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REVIEW

Cell Adhesion Molecules: Potential Therapeutic & Diagnostic Implications

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Abstract The role of cell adhesion molecules (CAM) and extracellular matrix proteins (ECM) in various pathological processes including angiogenesis, thrombosis, apoptosis, cell migration & proliferation are well documented. These processes can lead to both acute and chronic disease states such as ocular diseases, metastasis, unstable angina, myocardial infarction, stroke, osteoporosis, a wide range of inflammatory diseases, vascular remodeling, and neurodegenerative disorders. A key success in this field is evident from the potential role of the platelet GPIIb/IIIa integrin in the prevention and diagnosis of various thromboembolic disorders. Additionally, the use of soluble adhesion molecules as potential diagnostic markers for acute and chronic leukocyte, platelet, and endothelial cellular insult are increasingly utilized. The development of various therapeutic and diagnostic candidates based on the key role of CAM, with special emphasis on integrins in various diseases as well as the structure-function aspects of cell adhesion and signaling of the different CAM and ECM are highlighted.

Keywords Integrins · Selectins · Immunoglobulins · Soluble adhesion molecules · Angiogenesis · Inflammatory and immune disorders

Introduction

Several physiological processes including cell activation, migration, proliferation, differentiation, and many other processes require direct contact between cells or extracellular matrix proteins. Cell-cell and cell-matrix interactions are mediated through several different families of CAM including the Selectins, the integrins, the cadherins, and the Immunoglobulins. Newly discovered CAM, along with the discovery of new roles for integrins, selectins, and immunoglobulins in certain disease states, provide great opportunities to develop therapeutic and perhaps diagnostic modalities.

Intensified drug discovery efforts, directed at manipulating CAM activity through monoclonal antibodies, peptides, peptidomimetics, and non-peptide small molecules for diagnostic and therapeutics, continue to broaden the scope of key clinical applications. This chapter focuses on the current advances in the discovery and development of novel anti-integrins for potential therapeutic & diagnostic applications as well methods required in studying these different CAM members.

CAM plays a very significant and critical role in both normal and in different pathophysiological disease states. For this key reason, the selection of specific and relevant CAM to target certain disease condition without interfering with other normal cellular functions is a very important prerequisite for the ultimate success in developing truly active and safe therapeutic strategies [1, 2]. Exciting advances in our understanding of several CAMs, most notably the $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha 4\beta 1$, $\alpha 5\beta 1$ and $\alpha IIb/\beta 3$ integrin receptors and their direct relationships to different disease states represent a tremendous therapeutic and diagnostic opportunities [1–8]. A potential role of specific CAM in different disease states including: cardiovascular, cancer, inflammatory, ocular, pulmonary, bone, central nervous system, kidney, and gastrointestinal system have been implicated. For example, the role of the integrin α_{IIb}/β_3 in the prevention, treatment, and diagnosis of various

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thromboembolic disorders provide excellent proof of this concept [3–8]. Additionally, the potential prophylactic role of anti-selectins, the role of β_1 along with other leukointegrins in various inflammatory conditions, the potential utility of various soluble adhesion molecules as a surrogate markers for acute and chronic endothelial injury, and the potential role of $\alpha_v\beta_3$ in angiogenesis, and osteoporosis has been implicated [9, 10].

Selected Cell Adhesion Molecules

Selectins

Different families of cell adhesion receptors include: the selectins, consisting of three cell adhesion molecules unified structurally by the inclusion of lectin (L), EGF-like (E) and complement (C) binding-like domains (LEC-CAMs). Functionally the selectins are unified by their ability to mediate cell binding through interactions between their lectin domains and cell surface carbohydrate ligands [11]. These include the E-, L-, and P- selections. The P- and E-selections are calcium-dependent on platelets or endothelial cell surface lectins that mediate leukocyte adhesion by recognition of cell-specific carbohydrate ligands. L-Selectins are found on all leukocytes and bind to its counter receptors on endothelial cells (Gly-CAM-1), a mucin-like endothelial glycoprotein [12]. E-selectin is an endothelial adhesion molecule whose expression is induced by various inflammatory stimuli and which recognizes cell surface carbohydrate, Sialyl Lewis^x (SLe^x) [13]. P-selectin is found stored in alpha granules of platelet, as well as Weible-palade bodies of endothelial cells, and recognizes a carbohydrate that is closely related SLe^x[14].

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 myocardial ischemia/reperfusion injury model [15, 16]. Additionally, in primate model of carotid artery restenosis, GA-6 resulted in 25% reduction in the neointimal-medial ratio after 14 days [17]. L-selectin is involved in mediating neutrophil rolling interactions at sites of inflammation [18].

