Adhesion Molecules:

Overview and Approach to Inhibition

- Arthur Kavanaugh, M.D.

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"ὁ δ' ἄρα πρηνὴς ἐπὶ γαιῃ κεῖτο ταθείς,
ἐκ δ αἷμα μέλαν ῥέε, δεῦε δὲ γαῖαν."

- Homer, *The Iliad* XXI. 118-9

Arthur Kavanaugh. M.D

Associate Professor of Internal Medicine

Rheumatic Diseases Division

Areas of interest include:

Patient Care: Rheumatoid Arthritis, Systemic Lupus Erythematosus, primary immunodeficiencies, other Rheumatologic and Clinical Immunologic conditions, General Internal Medicine.

Research: Novel forms of treatment for Rheumatoid Arthritis, Systemic Lupus Erythematosus, Asthma, et al; Evaluation of mechanisms of immunomodulatory agents; Cost-benefit analyses for novel therapuetics; Creation of evidenced based guidelines for rheumatologic diagnosis and treatment; Creation of evidenced based guidelines for the optimal utilization of immunologic laboratory tests.

Teaching: Medical Ethics, Rheumatology, Clinical Immunology, Internal Medicine.

I. Overview of Adhesion Molecules

1. Introduction

Recent years have witnessed extraordinary advancements in our knowledge regarding cell surface adhesion molecules. These diverse molecules, which bind to specific ligands expressed on cells and extracellular matrix (ECM) molecules, have recently been the subject of intense investigation by numerous investigators.

Adhesion molecules have been demonstrated to be capable of effecting various prominent interactions. This includes the adhesion of circulating leukocytes to the vascular endothelium, the transendothelial migration of these cells, retention of cells at extravascular sites, and activation of immunocompetent cells (1-10). By mediating these events, adhesion molecules play a critical role in both inflammatory responses as well as immunosurveillance. Although these processes are essential to salubrious activities such as the elimination of infectious agents, they also underlie various pathologic states. Thus, adhesion molecule-mediated interactions are integral to the initiation and propagation of allergic diseases. Accompanying the substantial developments in our understanding of adhesion molecules has been the expectation that they may serve as valuable therapeutic targets. In addition to having considerable theoretical appeal, the concept of targeting adhesion receptors has been successfully tested in both animal models and human diseases.

2. History

Progress in our understanding of adhesion molecules has followed developments in the appreciation of the endothelium as a dynamic organ. In both fields, many years of slow progress have given way to the current exponential growth in both comprehension and interest.

It was over a millennium before William Harvey's description of the circulatory system supplanted Galen's archaic concept of the tidal ebb and flow of blood elements (11). Later in the 1600's, Malphigi identified the capillary bed. By the 18th century it became accepted that lymph was derived from blood. Although all components of the circulation were thus apparent by this time, the nature of its endothelial lining layer could not be properly appreciated until cell theory was promulgated, nearly a century later. During the 19th century, several seminal observations helped provide the foundation for studies that would ultimately lead to the discovery of adhesion molecules. Using intravital microscopy, Dutrochet, Metchnikoff, Cohnheim and other investigators described the interaction of circulating cells with the vessel wall (12). It was observed that leukocytes passing through post capillary venules at high velocity initially slow down and roll along the vessel wall. Subsequently these cells become flatter and more tightly adherent to endothelial cells. Finally some migrate through the vascular endothelium into the inflammatory site. While the mechanisms underlying these observations would not be defined until well into the next century, these investigators formed prescient hypotheses on the dynamic nature of the interaction between leukocytes and endothelial cells.

Approximately 40 years ago, it was appreciated that lymphocytes possess a pattern of recirculation distinct from other leukocytes; they can enter lymphoid tissue from the blood stream via postcapillary venules, traverse lymph vessels back to the circulation, and again recirculate to lymphoid tissue (13). Moreover, it was noted that this lymphocyte recirculation was organ specific. Lymphocytes isolated from a particular lymphoid tissue would return or

Table 1. Integrins

Receptor	Counter-Receptor	Distribution	MW	Regulation
β1 (CD29)		*Very Late Activation (VLA) Antigens*		
α1β1 / VLA-1 (CD49a/CD29)	**laminin, collagen†**	activated T cells, fibroblasts, mesangial Cells, hepatic sinusoids	210/ 130	expression increased by antigen, mitogen
α2β1 / VLA-2 (CD49b/CD29)	**collagen** (DGEA motif), **laminin†**, tenascin	activated T cells, endothelial cells, platelets, basophils	165/ 130	expression increased by antigen, mitogen
α3β1 / VLA-3 (CD49c/CD29)	**laminin, collagen** fibronectin†, epiligrin	glomerulus, thyroid, basement membrane; many Cell Lines	135/ 130	
α4β1 / VLA-4 (CD49d/CD29)	**VCAM-1** (domains 1&4), **Fn** (CS-1 domain; EILDV motif), (with activation, weaker binding to Fn via RGD motif on Hep II site)	lymphocytes, monocytes, eosinophils, basophils, mast cells, NK cells, (*not* PMN) - provides co-stimulation to T cells (in part by a focal adhesion kinase $pp125^{FAK}$) - ligation triggers T cell motility - supports T cell rolling on EC - ligation → T cell MMP production	150/ 130	expression/ activity increased by many stimuli (antigen, mitogen, etc)
α5β1 / VLA-5 (CD49e/CD29)	**fibronectin†**	lymphocytes, monocytes, endothelial cells, basophils, mast cells, fibroblasts - provides co-stimulation to T cells (in part by a focal adhesion kinase $pp125^{FAK}$) - ligation → T cell MMP production	160/ 130	activity increased by antigen
α6β1 / VLA-6 CD49f/CD29	**laminin†**	platelets, T cells, eosinophils, monocytes, endothelial cells	130/ 130	activity increased by antigen
α9β1	**tenascin** (at a non-RGD motif)	basal keratinocytes, hepatocytes, airway epithelial cells, smooth and skeletal muscle cells	130/ 130	
αvβ1 (CD 51/CD29)	**fibronectin, vitronectin†**	platelets, B cells	135/ 130	

