide, WWELR, was used which inhibited endothelial cell adhesion to laminin-1 and collagen types I and IV. Subsequent investigations identified an inhibitory motif consisting of two aromatic residues followed by an acidic residue, a hydrophobic residue and a basic residue[70]. Specific inhibitory sequences identified included FYFDLR and YGYYGDALR, the latter similar to the proposed motif. From examination of a series of peptides derived from the G domain of the A chain of murine laminin, four peptides were found that promoted the adhesion and spreading of HT-1080 human fibrosarcoma cells[71]. Adhesion to two of these peptides, KNLEISRSTFDLLRNSYGVRK and DGKWHTVKTEYIKRKAF, was inhibited by antibodies against the β_1 integrin subunit, suggesting that laminin has multiple sites comprising its integrin recognition domain. The sequence YIGSR of the β1 chain has been studied as an adhesion site[72]. The peptide RYVVLPRPVCFEKGMNYTVR from the β1 chain supported the adhesion of various cell lines[73]. Further work refined the location of the adhesion domain to the sequence RYVVLPR, which is located 265 amino acids from the sequence YIGSR (Ref. 74). Synthetic peptides from the β1 chain were examined for their ability to support adhesion of melanoma cells. Using this approach, the sequence PDSGR was identified as an adhesive site[75].

From the cDNA of the laminin receptor, the peptide IPCNNKGAHSVGLMWWMLAR was deduced and found to bind laminin and prevent the attachment of melanoma cells to bovine aortic endothelial cells and human umbilical vein endothelium[76].

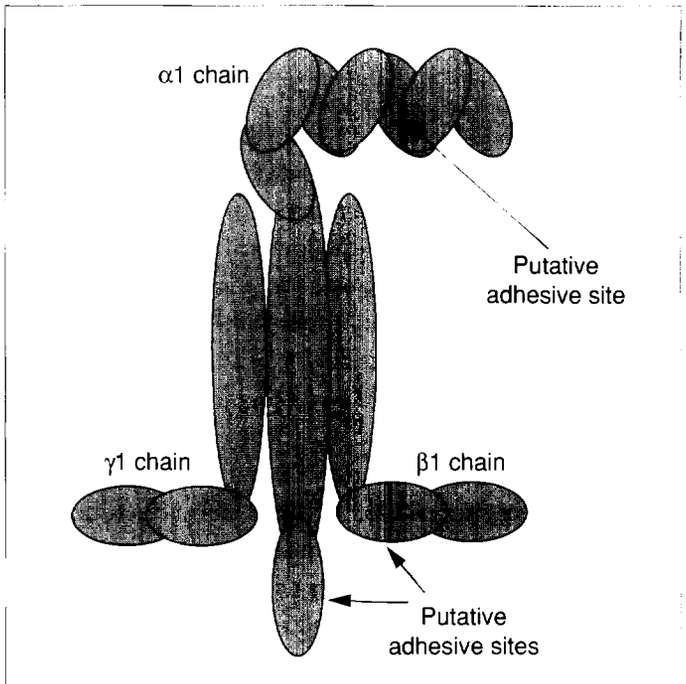

Figure 5. *A stylized rendering of the laminin structure consisting of three nonidentical associated chains.*

Table 4. Cell adhesion sequences involved in laminin-dependent adhesion

Molecule	Sequence	Ligand
Laminin	KGVSSRSYVGCIKNLEISRST	?
Laminin	IKVAV	?
Laminin	RGDN	?
EC with laminin[a]	WWELR	?
Laminin	FYFDLR	?
Laminin	YGYYGDALR	?
Laminin	KNLEISRSTFDLLRNSYGVRK	β1
Laminin	DGKWHTVKTEYIKRKAF	β1
Laminin	YIGSR	?
Laminin	RYVVLPRPVCFEKGMNYTVR (RYVVLPR)	?
Laminin	PDSGR	?
Laminin receptor (?)	IPCNNKGAHSVGLMWWMLAR	Laminin
Laminin	LRE	?

[a]EC, endothelial cell

Laminin-3 is concentrated in a subset of basal laminae and contains a neuronal attachment site in the C-terminus. Synthetic peptides were used to localize a primary adhesive site with the sequence LRE (Ref. 77).

Additional adhesive proteins

Several other proteins have been implicated in cell adhesion processes. Adhesive domains are given in Table 5.

Vitronectin. The plasma component vitronectin was found to contain the cell attachment sequence RGDV, which bound to $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_{IIb}\beta_3$ (Ref. 78).

