Recent advances in cell adhesion molecules and extracellular matrix proteins: potential clinical implications

Shaker A. Mousa and David A. Cheresh

The roles of cell adhesion molecules (CAMs) and extracellular matrix proteins (ECMs) in various pathological processes leading to both acute and chronic disease states have recently been documented. The authors report on the development of various therapeutic and diagnostic candidates based on the key role of CAMs in a range of diseases, and discuss the structure–function aspects of cell adhesion and signaling of the different CAMs/ECMs.

Several physiological processes, including cell activation, migration, proliferation and differentiation, require direct contact between cells or extracellular matrix proteins. Cell–cell and cell–matrix interactions are mediated through several different families of cell adhesion molecules (CAMs), including the selectins, the integrins, the cadherins and the immunoglobulins. Newly discovered CAMs, together with the discovery of new roles for integrins, selectins and immunoglobulins in certain disease states, provide great opportunities for the development of therapeutic, and perhaps diagnostic, modalities[1].

Intensified R&D efforts, directed at manipulating CAM activity through the use of monoclonal antibodies, peptides, peptidomimetics and nonpeptide small molecules, continue to broaden the scope of key clinical applications (Table 1). This article focuses on current advances in the discovery and development of novel anti-CAM agents for potential therapeutic and diagnostic applications.

Roles of CAMs in disease states

Cell adhesion molecules play highly significant roles both in normal and in various pathophysiological disease states. For this reason, the selection of a specific and relevant CAM to target a certain disease condition without interfering with other normal cellular functions is a very important prerequisite for ultimate success in developing truly active and safe therapeutic strategies[2,3]. Recent breakthroughs with animal models, the latest advances in the understanding of the signaling pathways, transcriptional regulation and the structure–function aspects of CAMs, as well as the role of CAMs and extracellular matrix proteins (ECMs) in cellular migration, spreading, proliferation and survival, have highlighted the potential for novel strategies based on CAM function.

There have been exciting advances in understanding several CAMs; most notably, the $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_4\beta_1$ and $\alpha_{IIb}\beta_3$ integrin receptors and their direct relationships to different disease states represent tremendous therapeutic and diagnostic opportunities[1–8]. Potential roles for specific CAMs in different disease states, including cardiovascular, cancer, inflammatory, ocular, pulmonary, bone, central nervous system, kidney and gastrointestinal system disorders, have been suggested, including the following examples:

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- The role of integrin $\alpha_{IIb}\beta_3$ in the prevention, treatment and diagnosis of various thromboembolic disorders[1,4–8].
- The potential role of antiselectins in the prophylaxis and prevention of different diseases states[1].
- The role of β_1, together with other leukointegrins, in various inflammatory conditions[9–14].
- The key role of certain signaling mechanisms in mediating specific CAM functions[15].
- The potential utility of various soluble adhesion molecules (sICAM, sVCAM, selectins) as surrogate markers for acute and chronic endothelial injury (i.e. acute and chronic inflammatory states)[16,17].
- The potential role of $\alpha_V\beta_3$ in angiogenesis and osteoporosis[18,19].

Selectins

The selectins, a family of cell adhesion receptors, consist of three cell adhesion molecules unified structurally by the inclusion of lectin (L), epidermal growth factor (EGF)-like (E) and complement binding-like (C) domains. Functionally, the selectins are unified by their ability to mediate cell binding through interactions between their lectin domains and cell surface carbohydrate ligands[20]. The family includes the E-, L- and P-selectins. The P- and E-selectins are calcium-dependent cell-surface lectins (on platelets or endothelial cells), which mediate leukocyte adhesion by recognition of cell-specific carbohydrate ligands. L-selectins are found on all leukocytes and bind to counter-receptors (Gly-CAM-1; a mucin-like endothelial glycoprotein[21]) on endothelial cells. The expression of E-selectin is induced by various inflammatory stimuli, and it recognizes cell-surface carbohydrate sialyl Lewis X (sLe^X)[22]. P-selectin is stored in alpha granules of platelets and is also found in Weibel–Palade bodies of endothelial cells; it recognizes a carbohydrate that is closely related to sLe^X (Ref. 23).

The selectin family of cell adhesion molecules plays a key role in the mediation of early neutrophil (PMN) rolling on, and adherence to, endothelial cells (EC). P-selectin on platelet (P) and EC surfaces and L-selectin on the leukocyte surface act in concert to promote PMN–EC and PMN–P interactions. Monoclonal antibodies, which neutralize either P-selectin or L-selectin, have been found to preserve endothelial and monocyte cell function in a myocardial ischemia/reperfusion injury model[24,25].

The potential role of P-selectin in thrombosis initiation and stabilization, various inflammatory disorders and in restenosis is under investigation[26]. In models of ischemia/reperfusion injury, it has been demonstrated that L- and P-selectins participate in the initial rolling of leukocytes, infiltration of further neutrophil pools and EC dysfunction[26]. Blockade of L- and P-selectins with monoclonal antibodies (DREG-200 and PB1.3) in a cat model of myocardial ischemia/reperfusion reduced endothelial dysfunction, decreased infarct size and improved myocardial function (reversal of myocardial stunning and restoration of myocardial contractility).

L-selectin is involved in mediating neutrophil rolling interactions at sites of inflammation[26]. Expression of L-selectin is regulated by cleavage at a membrane-proximal site by an unusual proteolytic activity. An inhibitor of L-selectin protease was shown to dramatically affect neutrophil rolling behavior under hydrodynamic flow by prolonging transient attachment times to immobilized L-selectin ligands[27]. These results suggest that L-selectin is cleaved in seconds – the time-course of these rolling interactions – and that shedding of L-selectin contributes to the velocity of leukocyte rolling.

Antiselectins in development

Various humanized monoclonal antibodies, oligosaccharides or small-molecule anti-E-, P- and L-selectins are under preclinical

Table 1. Targeting of cell adhesion molecules for therapeutic and diagnostic applications

Therapeutic area	Diseases	CAM
Cardiovascular	Thromboembolic disorders (therapeutic and diagnostic)	$\alpha_{IIb}\beta_3$, selectins
	Restenosis	$\alpha_V\beta_3$, selectins
	Atherosclerosis	β_1, β_2, VCAM-1
Inflammatory	Rheumatoid arthritis, osteoarthritis, transplantation	β_1, β_2, selectins, ICAM-1
Cancer	Metastasis	$\alpha_V\beta_3$, β_1, β_4, β_5
Ocular	Diabetic retinopathy, macular degeneration	$\alpha_V\beta_3$, $\alpha_V\beta_5$
Pulmonary	Asthma, allergy	$\alpha_4\beta_1$, VCAM-1
Bone	Osteoporosis	$\alpha_V\beta_3$, β_1, β_5
Central nervous system	Multiple sclerosis, neurological disorders	$\alpha_4\beta_1$, VCAM-1, ICAM-4
Gastrointestinal tract	Bowel diseases	β_1, β_4, β_6, β_7
Kidney	Renal failure	β_1

investigation for treatment of ischemia/reperfusion injury or various inflammatory disorders[26]. Furthermore, distinct myocardial protective efficacy for the oligosaccharide sLe^X has been shown in a canine model of ischemia/reperfusion injury and hence has potential as an adjunct to thrombolysis postangioplasty. Also, preclinical investigations with Cylexin (Cy1503) in a canine model of coronary thrombosis/rethrombosis have demonstrated the potential utility of Cylexin as an adjunct to thrombolysis in reducing the incidence of rethrombosis and in reducing infarct size[28].