Human Soluble Selectin Assays (sP-, sL-, or sE-selectins)

The most commonly used assay employs the quantitative sandwich immunoassay technique for the measurements of plasma or serum levels of soluble selectins. A monoclonal antibody specific for sP-, sL-, or sE-Selectins precoated onto a microplate. Standard, samples, and control are pipetted into the wells, together with a polyclonal antibody specific for sP-, sL-, or sE-Selectins conjugated to horseradish peroxidase (HRP). After removal of unbound conjugated antibody a substrate is added and color is developed which is proportional to sP-selectin concentration.

Integrins

Integrins are a widely expressed family of cell adhesion receptors via which cells attach to extracellular matrices, to each other's or to different cells. All integrins are composed of $\alpha\beta$ heterodimeric units, expressed on a wide variety of cells, and most cells express several integrins. The interaction of integrins with the cytoskeleton and extracellular matrix appears to require the presence of both subunits. The binding of integrins to their ligands is a cation-dependent. Integrins appear to recognize specific amino acid sequences in their ligands. The most well studied is the RGD sequence found within a number of matrix proteins including fibrinogen, vitronectin, fibronectin, thrombospondin, osteopontin, VWF, and others. However, other integrins bind to ligands via non-RGD binding domain such as the $\alpha4\beta1$ integrin receptors that bind and recognize the LDV sequence within the CS-1 region of fibronectin. There are at least 8 known β subunits and 14 α subunits [1, 2].

Coordinate Regulation of Cell Adhesion and Signaling through Integrins: Integrin adhesion receptors contain an extracellular face that engages adhesive ligands and a cytoplasmic face that engages intracellular proteins. These interactions are critical for cell adhesion and for anchorage-dependent signaling reactions in normal and pathological states. For example, platelet activation induces a confirmational change in integrin αIIb/β_3, thereby converting it into a high affinity fibrinogen receptor. Fibrinogen binding then triggers a cascade of protein tyrosine kinases and phosphatases and recruitment of numerous other signaling molecules into F-actin-rich cytoskeletal assemblies in proximity to the cytoplasmic tails of αIIb and $\beta3$. 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 αII/$\beta3$ are involved in integrin signaling, presumably through direct interactions with cytoskeletal and signaling molecules. Blockade of fibrinogen binding to the extracellular face of aIIb/b3 has been shown to be an effective way to prevent platelet-rich arterial thrombi after

coronary angioplasty [19]. Once proteins that interact with the cytoplasmic tails of αIIb/β3 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.

Anti-Integrins as Potential Drug Discovery Target: The commercial and therapeutic potential of cell adhesion molecules is on the rise. Newly discovered CAM along with the discovery of new roles for integrins, selectins, and immunoglobulins in certain disease states, provide great opportunities to develop therapeutic and diagnostic drugs. Integrin represents the best opportunity in achieving small molecule antagonist for both therapeutic and diagnostic utility in various key diseases with unmet medical need.

β1 Integrins

The largest numbers of integrins are members of the β1 integrins, which are also known as the VLA subfamily because of the late appearance of VLA after activation. There are at least seven receptors characterized from this subfamily, each with different ligand specificity. Among the most studied include the α4β1, α5β1, α6β1, αnβ1 receptors. The leukocyte integrin α4β1 (also known as VLA4 and CD49d/CD29) is a cell adhesion receptor, which is predominantly expressed on lymphocytes, monocytes, and eosinophil [20]. The leukocyte integrin α4β1 is a potential target for therapeutics in chronic inflammatory diseases.

Potent and Selective Small Molecule Antagonists of α4 Integrins: The α4 integrins are heterodimeric cell surface molecules central to leukocyte-cell and leukocyte-matrix adhesive interactions. The integrin α4β7, expressed on all leukocytes except neutrophils, interacts with the immunoglobulin (Ig) superfamily member VCAM-1, and with an alternately spliced form of fibronectin (Fn). The integrin α4β7 is also restricted to leukocytes and can bind not only to VCAM1 and Fn, but also to MAdCAM the mucosal addressin or homing receptor, which contains Ig-like domains related to VCAM-1. Certain monoclonal antibodies to the α4 chain and α4β7 can block their in vitro adhesive function. In vivo studies with these monoclonal antibodies in several species demonstrate that the interactions between these integrins and their ligands play a key pathophysiologic role in immune and inflammatory reactions. Thus, α4 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- α4 monoclonal antibody and small molecule antagonists were designed. These molecules demonstrated in vivo efficacy in several animal models [21, 22]. The mucosal vascular addressin, MAdCAM-1 is an immunoglobulin-like adhesion receptor preferentially expressed by venular endothelial cells defining sites of lymphocyte extravasation in mucosal lymphoid tissues and lamina popria. MAdCAM-1 binds lymphocyte integrin α4β7 [23]. A Peptide - based analogs based on various regions in the first and second domains of MAdCAM-1 for the binding to α4β7 were identified.