C-reactive protein. The dodecapeptide TKPLKAFTVCLH from C-reactive protein, an acute-phase blood protein that accumulates at sites of tissue damage, inhibited the adhesion of fibroblasts to fibronectin. Structure–activity studies have identified FTVCL as the minimum sequence for cell attachment or inhibition[79].

Cadherins. The cadherins are a family of transmembrane glycoproteins involved in calcium-dependent homotypic cell–cell adhesion at cellular junctions[80]. Using an immobilized peptide ELISA assay, the extracellular domains of E-cadherin (epithelial cadherin) were scanned for the epitope of a blocking anti-E-cadherin monoclonal antibody. Two peptide sequences were identified, one containing the cadherin conserved sequence HAV and the second containing the conserved putative calcium binding sequence DQND (Ref. 81). The synthetic peptide LRAHAVDVNG inhibits the compaction of mouse embryos and neurite growth by inhibiting cell–cell adhesion[82].

Table 5. Additional cell adhesion sequences involved in adhesion[a]

Molecule	Sequence	Ligand
C-reactive protein	TKPLKAFTVCLH (FTVCL)	?
E-cadherin	LRAHAVDVNG (HAV)	?
Collagen	DGEA	$\alpha_2\beta_1$
Collagen	GVKGDKGNPGWPGAP	?
Collagen	GRGDTP	?
Collagen	GEFYFDLRLKGDK	?
Kininogen	CNAEVYVVPWEKK	?
Kininogen	RGD	$\alpha_v\beta_3$
Entactin	SIGFRGDGQTC	$\alpha_v\beta_3$
vWF	CQEPGGLVVPPTDAP	GPIb
vWF	LCDLAPEAPPPTLPP	GPIb
vWF	DMMERLRISQKWVRV	GPIb
vWF	KDRKRPSELRRIASQ	GPIb
vWF	RMSRNFVRYVQGLKK	GPIb
GP80	YKLNVNDS	?
Plasmodium falciparum infected erythrocytes	KLIKIFQDHPLQKTYNYVL-MVPKQGPLPN and KPPKY-HPDVPYVKRVKTWRMH	?
Thrombospondin	CSVTCG	?
Amyloid P component	FTLCFR	?

[a]GP, glycoprotein; vWF, von Willebrand factor

Collagen. The collagen family consists of homo- or heterotrimeric structural glycoproteins containing at least one triple helical domain containing the repeated Gly-X-Y sequence. On some cell types, the $\alpha_2\beta_1$ integrin binds to a site within the α1(I)-CB3 fragment of type-I collagen. The sequence DGEA within type-I collagen was found to inhibit platelet–collagen adhesion mediated by the $\alpha_2\beta_1$ integrin but not by $\alpha_5\beta_1$ or $\alpha_6\beta_1$ (Ref. 83). The sequence GVKGDKGNPG-WPGAP from type-IV collagen supports melanoma cell adhesion[84]. Collagen also contains an RGD sequence (RGDT) involved in cell adhesion[85]. Although the synthetic peptide RGDS inhibited attachment of cells to fibronectin, it did not block cell adhesion to collagen; however, the peptide GRGDTP did inhibit collagen-dependent cell adhesion[86], showing that, while RGD is a common recognition sequence for cell adhesion, specificity can be conferred by adjacent residues. The attachment of human keratinocytes to collagen was studied to identify binding sites[87]. One peptide, GEFYFDLRLKGDK, was found to bind heparin and support keratinocyte adhesion.

Kininogen. Kininogen is a multidomain protein that binds to platelets, endothelial cells and neutrophils in the intravascular compartment through a binding site in domain 3 (Ref. 88). Mapping of the epitope of a monoclonal antibody that binds to domain 3 and blocks adhesion to platelets identified CNAEVYVVPWEKK as an adhesive site[89].

Entactin. Entactin is a sulfated glycoprotein basement membrane component that binds avidly to both laminin and type-IV collagen and has been shown to support neutrophil adhesion. It consists of three globular domains and a rigid stalk that generate an asymmetric dumbbell-like structure. Globular domains G1 and G2, located at the N-terminal portion of the molecule, are attached by a thread-like structure. These are connected by the stalk, consisting of four EGF-like repeats and a thyroglobulin-like repeat, to globular domain G3 at the C-terminal end of the molecule[90]. The first EGF domain contains an RGD motif within the sequence

SIGFRGDGQTC that is involved in cell adhesion and is recognized by the $\alpha_v\beta_3$ integrin receptor[91].

von Willebrand factor. Two discontinuous but spatially adjacent sequences, CQEPGGLVVPPTDAP and LCDLAPEAPPPTLPP binding to GPIb were identified[92]. Proteolytic fragments and overlapping synthetic peptides defined additional binding domains within sequences DMMERLRISQKWVRV, KDRKRPSELRRIASQ and RMSRNFVRYVQGLKK. A distinct binding site on vWF for $\alpha_{IIb}\beta_3$ has been partially characterized[93].