The potential of P-selectin antagonists (GA6 and Cy1418) in the initiation and stabilization of arterial and venous thrombosis, as well as in restenosis, has been suggested to be mediated via tissue factor blockade with the subsequent inhibition of fibrin deposition[29]. In a primate model of acute thrombosis, administration of GA6 before streptokinase resulted in enhanced thrombolysis. In canine and primate models of deep venous thrombosis, the antithrombotic efficacy of Cy1747–1748 (1.0 mg/kg, intravenous dose) compared favorably with heparin in preventing the incidence of thrombosis, reducing thrombus weight and in improving vessel patency. In a primate model of carotid artery restenosis, GA6 achieved a 25% reduction in the neointimal/medial ratio after 14 days[30].

A structural model of selectin–sLe^X interactions was used in the design and synthesis of nonoligosaccharide selectin inhibitors[26]. The compounds developed have greater *in vitro* potency than the parent sLe^X tetrasaccharide, are efficacious in large animal models at low doses, show no overt toxicity even at very high doses and are currently in preclinical development. While there have been major advances in the design of peptidomimetics, this work may represent the first successful report of glycomimetic rational design.

Integrins

Integrins are a widely expressed family of cell adhesion receptors that mediate either homotypic or heterotypic cell-to-cell adhesion or cell-to-ECM adhesion. All integrins are composed of αβ heterodimeric units, which are expressed on a wide variety of cells, most cells expressing several integrins (Figure 1). The interaction of integrins with the cytoskeleton and extracellular matrix appears to require the presence of both subunits. Binding of integrins to their ligands is cation-dependent, and they appear to recognize specific amino acid sequences. The most well studied is the RGD sequence found in some matrix proteins, including fibrinogen, vitronectin, fibronectin, thrombospondin, osteopontin and von Willebrand factor. However, other integrins bind to ligands via a non-RGD-binding domain, such as the $\alpha_4\beta_1$ integrin receptors that bind and recognize the LDV sequence within the CS-1 region of fibronectin. There are at least eight known β subunits and 14 α subunits[2,3]. Although combinations of the eight and 14 different β and α subunits could theoretically give rise to more than 100 integrins, the actual diversity is much more restricted (Figure 1). A model of integrin $\alpha_v\beta_3$ heterodimeric structure is shown in Figure 2.

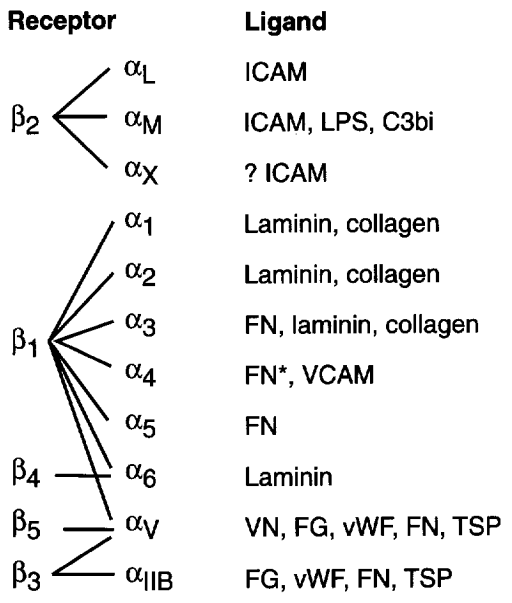

Figure 1. *Integrins and corresponding ligands. The integrin family of CAMs consist of multiple heterodimeric forms of α and β subunits associated as shown. The α_4, α_6 and α_V subunits can associate with other β subunits such as β_7, β_4 and β_5, β_6, β_8, respectively. LPS, lipopolysaccharide; FN, fibronectin; FG, fibrinogen; TSB, thrombospondin; VWF, von Willebrand factor.*

Coordinated regulation of cell adhesion and signaling

Integrin adhesion receptors contain an extracellular face, which engages adhesive ligands, and a cytoplasmic face, which engages intracellular proteins (Figure 2). These interactions are critical for cell adhesion and for anchorage-dependent signaling reactions in normal and pathological states (Figure 3). For example, platelet activation induces a conformational change in integrin $\alpha_{IIb}\beta_3$, thereby converting it into a high-affinity fibrinogen receptor. Fibrinogen binding then triggers a cascade of protein tyrosine kinases and

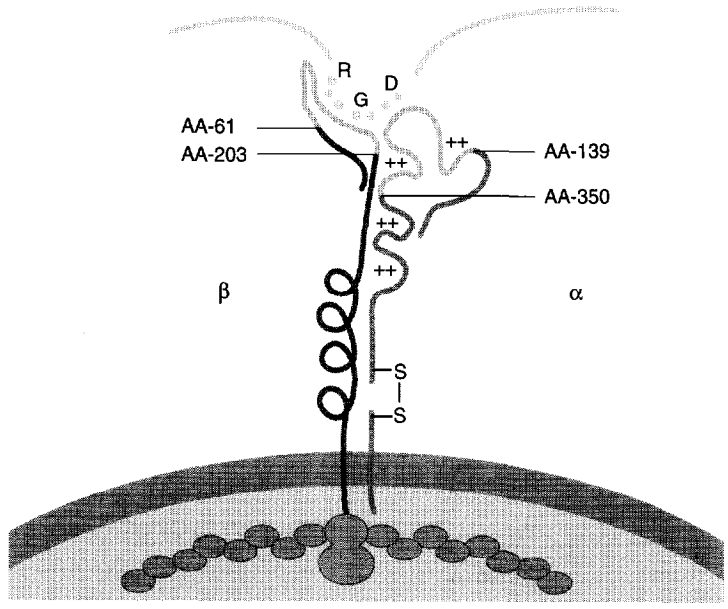

Figure 2. *Model of $\alpha_V\beta_3$ integrin in a cell membrane, as exemplified by the vitronectin receptor.*