The Leukocyte Integrin α4β1 as a Potential Target for Therapeutics: These leukocyte populations primarily mediate chronic inflammatory disease (e.g., rheumatoid arthritis, asthma, and psoriasis and allergy). In contrast, VLA-4 is not present on circulating unstimulated neutrophils, which constitute a first line of defense against acute infections. Eosinophils selectively accumulate at sites of chronic allergic diseases such as bronchial asthma. The role of β1 integrins and its regulations by cytokines and other inflammatory mediators during eosinophil adhesion to endothelium, extracellular matrix proteins, and transendothelial migration has been well documented [24, 25]. The interactions of VLA-4 with alternatively spliced fibronectin containing CS-1 to design receptor blockers which bind to the VLA-4 integrin receptor has been utilized in targeting small molecule inhibitors. Evaluation of these analogs in animal models of disease indicate that VLA-4 blockade has the potential of achieving dramatic in vivo effects in a variety of chronic inflammatory disorders [20–22].

Antibodies Against α4 Integrin Prevent Immune Cell Infiltration of the CNS and Reverse Progression of Experimental Autoimmune Encephalomyelitis (EAE): Infiltration of circulating immune cells into the central nervous system (CNS), resulting in edema, myelin damage and paralysis has been documented [26]. The role for the adhesion molecule α4β1 integrin in this process has been demonstrated. When administered to animals with EAE, antibodies against α4 integrin prevented the adhesion of lymphocytes and monocytes to inflamed endothelium within blood vessels of the CNS, and prevented immune cell infiltration. 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 reduced permeability of the blood brain barrier to Gd-DPTA, while histological analysis demonstrated that treatment prevented the destruction of myelin. Remarkable, anti-α4 integrin reversed the accumulation of lymphocytes and monocytes within the CNS, while not affecting the level of the cells in the circulation. These results suggest that the active disease process requires an ongoing recruitment of circulating cells into the CNS, and

that anti-α4 integrin prevents this recruitment and reverses disease progression.

β1 Integrins in Gastrointestinal Diseases: The inflammatory bowel diseases (IBD), Crohn's disease, and ulcerative colitis (UC) are immunologically mediated illnesses. Using antibodies to the β family of integrins, isolated intestinal lamina mononuclear cells from IBD and normal intestine express a pattern of integrins found on normal solid organs with more β_7 expression than IBD. Crohn's CD3 + cells express more $\beta 1$ than normal, supporting separate β integrin systems in GI diseases. However, $\beta 1$ and $\beta 7$ integrins in particular remain as a potential therapeutic target for GI inflammatory disease [27].

α5β1 Integrin in Angiogenesis: Recent evidence suggested the role for this integrin in the modulation of angiogenesis [28]. Thus antagonist for $\alpha 5\beta 1$ might have potential utility in various angiogenesis-mediated disorders [28].

α5β1 Integrin & Bacterial Infection: Recent studies suggested a key role for $\alpha 5\beta 1$ integrin in mediating certain bacterial invasion into human host cells leading to antibiotic resistance [29].

β2 Integrins

The leukocyte restricted β_2 (CD18) integrins promote a variety of homotypic and heterotypic cell adhesion events required for normal and pathologic functioning of the immune system [30]. Several physiological processes including cell adhesion, activation, migration, transmigration require direct contact between cells or extracellular matrix proteins via CAM receptors. To date only three members of this integrin subfamily have been identified including: CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), and CD11c/CD18 (P150,95). A molecularly cloned cDNA encoding a fourth alpha chain, designated a_d, that associates with CD18 in normal leukocytes and upon co-transfection into CHO cells have been identified. In vitro studies have shown that LFA-1(CD11a/CD18) and Mac-1 on neutrophils can be differentially activated for distinct function [30]. In addition, investigations in vivo, including studies in CD11b deficient mice further underscore the biologic significance of the distinct contributions of LFA-1 and Mac-1 to neutrophil-dependent tissue injury.

β3 Integrins

αIIb/β3 Integrin: Intravenous Platelet αIIb/β3 Receptor Antagonists: The realization 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 utilities. Platelet $\alpha IIb/\beta 3$ receptor blockade represents a very promising therapeutic and diagnostic strategy of thromboembolic disorders. 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.