GP80. The cell surface glycoprotein GP80 mediates cell–cell binding important in normal morphogenesis during the development of *Dictyostelium discoideum*. Activity was initially mapped to a 51-mer located within the first globular repeat of the N-terminal domain and localized to the sequence YKLNVNDS (Ref. 94).

Pertussis toxin. Peptides from the putative carbohydrate recognition domains of pertussis toxin inhibit *B. pertussis* adherence to macrophages[95]. The dipeptides LD-NH_2 and LN-NH_2 were found to inhibit platelet aggregation induced by collagen, ADP or adrenaline[96].

Plasmodium falciparum. Peptides from a membrane protein of *P. falciparum* infected erythrocytes were shown to inhibit adhesion of these cells to C32 amelanotic melanoma cells[97]. Active sequences are contained within the peptides KLIKIFQDHPLQKTYNYVLMVPKQGPLPN and KPPKYHPDVPYVKRVKTWRMH.

Thrombospondin. Thrombospondin supports attachment of several cell types. The thrombospondin hexapeptide CSVTCG was shown to support CD36-dependent tumor cell adhesion[98].

Amyloid P. The peptide FTLCFR, a sequence of amyloid P component, was found to support the adhesion of a wide variety of cell types, including osteoblasts, fibroblasts and melanoma cells[99].

Compounds used to inhibit the cell adhesion process

Although there are numerous inhibitors of various cell adhesion processes under clinical investigation, only one compound, ReoPro, has been approved. ReoPro (7E3), a Fab fragment of a human/murine chimeric IgG monoclonal antibody that binds to integrin $\alpha_{IIb}\beta_3$ and inhibits platelet aggregation, has been shown to be effective in high-risk patients in preventing cardiovascular ischemic complications and late restenosis after percutaneous coronary revascularization[100].

Potential applications and therapeutic targets

There are no clinical conditions in which overexpression of adhesion molecules is the primary causative factor, although genetic disorders have been identified in which the synthesis or expression of certain adhesion molecules is reduced. Upregulation of membrane-bound adhesion molecules and increases in soluble levels have been observed in numerous conditions, including inflammation, transplantation, infection and cancer. It has been suggested that, in some instances, this increase in soluble levels is an attempt to downregulate membrane-bound adhesion. In such cases, cell adhesion antagonists could be useful in affecting disease progression. Peptides, carbohydrates, soluble adhesion molecules, chimeric constructs and monoclonal antibodies have been evaluated in animal models involving cell adhesion.

Adhesion molecules have been shown to play a role in synovial pannus formation in rheumatoid arthritis[101]. An antibody against CD11a showed increased survival in rats in a model of pulmonary ischemia–reperfusion[102]. An antibody against CD11b afforded increased graft survival in a pig-to-dog renal xenograft model[103]. Peptides from the CS-1 and RGD domains of fibronectin as well as peptides from the heparin binding domain were found to inhibit leukocyte recruitment and the progression of arthritis in a rat model[104]. A monoclonal antibody blocking PECAM-1 was effective in a murine model of acute inflammation[105]. Apoptosis in tumor cell lines to promote tumor regression has been demonstrated with the peptide cyclo(RGDFV)[106], an $\alpha_v\beta_3$ antagonist, and a 16-mer branched construct of the laminin peptide YIGSR (Ref. 107). Monoclonal antibodies against integrins have been shown to regulate *in vitro* adhesion of human breast cancer cells[108], sugesting that cell adhesion antagonists may be useful as antitumor agents.

Summary

The study of adhesion molecule structure has led to the identification of a number of active site motifs, as well as totally unrelated sequences that support or block cell adhesion. These have increased the understanding of cell adhesion on a molecular level and have provided targets for the design of modulators of various adhesive processes. The use of the monoclonal antibody ReoPro, an inhibitor of $\alpha_{IIb}\beta_3$-dependent platelet aggregation approved for use in high-risk angioplasty, has validated the concept that compounds that inhibit cell adhesion are important additions to the physician's armamentarium.

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