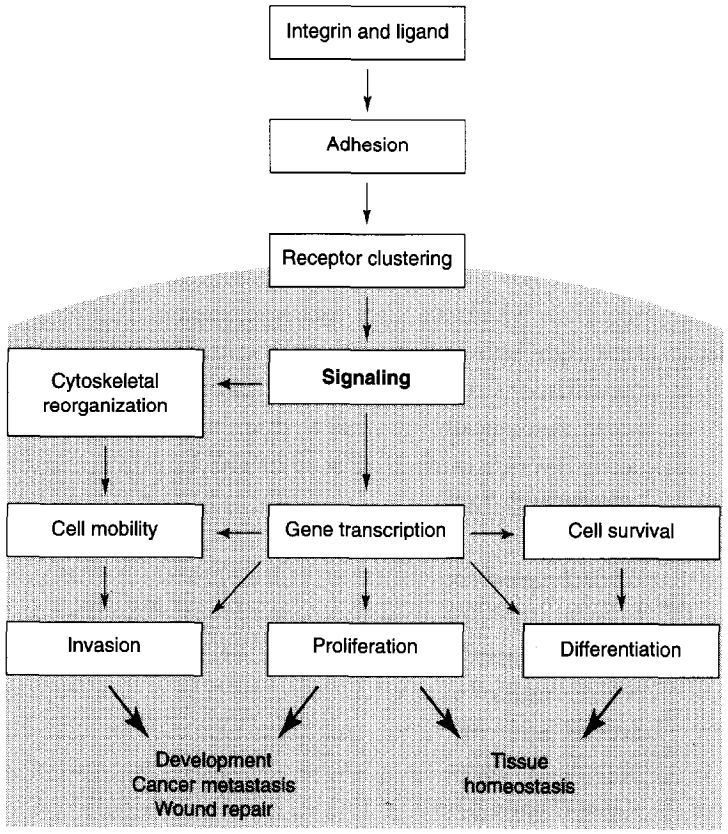

Figure 3. *Outside–in signaling.*

phosphatases and recruitment of numerous other signaling molecules into F-actin-rich cytoskeletal assemblies in proximity to the cytoplasmic tails of α_{IIb} and β_3. These dynamic structures appear to influence platelet functions by coordinating signals emanating from integrins and G-protein-linked receptors. Studies of integrin mutations confirm that the cytoplasmic tails of $\alpha_{IIb}\beta_3$ are involved in integrin signaling, presumably through direct interactions with cytoskeletal and signaling molecules. Blockade of fibrinogen binding to the extracellular face of $\alpha_{IIb}\beta_3$ has been shown to be an effective way to prevent the formation of platelet-rich arterial thrombi after coronary angioplasty[31]. Once proteins that interact with the cytoplasmic tails of $\alpha_{IIb}\beta_3$ are fully identified, it may also be possible to develop selective inhibitors of integrin adhesion or signaling whose locus of action is inside the cell.

α_4 integrins

The α_4 integrins are heterodimeric cell surface molecules that are central to leukocyte–cell and leukocyte–matrix adhesive interactions. The integrin $\alpha_4\beta_7$, which is expressed on all leukocytes except neutrophils, interacts with the immunoglobin (Ig) superfamily member VCAM-1, and with an alternately spliced form of fibronectin (FN). The integrin $\alpha_4\beta_7$ is found only on leukocytes and can bind not only to VCAM-1 and FN, but also to MAdCAM, a mucosal vascular addressin that is preferentially expressed by venular endothelial cells and contains Ig-like domains related to VCAM-1. Peptide-based analogs based on various regions in the first and second domains of MAdCAM-1 (which binds the lymphocyte homing receptor for Peyer's patches[12]) for binding to $\alpha_4\beta_7$ have been identified.

Certain monoclonal antibodies to the α_4 chain and $\alpha_4\beta_7$ can block their *in vitro* adhesive function. *In vivo* studies with these antibodies in several species have shown that the interactions between these integrins and their ligands play a key pathophysiological role in immune and inflammatory reactions. Thus, α_4-integrin-dependent adhesive interactions with VCAM-1, MAdCAM and FN appear to play a central role in the recruitment, priming,

activation and apoptosis of certain leukocyte subsets, and offer novel targets for drug intervention. To this end, a selective and potent anti-α_4 monoclonal antibody and small-molecule antagonists were designed. *In vivo* efficacy of these molecules has been demonstrated in several animal models[10,11].

Infiltration of circulating immune cells into the CNS, resulting in edema, myelin damage and paralysis, has been documented[32]. The role for the adhesion molecule $\alpha_4\beta_1$ integrin in this process has been demonstrated. When administered to animals with experimental autoimmune encephalomyelitis, antibodies against α_4 integrin prevented the adhesion of lymphocytes and monocytes to inflamed endothelium within blood vessels of the CNS, and prevented immune cell infiltration[33]. Even when administered to animals after the onset of paralysis, anti-α_4 integrin reversed all clinical signs of disease. MRI analysis of these animals showed that antibody treatment reduced edema and permeability of the blood–brain barrier, while histological analysis demonstrated that treatment prevented the destruction of myelin. Remarkably, anti-α_4 integrin reversed the accumulation of lymphocytes and monocytes within the CNS, but did not affect the level of the cells in the circulation. The results suggest that the active disease process requires an ongoing recruitment of circulating cells into the CNS, and that anti-α_4 integrin prevents this recruitment and reverses disease progression. AN100226 (Athena Neurosciences) is a humanized antibody against α_4 integrin that might have potential for the treatment of multiple sclerosis[34].

β_1 integrins

Most integrins are members of the β_1 integrin family, which is also known as the VLA subfamily because of the late appearance of VLA (very late antigen) after activation. At least seven receptors of this subfamily have been characterized, each with different ligand specificities. Among the most studied are the $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$ and $\alpha_N\beta_1$ receptors.

The leukocyte integrin $\alpha_4\beta_1$ (also known as VLA-4 and CD49d/CD29) is a cell adhesion receptor that is predominantly expressed on lymphocytes, monocytes and eosinophils[9], and is a potential therapeutic target in chronic inflammatory diseases. This leukocyte primarily mediates chronic inflammatory diseases, such as rheumatoid arthritis, asthma, psoriasis and allergy. In contrast, it is not present on circulating unstimulated neutrophils, which constitute a first line of defense against acute infections. The interaction of VLA-4 with alternately spliced FN containing CS-1 has been utilized in targeting small-molecule inhibitors to the VLA-4 integrin receptor. Evaluation of these analogs in animal models of disease indicates that VLA-4 blockade has the potential to achieve dramatic *in vivo* effects in a range of chronic inflammatory disorders[9–11]. Examples of $\alpha_4\beta_1$ antagonists under either preclinical or clinical investigation are given in Table 2.