The clinical utility of Abciximab (ReoPro, c7E3 Fab) based on several trials involving coronary artery intervention procedures [31–33]. The potent, rapid, and sustained blockade of platelet GPIIb/IIIa receptors and perhaps its $\alpha v\beta 3$ blockade might be the key aspect contributing to the dramatic early antithrombotic benefits. Early benefits were maintained for over 3 years in-patients receiving 12-h Abciximab treatment in the EPIC trial. These unique pharmacological characteristics may also provide benefits in other thrombotic conditions such as stroke, unstable angina and acute myocardial infarction. Coronary Intervention: Epic (high-risk abrupt closure), Epilog (broad entry criteria), Capture (refractory unstable angina), Epistent (stent), and Rapport (direct angioplasty). Integrilin is a cyclic heptapeptide KGD analog. Impact II (coronary intervention—broad entry criteria) and Pursuit (Unstable angina—chest pain $\leq$ 24 h ischemic ECG changes) both demonstrated significant clinical benefits [34]. Tirofiban: the Restore (Coronary intervention—high risk of abrupt closure per clinical and anatomic criteria), Prism and Prism plus trials (unstable angina, chest pain 24 and 12 h) demonstrated significant clinical benefits. Lamifiban: the Paragon trial demonstrated significant clinical benefits [35]. Studies with Lamifiban in Canada were stopped due to lack of efficacy and nuisance bleeding [36].

Orally Active GPIIb/IIIa Antagonists: A high level of platelet antagonism has been required when GPIIb/IIIa antagonists have been employed for acute therapy of coronary arterial disease. However, the requirements for chronic therapy using orally active agents are only now becoming determined. Interaction with aspirin and other antiplatelet and anticoagulant drugs lead to shifts in the dose-response curves for both efficacy and unwanted side effects, such as increased bleeding time [37–39]. More recently, both Xemilofiban (EXCITE), Orbofiban (OUPIS) sponsored by Searle and Sibrafiban sponsored by Roche were withdrawn because of a disappointing outcome. This raises a lot of serious questions with regard to the potential of oral GPIIb/IIIa antagonists [40, 41].

Issues in Clinical Development: These include thrombocytopenia, monitoring, bleeding risk, and drug interactions [42, 43].

Role of Platelet Integrin GPIIb/IIIa Receptor Antagonists in the Rapid Diagnosis of Thromboembolic Events: The role of the platelet integrin GPIIb/IIIa receptor and its potential utility as a radiodiagnostic agent in the rapid detection of thromboembolic events has been demonstrated [44].

αvβ3 Integrin: *The Role of Integrin αvβ3 and Matrix Proteins in Vascular Remodeling via Endothelial and Smooth Muscle Cell Actions:* Vascular remodeling processes plays a key role in the pathological mechanisms of atherosclerosis and restenosis. In response to vascular injury such as by PTCA, matrix proteins like osteopontin, vitronectin are rapidly up-regulated [45]. Osteopontin stimulates smooth muscle cell migration via its action on the integrin αvβ3 and thereby contributes to neointima formation and restenosis [46, 47]. In addition, the matrix protein osteopontin and vitronectin induce angiogenesis, which may support neointima formation and arteriosclerosis [48]. Thus, specific matrix proteins via selected integrins and especially αvβ3 may be important targets for selective antagonists aimed at blocking the pathological processes of restenosis [45]. See Figs. 1 and 2 for selected peptidomimetic and non-peptide antagonists.

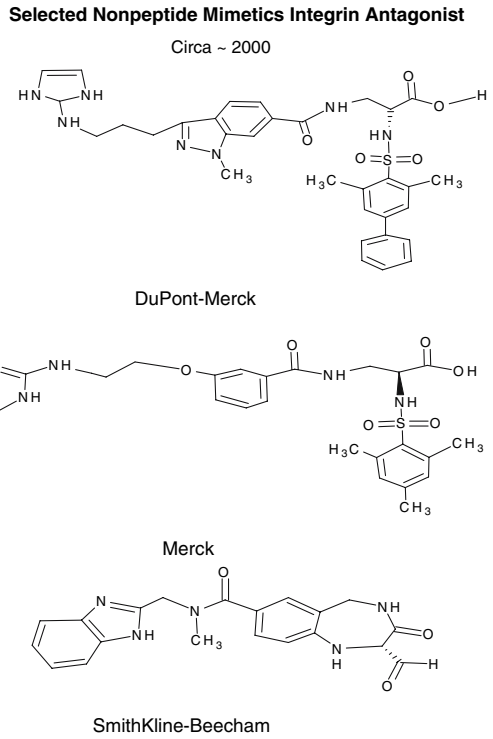

Fig. 2 Selected Non-peptide Integrin antagonists

Immunoglobulin (Ig)

ICAMs and VCAMs are members of the Ig superfamily. At this point, most of the effort in targeting the Ig superfamily is focused on the development of specific monoclonal antibodies and/or anti-sense oligonucleotide and small molecules that might specifically block gene transcriptional factors. Strategies for designing small molecular weight inhibitors for the Ig superfamily are somewhat more difficult. However, with current advances in molecular modeling, crystal structure information it might be possible to develop cyclic peptides and peptidomimetics Ig antagonist.