Eosinophils selectively accumulate at sites of chronic allergic diseases, such as bronchial asthma. The role of β_1 integrin and its regulation by cytokines and other inflammatory mediators during eosinophil adhesion to endothelium, extracellular matrix proteins and transendothelial migration have been well documented[13,14].

The inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis, are immunologically mediated illnesses. Expression of the β family of integrins in isolated intestinal lamina propria mononuclear cells from IBD and normal intestine demonstrated a pattern of integrins with more β_1 than is normal in Crohn's disease[35].

Secretion of extracellular matrix and increased suprabasilar expression of certain β_1 and β_4 integrins occurs with tumor formation of benign and malignant squamous cell neoplasms of the upper aerodigestive tract[36]. Increased suprabasilar expression is associated with early recurrence and is retained during tumor progression. These integrins are likely candidates for a functional role in the abnormal behavior of epithelial neoplasms and as targets for therapy, using integrin antagonists.

β_2 integrins

The leukocyte restricted β_2 (CD18) integrins promote a variety of homotypic and heterotypic cell adhesion events that are required for normal and pathological functioning of the immune system[15]. Several physiological processes, including cell adhesion, activation, migration and transmigration, require direct contact between cells or extracellular matrix proteins via CAM receptors (Figure 4). Only three members of this integrin subfamily have been identified: CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1) and CD11c/CD18 (P150,95). A molecularly cloned cDNA encoding a fourth α chain, designated α_d, which associates with CD18 in normal leukocytes upon cotransfection into Chinese hamster ovary cells, has recently been identified[37]. Its restricted display on specific leukocyte subsets in normal and pathological tissues suggests that expression of this α chain is distinct from that

Table 2. Selected companies with cell adhesion molecule programs indicating development status of pipeline products[a,b]

Company	Target	Product[c]	Development status
Athena Neurosciences/ Wyeth Ayerst	Multiple sclerosis	AN00226 humanized mAb	Phase II
Biogen	Inflammatory diseases	Humanized mAb to VLA-4 integrin	Phase I
Boehringer Ingelheim	Kidney and liver transplant, rheumatoid arthritis	Anti-ICAM-1 murine mAb	Clinical
Cell Genesys	Inflammatory diseases	Murine mAb to L-selectin	Preclinical
Centocor/Lilly	Antiplatelet: arterial thrombosis, unstable angina, adjunct to angioplasty	ReoPro, 7E3 mAb (IV)	Approved December 1994 for angioplasty; Phase III for other indications
COR Therapeutics	Antiplatelet: arterial thrombosis, unstable angina, acute myocardial infarction	Integrelin™ (IV)	Phase III for angioplasty; Phase II for unstable angina
Corvas International	Acute inflammatory diseases, ischemic stroke	Neutrophil inhibitory factor	Preclinical
Cytel	Reperfusion injury	Cylexin™	Phase II for myocardial infarction and surgical removal of clots
	Trauma	Cy1747–1748 humanized mAb to P-selectin	IND 1995
	Chronic inflammatory diseases	Analog of CS-1 fibronectin to VLA-4 integrin	Discovery
DuPont Merck	Coronary artery disease	DPM728 cyclic peptide $\alpha_{IIb}\beta_3$ (GPIIb–IIIa) antagonist (IV/oral)	Phase I
		DPM754	Clinical
Genentech	Trauma	Humanized mAb to CD18 subunit of Mac-1 integrin	Preclinical
Genetics Institute	Inflammatory diseases	Recombinant glycoprotein to block selectin	Preclinical
Glycomed	Adult respiratory distress syndrome	Celadin™	Preclinical
		Glycomimetics	Research
Hyal Pharmaceutical	Basal cell carcinoma, actinic keratosis, pain	Hyaluronic acid as drug delivery vehicle	Phase III
	Restenosis, angiogenesis, cancer, ischemia/reperfusion injury	Hyaluronic acid as drug delivery vehicle	Phase II
Hoffmann-La Roche	Antiplatelet: arterial thrombosis, unstable angina, adjunct to angioplasty	Ro449883, Lamifiban (IV)	Phase III
		Ro483657 (oral)	Phase II
ICOS	Multiple sclerosis	Hu23F2G humanized mAb	Phase I
	Organ transplantation	ICAM-3 modulator	Preclinical
	Atherosclerosis	α_d modulator	Preclinical
	Neurologic diseases	ICAM-4 modulator	Research
Isis Pharmaceuticals	Cardiac transplant rejection	ISIS 2302 antisense oligonucleotide to block ICAM-1	Phase I
IXSYS	Cancer	LM609 (Vitaxin)	Phase I
Merck	Antiplatelet: arterial thrombosis, unstable angina, adjunct to angioplasty	MK383 (IV)	Phase II/III
	Thyroid allograft rejection	Anti-LFA-1/CD11a	Discontinued
Microcarb (now Antex Biologics)	*H. pylori*, *S. pneumoniae*, *H. influenzae*	Carbohydrate-based adhesion vaccines	Preclinical

Monsanto/Searle	Antiplatelet: arterial thrombosis, unstable angina, adjunct to angioplasty	SC54684A, Xemilofiban (oral) Orbofiban (oral)	Phase III Phase II
Neose	Peptic ulcer, inflammatory disorders	NE0080 NE1405 oligosaccharide P-selectin ligand NE1704 oligosaccharide E-selectin	IND 1994 Research Research
Oxford GlycoSystems	Inflammatory disorders	Carbohydrate mimetics of sLe^X-like compounds	Preclinical
Protein Design Labs	Inflammatory disorders	SMART™ (proprietary antibody technology, also utilized to develop antivirals) humanized anti-L-, E-, P-selectins, anti-CD integrin subunit	Preclinical
Repligen/Eli Lilly	Inflammatory disorders, thoracoabdominal aortic aneurysm, coronary-pulmonary bypass, stroke, head injury	m60.1 murine mAb to leukocyte integrin	Phase I/II; humanized forms under evaluation
Scios Nova	Inflammatory disorders	Small-molecule inhibitors of leukocyte adhesion	Research
Telios/Eli Lilly	Antithrombosis, restenosis after angioplasty, stroke, organ transplantation, osteoporosis	TP9201 synthetic peptide binds to $\alpha_{IIb}\beta_3$ (GPIIb–IIIa) platelet receptor;RGD-containing compounds to block integrin receptors onosteoclasts	Preclinical
Texas Biotechnology	Inflammatory disorders (acute cardiovascular)	Synthesized small molecules to block E/P-selectin; small peptide analogs of VCAM–$\alpha_4\beta_1$ interaction	
Tularik/Yamanouchi	Inflammatory disorders	Small molecules to block transcription factors that activate CAM gene expression	Discovery
Karl Thomae, Zeneca, SmithKline Beecham, Glaxo, Sandoz, Takeda, Sanofi	Antiplatelet: arterial thrombosis, unstable angina, adjunct to angioplasty, stroke and peripheral artery diseases		Preclinical to Phase II clinical

[a]Includes platelet $\alpha_{IIb}\beta_3$ (GPIIb–IIIa) antagonists.
[b]Adapted from *Drug Market Dev.* (1995) 6, 15–16.
[c]mAb, monoclonal antibody; IV, intravenous.

of the other leukointegrins, and suggests a role in specific disease states in which macrophages figure prominently. The α_d protein incorporates an I domain homologous to that implicated in ligand binding for the other β_2 integrins, and displays selective recognition of specific ICAM family members, such as ICAM-3.

Recent studies *in vitro* have shown that LFA-1 (CD11a/CD18) and Mac-1 on neutrophils can be differentially activated for distinct functions[15]. In addition, investigations *in vivo*, including studies in CD11b-deficient mice, further underscore the biological significance of the distinct contributions of LFA-1 and Mac-1 to neutrophil-dependent tissue injury. A comparison of the ligand-binding properties and affinity regulation of α_d relative to other leukointegrins has been demonstrated. Examples of leukocyte integrin antagonists are given in Table 2.

β_3 integrins: $\alpha_{IIb}\beta_3$ integrin receptor antagonists

There is an urgent need for more efficacious antithrombic drugs that are superior to aspirin or ticlopidine for use in the prevention and treatment of various cardiovascular and cerebrovascular thromboembolic disorders. The realization

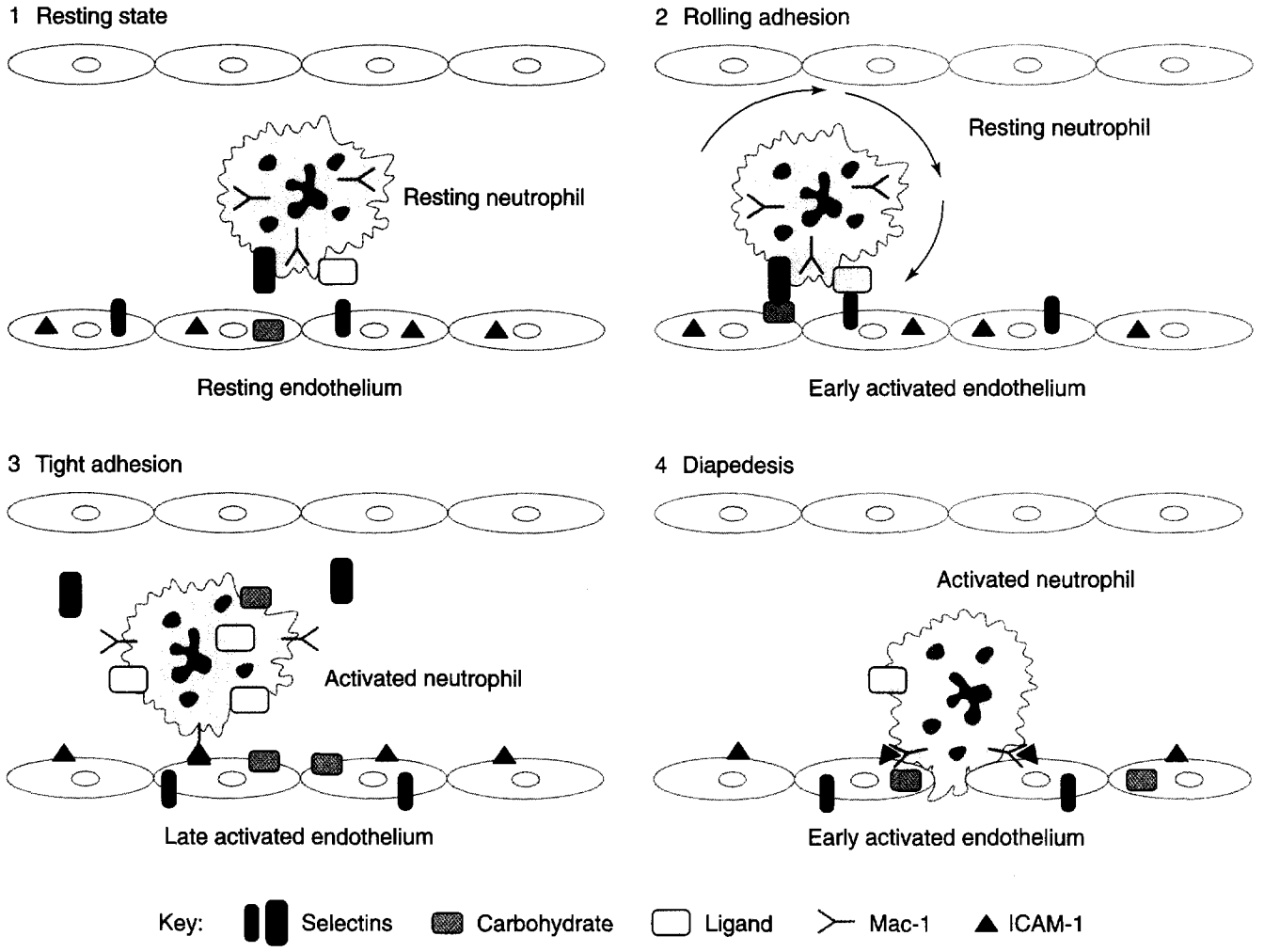

Figure 4. *The leukocyte–endothelial adhesion cascades, including: (1) resting state, (2) rolling adhesion, (3) tight adhesion and (4) diapedesis.*

that the platelet integrin $\alpha_{IIb}\beta_3$ is the final common pathway for platelet aggregation regardless of the mechanism of action, prompted the development of several small-molecule $\alpha_{IIb}\beta_3$ receptor antagonists for intravenous and/or oral antithrombotic applications[38]. Clinical experiences (efficacy/safety) gained with injectable $\alpha_{IIb}\beta_3$ antagonists will provide valuable insights into the potential of long-term chronic usage of oral $\alpha_{IIb}\beta_3$ antagonists. At this point, there are still many unanswered questions, and careful studies will be needed to elucidate the safety and efficacy of this mechanism either alone or in combination with antiplatelet/anticoagulant therapies[38]. However, platelet $\alpha_{IIb}\beta_3$ receptor blockade represents a very promising therapeutic and diagnostic strategy of thromboembolic disorders.

c7E3 (ReoPro). Following coronary angioplasty, patients are at risk of abrupt vessel closure and acute myocardial infarction, and may need repeated percutaneous transluminal angioplasty (PTCA) or coronary artery bypass grafting (CABG) procedures. The binding and aggregation of platelets at the angioplasty site is the crucial initiating event associated with these complications.