Several investigations with monoclonal antibodies to ICAM-1 demonstrated anti-inflammatory properties with

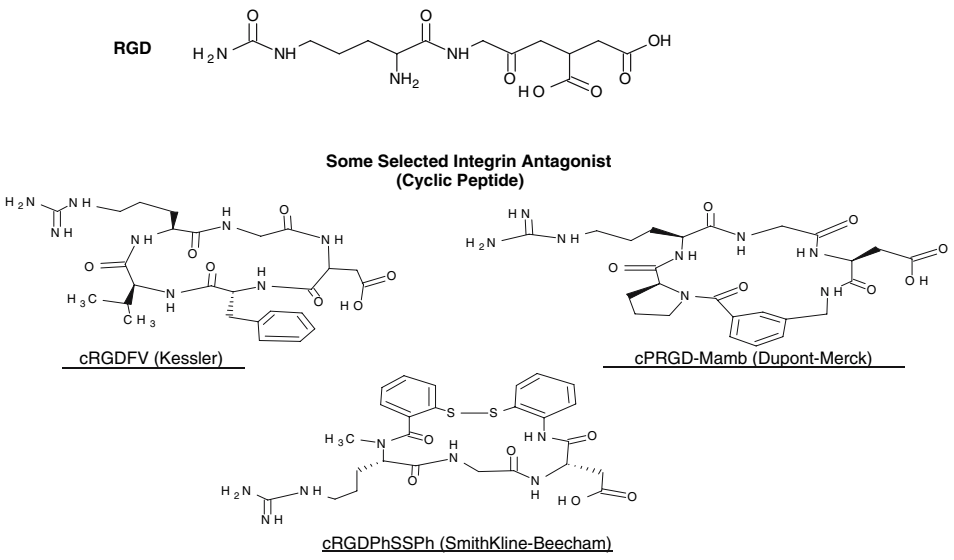

Fig. 1 Selected peptidomimetic integrin antagonists

tremendous therapeutic potential in liver and kidney transplant as well as in rheumatoid arthritis [49, 50]. In contrast to current immuno-suppressants, which demonstrated efficacy in organ transplant along with major adverse effects, the use of anti-CAM as a strategy might proven to be effective and safer.

Role of PECAM-1 in Regulating Transendothelial Migration of PMN's in Disease States

PMN's 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 endothelial cells. 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 by inhibiting their transendothelial migration [51].

Soluble Immunoglobulin Adhesion Molecules as a 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 resulted in a significant reduction of myocardial infarct size in experimental myocardial/ischemia reperfusion injury models [52, 53]. Additionally, soluble isoforms of these CAMs thought to be shed from the surface of activated cells, which can be quantified in peripheral blood [54, 55]. Increased serum concentrations of soluble CAMs have been observed in a variety of diseases [54, 55]. Recent studies suggested the prognostic and diagnostic potential for various soluble adhesion molecules in various vascular and cardiovascular diseases. See Fig. 3 for diagrammatic sketch of soluble immunoglobulins.

Human Soluble VCAM-1, ICAM-1, or PECAM Assays

This involves the simultaneous reaction of sVCAM-1 present in the sample or standard to two antibodies directed against different epitopes on the sVCAM-1 molecule. One antibody is coated onto the walls of the microtiter wells and the other is conjugated to the enzyme horseradish peroxidase (HRP). Any sVCAM-1 present forms a bridge between the two antibodies. The same concept applies to sICAM-1 or PECAM.

After removal of unbound material by aspiration and washing, the amount of conjugate bound to the well is detected by reaction with a substrate specific for the enzyme which yields a colored product proportional to the amount of conjugate (and thus sVCAM-1 in the sample). The colored product can be quantified photometrically.

By analyzing standards of known sVCAM-1 concentration coincident with samples and plotting a curve of signal versus concentration, the concentration of unknowns can be determined.

In Conclusion: It is very clear that several members in the CAM superfamilies and in particular the integrin family will serve as potential therapeutic and diagnostic strategies for a number of diseases with unmet medical needs. The selection of a certain CAM that is associated with specific pathophysiological aspect of certain disease processes as well as the ease of achieving small anti-CAM molecules will determine the ultimate success in the discovery and development of therapeutics and diagnostic drugs.