The monoclonal antibody c7E3 (ReoPro; Centocor/Lilly) binds to the $\alpha_{IIb}\beta_3$ receptor on platelets and inhibits aggregation, yet allows platelet adhesion to the injury site. It associates with the β_3 unit and thereby inhibits the integrin molecules $\alpha_{II}\beta_3$ and $\alpha_v\beta_3$. Recently completed Phase III trials have demonstrated effective reduction in postangioplasty occlusive events, as well as late events associated with restenosis[31]. The antibody was studied in

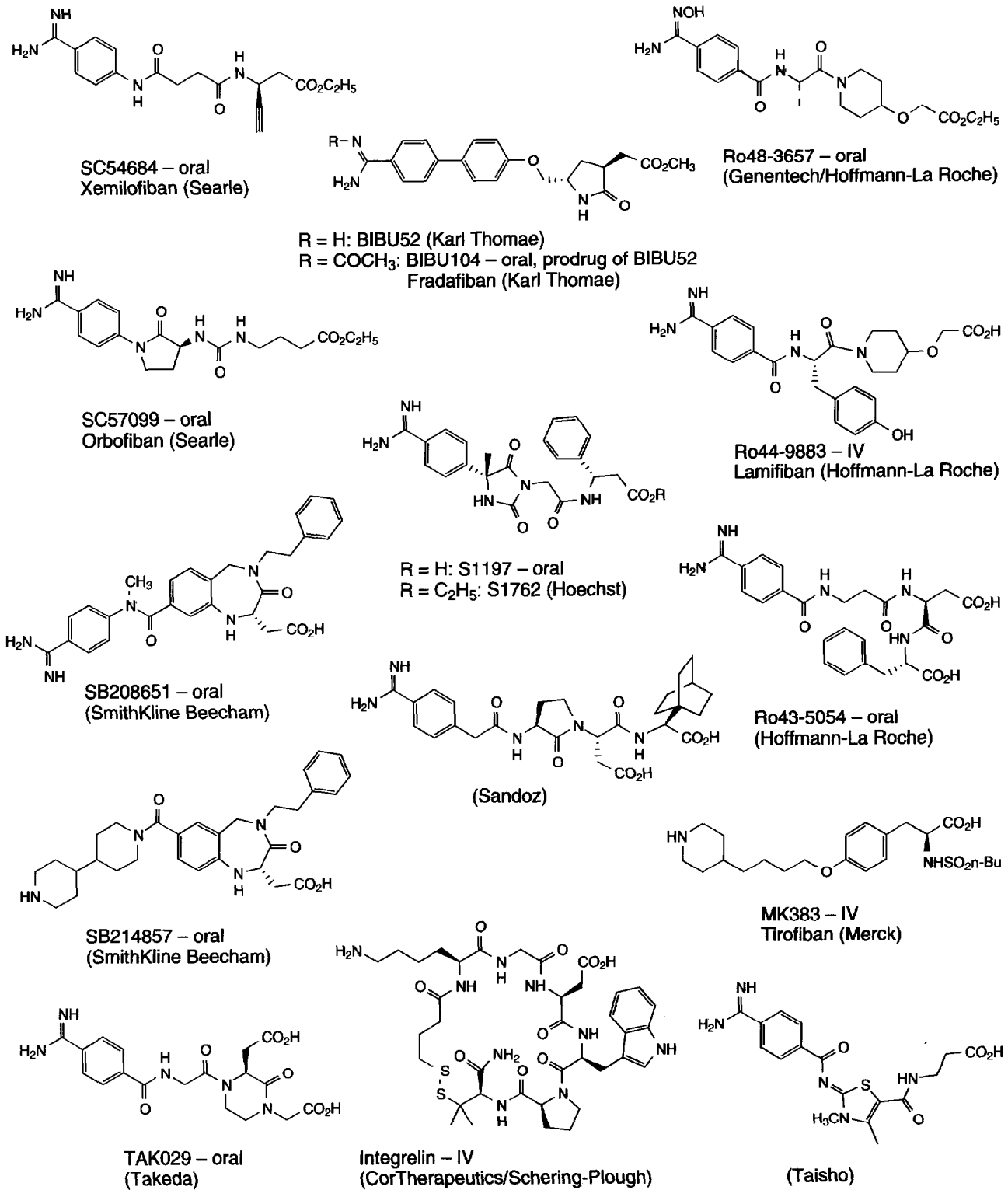

Figure 5. *Chemical structures of some platelet $\alpha_{IIb}\beta_3$ (GPIIb–IIIa) receptor antagonists that are currently in preclinical or clinical trials (see Table 2 for development status and disease target). The chimeric monoclonal antibody c7E3 (ReoPro) is the first FDA-approved $\alpha_{IIb}\beta_3$ antagonist.*

2,100 high-risk patients undergoing coronary angioplasty, where blockade of $\alpha_{IIb}\beta_3$ is known to prevent platelet aggregation[38]. Postangioplasty thrombotic events, including myocardial infarction, urgent re-PTCA or CABG, were reduced by over 30%. At six-months' follow-up, those patients treated with high-dose c7E3 had reduced clinical events consistent with a decrease in 'restenosis'. It is thought that blockade of $\alpha_V\beta_3$, the integrin associated with migration of endothelial cells and vascular smooth muscle cells, may be responsible for the observed reduction in six-month events. The scientific rationale for blocking this integrin receptor and clinical results have been documented for the early thrombotic events[8,31,39] (see Figure 5 and Table 2).

Cyclic peptide $\alpha_{IIb}\beta_3$ (GPIIb–IIIa) antagonists. A series of constrained RGD analogs with high affinity and specificity for the platelet $\alpha_{IIb}\beta_3$ receptors has been identified[7,8,38]. The pharmacological profiles and the potential therapeutic implications of a representative analog, namely DMP728, after intravenous or oral administration in cerebrovascular, cardiovascular and peripheral artery thromboembolic animal models of coronary, carotid and peripheral artery thrombosis have been documented[7,8].

MK383 (Tirofiban). This nonpeptide, small-molecular-weight $\alpha_{IIb}\beta_3$ inhibitor is active orally, but not by intravenous administration. It inhibits fibrinogen binding and hence platelet aggregation mediated by any of the known mechanisms of platelet activation. The *in vitro* and *in vivo* properties of MK383 as an antiplatelet and antithrombotic have been documented[38]. Additionally, clinical results with this agent (IV) in Phase II/III also illustrate the potential of such an inhibitor in acute thromboembolic disorders[39].

Orally active $\alpha_{IIb}\beta_3$ antagonists. A high level of platelet antagonism has been required when $\alpha_{IIb}\beta_3$ antagonists have been used for acute therapy in coronary arterial disease. However, the requirements for chronic therapy using orally active agents are only now being determined. Interaction with aspirin and other antiplatelet and anticoagulant drugs leads to shifts in the dose–response curves for both efficacy and unwanted side effects, such as increased bleeding time. As experience is gained with this new class of agents, the benefits and pitfalls associated with their use will become clearer (see Figure 5 and Table 2).

Platelet integrin $\alpha_{IIb}\beta_3$ receptor antagonists in the rapid diagnosis of thromboembolic events. Adhesion molecules play a major role in the pathogenesis of many disease states through their cell–matrix interactions[38]. They alter cellular functions upon binding to matrix proteins, which can be regulated by other pathogenic stimuli acting on the cell. Thus, targeting these receptors should have utility in the diagnosis of diseases characterized by cellular injury. In order for an agent to be useful as a diagnostic agent, it must be specific for the disease, as well as possess adequate contrast. Contrast imaging quality is the result of the combination of the rapid clearance of tissue background and the retention at the desired receptor site. The role of the platelet integrin $\alpha_{IIb}\beta_3$ receptor and its potential utility as a radiodiagnostic agent in the rapid detection of thromboembolic events has been demonstrated[40].

β_3 integrins: $\alpha_V\beta_3$ integrin

Examples of $\alpha_V\beta_3$ antagonists under preclinical investigation in different therapeutic areas are given in Table 2.

Role of integrin $\alpha_V\beta_3$ and matrix proteins in vascular remodeling. Vascular remodeling processes play a key role in the pathological mechanisms of atherosclerosis and restenosis. In response to vascular injury, for example by PTCA, matrix proteins such as osteopontin and vitronectin are rapidly upregulated[41]. Osteopontin stimulates smooth muscle cell migration via its action on the integrin $\alpha_V\beta_3$ and thereby contributes to neointima formation and restenosis[42,43]. In addition, the matrix proteins osteopontin and vitronectin induce angiogenesis, which may support neointima formation and arteriosclerosis[44]. Thus, specific matrix proteins via selected integrins, and especially $\alpha_V\beta_3$, may be important targets for selective antagonists aimed at blocking the pathological processes of restenosis[41].

Integrin $\alpha_V\beta_3$ antagonists and tumor regression. A single intravascular injection of a cyclic-peptide or monoclonal-antibody antagonist of integrin $\alpha_V\beta_3$ disrupts ongoing angiogenesis on the chick chorioallantoic membrane[18,19]. This leads to the rapid regression of histologically distinct human tumors transplanted onto this membrane. In fact, $\alpha_V\beta_3$ antagonists also prevent the spontaneous pulmonary metastasis of human melanoma cells. All human tumors examined in this model are $\alpha_V\beta_3$-negative, which suggests that these antagonists have no direct effect on the tumor

cells themselves. Induction of angiogenesis by a tumor or cytokine promotes entry of vascular cells into the cell cycle and expression of integrin $\alpha_v\beta_3$. After angiogenesis is initiated, antagonists of this integrin induce apoptosis of the proliferative angiogenic vascular cells, leaving pre-existing quiescent blood vessels unaffected.

These studies are supported by *in vitro* results[18,19]. Specifically, cultured human endothelial cells are protected from apoptosis when they are allowed to attach to immobilized anti-$\alpha_v\beta_3$ monoclonal antibody LM609 (Refs 9,10). The adhesion event appears to decrease expression of *p53* and *bax* while increasing that of *Bc1-2*. Ligation of $\alpha_v\beta_3$ is required for the survival and maturation of newly forming blood vessels, an event essential for the proliferation and metastatic properties of human tumors. Integrin $\alpha_v\beta_3$ is preferentially expressed on blood vessels undergoing angiogenesis. Antibody or peptide antagonists of this integrin have been shown to block angiogenesis in response to human tumors or purified cytokines in several preclinical models[18,19]. These inhibitors of $\alpha_v\beta_3$ promote selective apoptosis of newly sprouting vessels, preventing their maturation. These findings indicate that antibody or peptide antagonists of integrin $\alpha_v\beta_3$ may have a profound therapeutic value in the treatment of diseases associated with angiogenesis[18,19].

Potential role of $\alpha_v\beta_3$ antagonists in osteoporosis

RGD analogs have been shown to inhibit the attachment of osteoclasts to bone matrix and to reduce bone resorptive activity *in vitro*. The cell surface integrin, $\alpha_v\beta3$, appears to play a role in this process. Peptidomimetic antagonists of $\alpha_v\beta_3$, based on the RGD recognition sequence, were synthesized and evaluated in several assay systems. These compounds inhibited the binding of vitronectin to isolated $\alpha_v\beta_3$, inhibited $\alpha_v\beta_3$-dependent cell adhesion to vitronectin and reduced the hypercalcemic response to parathyroid hormone in parathyroidectomized rats. RGD analogs may represent a new approach to modulating osteoclast-mediated bone resorption and may be useful in the treatment of osteoporosis[45,46].

Integrin–matrix interactions in vascular injury

Smooth muscle cell (SMC) migration is a significant component of the pathophysiological response of the arterial wall to mechanical injury. Cell surface integrin–extracellular matrix interactions may play a vital role in regulating this migratory process. *In vitro* and *in vivo* studies, examining the effect of inhibition of β_3 integrin–matrix interactions on SMC migration and arterial wall thickening following PTCA/stent injury have been carried out[41]. Local manipulation of dominant cell integrin–matrix interactions involved in arterial-wall healing after injury may be a valuable approach in limiting restenosis.

Function of epithelial integrins

In vitro, epithelial integrins contribute to cell attachment, spreading, proliferation and survival, but the significance of these effects to the *in vivo* function of epithelial organs is poorly understood. To address this problem, a line of mice expressing null mutations of integrins that are expressed in epithelial cells was developed[47]. Mice expressing a null mutation of the β_6 subunit developed functionally significant inflammation in the lungs and skin, suggesting a role for β_6 integrin in downmodulation of inflammatory responses in these organs[47].