References

1. Cox, D., Aoki, T., Seki, J., Motoyama, Y., & Yoshida, K. (1994). The pharmacology of the integrins. *Medicinal Research Reviews, 14*(2), 195–228.
2. Albelda, S. M., & Buck, C. A. (1990). Integrins and other cell adhesion molecules. *The FASEB Journal, 4*, 2868–2880.
3. Cook, N. S., Kottirsch, G., & Zerwes, H. (1994). Platelet glycoprotein IIb/IIIa antagonists. *Drugs Future, 19*(2), 135–159.
4. Gold, H., Gimple, L. W., & Yasuda, T., et al. (1990). Pharmacodynamic study of F(ab')2 fragments of murine monoclonal antibody 7E3 directed against human platelet glycoprotein IIb/IIIa in patients with unstable angina pectoris. *The Journal of Clinical Investigation, 86*, 651–659.
5. Mousa, S. A., & Ahmad, S. (2007). Platelet GPIIb/IIIa antagonist, XV459, in heparin-induced thrombocytopenia. *American Journal of Hematology, 82*(4), 276–282.
6. Mousa, S. A., Bozarth, J. M., & Forsythe, M. S., et al. (1993). Anti-platelet efficacy and specificity of DMP 728, a novel platelet GPIIb/IIIa receptor antagonist. *Cardiology, 83*, 374–382.
7. Mousa, S. A., Bozarth, J. M., & Forsythe, M. S., et al. (1994). Antiplatelet and antithrombotic efficacy of DMP 728, a novel platelet GPIIb/IIIa receptor antagonist. *Circulation, 89*(1), 3–12.
8. Mousa, S. A., & Topol, E. (1997). Novel antiplatelet therapies: Recent advances. In Patrick, W., Serruys, & David Holmes (Ed.), The development of platelet GPIIb/IIIa receptor antagonists. *Current Review of Interventional Cardiology* (3rd ed., vol. 13, pp. 114–129). Philadelphia, PA: Current Medicine.
9. Brooks, P. C., Clark, R. A. F., & Cheresh, D. A. (1994). Requirement of vascular integrin $\alpha v\beta 3$ for angiogenesis. *Science, 264*, 569–571.
10. Brooks, P. C., Montgomery, A. M. P., & Rosenfield, M., et al. (1994). Integrin $\alpha v\beta 3$ antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell, 79*, 1157–1164.
11. Brandley, B, Swiedler, S., & Rabbins, P. (1990). Carbohydrate ligands for the LEC cell adhesion molecules. *Cell, 63*, 861–863.
12. Lasky, L. A., Singer, M., & Dowbenko, D., et al. (1992). An endothelial ligand for L-selectin is a novel mucin-like molecule. *Cell, 69*, 927–938.

13. Lasky, L. A. (1992). Selectins: Interproteins of cell-specific carboyhdrate information during inflammation. *Science, 258*, 964–968.
14. Phillips, M. L., Nudelman, E., & Gaeta, F. A., et al. (1990). ELAM-1 mediate cell adhesion by recognition of a carbohydrate ligand, sialyl-Lex. *Science, 250*, 1130–1132.
15. Weyrich, A. S., Ma, X.-L., Leter, D. J., Albertine, K. H., & Lefer, A. M. (1993). In vivo neutralization of P-selectin protects Feline heart and endothelium in myocardial ischemia and reperfusion injury. *The Journal of Clinical Investigation, 91*, 2620–2629.
16. Mulligan, M. S., Paulson, J. C., & De Frees, S., et al. (1993). Protective effects of oligo-saccharides in P-selectin -dependent lung injury. *Nature, 364*, 149–151.
17. Hullinger, T. G., De Graaf, G. L., Hartman, J. C., & Shebuski, R. J. (1995). The effect of P-selectin blockade on neointimal lesion development in a primate carotid injury model. *FASEB, 9*, 4897.
18. Paulson, J. C. (1992). Selectin/carbohydrate-mediated adhesion of leukocytes. In J. M. Harlan, & D. Y. Liu (Ed.), *Adhesion: Its role in inflammatory disease. Chapter 2* (pp. 19–42). W.H. Freeman and Company.
19. Topol, E. J., Califf, R. M., & Weisman, H. F., et al. (1994). Randomized trial of coronary intervention with antibody against platelet IIb/IIIa integrin for reduction of clinical restenosis: results at six months. *Lancet, 343*, 881–886.
20. Hamann, A., Andrew, D. P., Jablonski-Westrich, D., Holzmann, B., & Butcher, E. C. (1994). Role of α4-integrins in lymphocyte homing to mucosal tissues in vivo. *Journal of Immunology, 152*, 3282–3293.
21. Issekutz, T. B. (1991). Inhibition of in vivo lymphocyte migration to inflammation and homing to lymphoid tissues by TA-2 monoclonal antibody. *Journal of Immunology*, *147*, 4178–4184.
22. Elices, M. J., Osborn, L., & Takada, Y., et al. (1990). VCAM-1 on activated endothelium interacts with leukocyte integrin VLA-4 at asite distinct from the VLA-4/fibronectin binding site. *Cell, 60*, 577–578.
23. Berlin, C., Berg, E. L., Briskin, M. J., & Andrew, D. P., et al. (1994). α4β7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell, 74*, 185–195.
24. Yednock, T. A., Cannon, C., Fritz, L. C., Sanchez-Madrid, F., Steinman, L., & Karin, N. (1992). Prevention of experimental autoimmune encephalomyelitis by antibodies against α4β1 integrin. *Nature, 356*, 63–66.
25. Springer, T. A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration. The multistep paradigm. *Cell, 76*, 301–314.
26. Cannella, B., & Raine, C. S. (1995). The adhesion molecule and cytokine profile of multiple sclerosis lesions. *Annals of Neurology, 37*, 424–435.
27. Cerf-Bensussan, N., Jarry, A., & Lisowska-Grospierre, B., et al. (1987). A monoclonal (HML-1) defining a novel membrane molecule present on human intestinal lymphocytes. *European Journal of Immunology, 17*, 1279–1285.
28. Varner, J., Mousa, S. A. (1998). Antagonists of vascular cell integrin α5β1 inhibit angiogenesis. *Circulation, 98*(Suppl. I), I-795, 4166.
29. Cue D, Southern, S., Southern, P., Jadhav, P. K., Lorelli, W., Smallheer, J., Mousa, S., & Cleary, P. (2000). A nonpeptide integrin antagonist can inhibit epithelial cell ingestion of streptococcus pyogenes by blocking formation of integrin α5β1 – fibronectin –M1 protein complex. *Proceedings of the National Academy of Sciences, 97*(6), 2858–2863.
30. Figdor, C. G., & Kooyk, Y. V. (1992). Regulation of cell adhesion. In J. M. Harlan, & D. Y. Liu (Ed.), Chapter 7 (pp. 151–182). W.H. Freeman and Company.
31. Tcheng, J. E., Ellis, S. G., & George, B. S., et al. (1994). Pharmacodynamics of chimeric glycoprotein IIb/IIIa integrin antiplatelet antibody Fab 7E3 in high-risk coronary angioplasty. *Circulation, 90*, 1757–1764.
32. EPIC Investigators. (1994). Use of a monoclonal antibody directed against the platelet glycoprotein IIb/IIIa receptor in high-risk coronary angioplasty. *The New England Journal of Medicine, 330*, 956–961.
33. Kleiman, N. S., Ohman, E., & Califf, R. M., et al. (1993). Profound inhibition of platelet aggregation with monoclonal antibody 73E Fab after thrombolytic therapy: Results of the Thrombolysis and Angioplasty in Myocardial Infarction (TAMI) 8 Pilot Study. *Journal of the American College of Cardiology, 22*, 381–9.
34. Tcheng, J. E., Harrington, R. A., & Kottke-Marchant K., et al. (1995). Multicenter, randomized, double-blind, placebo-controlled trial of the platelet integrin glycoprotein IIb/IIIa blocker, Integrilin in elective coronary intervention. IMPACT Investigators. *Circulation, 91*(8), 215–217.
35. Peerlinck, K., De Lepeleire, I., & Goldberg, M., et al. (1993). MK383 (L-700462), a selective nonpeptide platelet glycoprotein IIb/IIIa antagonist, is active in man. *Circulation, 88*(4 pt 1), 1512–1517.
36. Théroux, P., Kouz, S., & Knudtson, M. L., et al. (1994). A randomized double-blind controlled trial with the non-peptide platelet GPIIb/IIIa antagonist RO 44–9883 in unstable angina [abstract]. *Circulation, 90*, 1–232.
37. Kottke-Marchant, K., Simpfendorfer, C., Lowrie, M., Burns, D., & Anders, R. J. (1995). Sustained but variable inhibition of platelet aggregation with Xemilofiban, an oral GPIIb/IIIa receptor antagonist, in patients with unstable angina. *Circulation*, 92(8 Suppl.), I-488.
38. Muller, T. H., Weisenberger, H., Brickl, R., Narjes, H., Himmelsbach, F., & Krause, J (1997). Profound and sustained inhibition of platelet aggregation by Fradafiban, nonpeptide platelet glycoprotein IIb/IIIa antagonist, and its orally active prodrug, Lefradafiban, in men. *Circulation, 96*(4), 1130–1138.
39. Cannon, C. P., McCabe, C. H., Borzak, S., Henry, T. D., Tischler, M. D., Mueller, H. S., Feldman, R., & Palmeri, S. T., et al. (1998). Randomized trial of an oral platelet glycoprotein IIb/IIIa antagonist, sibrafiban, in patients after an acute coronary syndrome: results of the TIMI 12 trial. Thrombolysis in myocardial infarction. *Circulation, 97*(4), 340–349.
40. Mousa, S. A., & Wityak, J. (1998). Orally active Isoxazoline GPIIb/IIIa antagonists. *Cardiovascular Drug Reviews, 16*(1), 48–61.
41. Harrington, R. A., Graffagnino, C., Armstrong, P. W., Joseph, D., & Card, T. L., et al. (1998). Dose-finding and tolerability of a new oral platelet GPIIb/IIIa inhibitor, SB 214857, in patients with coronary artery and cerebrovascular disease: The APLAUD results. *Circulation, 98*(17 suppl.), I-251, P 1303.
42. Vorchheimer, D. A., & Fuster, V. (1998). Oral platelet glycoprotein IIb/IIIa receptor antagonists: the present challenge is safety [editorial; comment]. *Circulation, 97*(4), 312–314.
43. Quinn, M., & Fitzgerald, D. J. (1998). Long-term administration of glycoprotein IIb/IIIa antagonists. *American Heart Journal, 135*(5 Pt 2 Su), S113–S118.
44. Mousa, S., Bozarth, J., Edward, S., Carroll, T., & Barrett, J. (1998). Novel Technetium-99m Labelled Platelet GPIIb/IIIa Receptor Antagonists For Imaging Venous & Arterial Thrombosis. *Coronary Artery Disease, 9*(2/3), 1–11.
45. Srivasata, S., Reilly, T., Shwartz, R., Holmes, D., & Mousa, S. (1996). Selective αvβ3 integrin blockade limits neointima hyperplasia and lumen stenosis in stented porcine coronary artery injury in Pig. *Circulation, 94*(8), I-41, 0231 (abst.).
46. Yue, T.-L., McKenna, P. J., & Ohlstein, E. H., et al. (1994). Osteopontin-stimulated vascular smooth muscle cell migration is mediated by β3 integrin. *Experimental Cell Research, 214*, 459–464.