Selectin–integrin models for leukocyte recruitment

The specific recruitment of leukocytes to venules is central to their rapid localized accumulation during local inflammatory responses[48]. In acute-phase responses, initial leukocyte rolling on the endothelium can be mediated by leukocyte L-selectin and the inducible vascular adhesion proteins P- and E-selectin[26]. In late-phase inflammation, as well as during lymphocyte recirculation, lymphocyte L-selectin and the peripheral or mucosal vascular addressins can facilitate initial attachment. Activated $\alpha_4\beta_7$ integrin (LPAM-1) can also initiate lymphocyte attachment, as well as support the slowing of rolling and tight endothelial adhesion. Activation-dependent tight adhesion supported by leukocyte β_2 integrins can occur through G-protein-receptor signaling. The temporal role of these adhesion and associated receptors is under investigation[49].

Immunoglobulins

ICAMs and VCAMs are members of the Ig superfamily. Most current effort in targeting the Ig superfamily is focused on the development of specific monoclonal antibodies and/or antisense oligonucleotides and small molecules that might specifically block gene transcriptional factors[14,37]. Strategies for designing small-molecular-weight inhibitors for the Ig superfamily are somewhat more difficult. However, with current advances in molecular modeling and crystal structure information it might be possible to develop cyclic peptides and peptidomimetic Ig antagonists.

Examples of anti-Ig agents undergoing preclinical or clinical investigation are given in Table 2.

Several investigations with monoclonal antibodies to ICAM-1 have demonstrated anti-inflammatory properties, with tremendous therapeutic potential in liver and kidney transplant as well as in rheumatoid arthritis[50,51]. In contrast to current immunosuppressants, which have demonstrable efficacy in organ transplant but are accompanied by major adverse effects, the use of anti-CAMs as a strategy might prove to be effective and safer.

Leukocyte migration and inflammatory diseases

Local inflammation dramatically increases the recruitment of circulating leukocytes. This is in part the result of induced expression of VCAM-1 and ICAM-1 by the vascular endothelium. Cellular migration mediated by endothelial VCAM-1/ICAM-1 and their leukocyte ligands $\alpha_4\beta_1/\alpha_L\beta_2$ integrins and the adhesion molecule pair PECAM-1–$\alpha_V\beta_3$ integrin has recently been investigated[49,52,53]. The speed of leukocyte locomotion was studied by using recombinant soluble molecules and videomicroscopy. The data suggest that the function of the different pairs of adhesion molecules is influenced by each molecule individually. Blocking either of the inflammatory adhesion molecules may bring the massive influx of hematopoietic cells to a halt.

Implications of a new IgSF–integrin interaction: CD31–$\alpha_V\beta_3$

CD31 has been implicated in several biological events, primarily involving interendothelial adhesion and leukocyte–endothelial adhesion and transmigration[52]. Animal model studies, using CD31 antibodies, have shown that CD31 is involved in monocyte and neutrophil recruitment during inflammation. CD31 is expressed by monocytes and vascular endothelium and exhibits both homotypic and heterotypic adhesive properties. The homotypic mode involves antiparallel interdigitation of opposing CD31 molecules. Recently a heterotypic binding to the integrin $\alpha_v\beta_3$ with PECAM-1 has been described[53].

The $\alpha_v\beta_3$ integrin has recently been implicated as a key controller of endothelial cell function. Blocking $\alpha_v\beta_3$ either by monoclonal antibodies or peptide antagonists has been shown to inhibit angiogenesis and promote apoptosis. The CD31–$\alpha_v\beta_3$ receptor–ligand pair might have important implications for both monocyte and endothelial migration.

PECAM-1 and transendothelial migration of PMNs

PMNs adhere to the inflamed vascular endothelium, eventually undergoing transendothelial migration. This latter process is largely regulated by PECAM-1, which is expressed on platelets, leukocytes and at the intercellular junctions of ECs. Specific antibodies neutralizing PECAM-1 selectively block PMN migration and markedly attenuate injury to ischemic-reperfused myocardium and coronary endothelium. Intravital microscopy confirms that the protective mechanism of PECAM-1 blockade is not via reduced leukocyte rolling or adherence, but rather by inhibition of transendothelial migration[54].

Extracellular matrix proteins

Osteopontin and α_V-integrin interactions in vascular cells

Osteopontin is an RGD-containing adhesive glycoprotein that has recently been identified as an important component of many chronic inflammatory and fibrotic diseases, including atherosclerosis and restenosis[55]. Osteopontin interacts with multiple receptors on vascular cells, including $\alpha_v\beta_3$, which mediate its adhesive and migratory functions. Recent *in vitro* and *in vivo* studies suggest that osteopontin and its receptors may play important roles in neointima formation and endothelial regeneration[41–44].

Fibronectin, thrombospondins and vitronectin

The metabolism of adhesive proteins of the extracellular matrix, particularly how these proteins become insolubilized in tissues and how tissue levels are controlled, is under extensive investigation. Fibronectin, which is highly soluble in blood, binds to cell surfaces – where strong noncovalent interactions form – and is controlled by cell shape and enhanced by lysophosphatidic acid. In contrast, thrombospondin-1 binds passively to existing matrix. Content of matrix thrombospondin-1 is controlled by cellular secretion and receptor-mediated degradation of the secreted protein. Matrix content of vitronectin is controlled, at least in part, by the conformational change that occurs when vitronectin complexes to thrombin–serpin complexes[56,57].

Soluble adhesion molecules as surrogate markers

CAMs are well recognized as adhesive receptors to facilitate adhesion, migration and transmigration of circulating cells into damaged vascular tissues. Recent studies have demonstrated expression of ICAM-1 on human atherosclerotic plaques, and treatment with an anti-ICAM-1 monoclonal

antibody results in a significant reduction of myocardial infarct size in experimental myocardial/ischemia reperfusion injury models[58,59]. Soluble isoforms of these CAMs, which are thought to be shed from the surface of activated cells, can be quantified in peripheral blood[16,17]. Increased serum concentrations of soluble CAMs have been observed in a variety of diseases[16,17].

Conclusion

It is clear that several members of the CAM superfamilies will serve as potential therapeutic and diagnostic strategies for a number of diseases with unmet medical needs. Key examples include the potential role of the platelet $\alpha_{II}\beta_3$ integrin in the prevention, treatment and diagnosis of various thromboembolic disorders, and the use of soluble adhesion molecules as potential *in vitro* diagnostic and prognostic markers for acute and chronic leukocyte, platelet and endothelial cellular insult.

A number of monoclonal antibodies as well as small-molecule inhibitors for various CAMs are in advanced clinical trials. The first FDA-approved monoclonal antibody for these strategies is the platelet $\alpha_{II}\beta_3$ antagonist ReoPro (c7E3).

The selection of a CAM that is associated with specific pathophysiological aspects of certain disease processes, as well as the ease of generating small anti-CAM molecules, will determine ultimate success in the discovery and development of therapeutics and diagnostic agents.

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