47. Liaw, L., Skiner, M. P., & Raines, E. W., et al. (1995). The adhesive and migratory effects of osteopontin are mediated via distinct cell surface integrins. The *Journal of Clinical Investigation, 95*, 713–724.
48. Zee, R., Passeri, J., Barry, J., Cheresh, D., & Isner, J. (1996). A neutralizing antibody to the alpha v Beta 3 integrin reduces neointimal thickening in a balloon-injured iliac artery. *Circulation, 94*(8), 1505 (abst.).
49. Flavin, T., Rothlein, R., Faanes, R., Ivens, K., & Starnes, V. A. (1991). Monoclonal antibody against intercellular adhesion molecule (ICAM)-1 prolongs cardiac allograft survival in cynomologus monkey. *Transplantation Proceedings, 23*, 533–534.
50. Haug, C. E., Colvin, R. B., & Delmonico, F. L., et al. (1993). A phase I trial of immunosuppressant with anti-ICAM-1 (CD54) mAb in renal allograft recipient. *Transplantation, 55*(4), 766–773.
51. Rosenblum, W. I., Nelson, G. H., Wormley, B., Werner, P., Wang, J., & Shih, C.Y. (1996). Role of platelet-endothelial cell adhesion molecule (PECAM) in platelet adhesion/aggregation over injured but not denuded endothelium in vivo and ex vivo. *Stroke, 27*, 709–711.
52. Yamazald, T., Seko, Y., & Tamatani, T., et al. (1993). Expression of intracellular adhesion molecule-1 in rat heart with ischemia/reperfusion and limitation of infarct size with antibodies against cell adhesion molecules. *The American Journal of Pathology, 143*, 410–418.
53. Simpson, P. J., Todd, R. F., & Micelson, J. K., et al. (1990). sustained limitation of myocardial reperfusion injury by a monoclonal antibody that alter leukocyte function. *Circulation, 81*, 226–237.
54. Newman, W., Dawson, B. L., & Carson, C. W., et al. (1993). Soluble E-selectins is found in supernatants of activated endothelial cells and is elevated in serum of patients with septic shock. *Journal of Immunology, 150*, 644–654.
55. Gearing, A. J. H., & Newman, W. (1993). Circulating adhesion molecules in disease. *Immunology Today, 14*(10), 506–512.