Receptor	Counter-Receptor	Distribution	MW	Regulation
β2 (CD18)		*Leukocyte Integrins*		
LFA-1 (CD11a/CD18)	**ICAM-1, 2, 3** E-Selectin	all leukocytes provides critical co-stimulation to T cells (inhibition can → anergy)	180/ 95	expression, activity increased by many stimuli (antigen, mitogen, etc)
Mac-1, CR3 (CD11b/CD18)	**ICAM-1,** fibrinogen iC3b, LPS, Factor X, CD23, haptoglobin, ICAM-2 (A domain)	granulocytes, monocytes, large granular lymphocytes - ligation can activate monocytes	170/ 95	expression/ activity increased by cytokines, other stimuli
p150/95, CR4 (CD11c/CD18)	**iC3b, fibrinogen,** CD23	monocytes, granulocytes, large granular lymphocytes, B cell subsets, platelets	150/ 95	expression increased by TNF-α
α_D/CD18	**ICAM-3**	tissue macrophages	160/ 95	constitutive
β3 (CD61)		*Cytoadhesins*		
gp IIb/IIIa CD41/CD61	**fibrinogen,** vitronectin, fibronectin, von Willebrand Factor †	platelets, endothelial cells	145/ 105	expression increased by many stimuli
αv/IIIa CD51/CD61	**vitronectin,** fibronogen, von Willebrand Factor, laminin, fibronectin, thrombospondin, tenascin,osteopontin†	platelets; many non-hematopoietic cells	160/ 105	expression increased by various stimuli
β4				
α6β4 CD49f/CD104	**laminin** (E8 region), **epiligrin**	epithelial cells, endothelial cells	150/ 205	
β7				
α4β7‡, LPAM-1* CD49d/β7	**MAdCAM-1‡, VCAM-1, fibronectin** (CS-1 domain)	subset of memory T cells, eosinophils, basophils, endothelial cells	150/ 120	
$\alpha^E\beta7$ CD103/β7	**E-cadherin,** other ligands	>95% intestinal intraepithelial lymphs, pulmonary T cells, (<7% circulating T cells)	160/ 120	expression ↑ by TGF-β; ↓ by TNF-α, IL-1, IFN-γ)

† = binds the amino acid sequence RGD (arginine-glycine-aspartic acid). The RGD motif is part of the recognition site for several ECM molecules, including: fibronectin, fibrinogen, vWF, vitronectin, collagen, and osteopontin.
‡ α4β7, which mediates homing to Peyer's patch and mesenteric lymph nodes, recognizes a protein based epitope of MAdCAM-1 (the MECA-367 mAb determinant): * LPAM-1 = lymphocyte Peyer's patch adhesion molecule-1
- Other integrins include: α7β1, α8β1, αVβ5, αVβ6, αVβ8

'home' back to that same tissue after intravenous injection. This initiated the concept of specific 'homing receptors' on lymphocytes which interact with distinct 'vascular addressins' on the vascular endothelium within particular organs, thus directing this specific recirculation.

The initial descriptions of cell surface 'adhesion molecules' in the early 1980's ushered in an era of exponential discovery (14). Since then, not only have numerous adhesion molecules been defined, but myriad functions and diverse interactions mediated by these versatile molecules continue to be described.

3. Individual Adhesion Molecules / Families

On the basis of structural homology, many adhesion molecules can be classified into one of three important families: the integrins, the selectins, and the immunoglobulin superfamily members.

a. Integrins

The integrins (Table 1) are large, heavily glycosylated, heterodimeric proteins composed of one of at least 15 distinct α-subunits in noncovalent linkage with one of at least 8 β-subunits. These adhesion receptors, which can bind ligands expressed on cell surfaces, ECM molecules, and soluble molecules, are phylogenetically ancient. Integrins can be subcategorized based upon their β-subunit usage (Table 1).

Although their intracytoplasmic segments are relatively short, integrins interact with extracellular cytoskeletal components, such as actin and talin. The name integrins derives from the concept that these molecules 'integrate' information from the extracellular milieu to intracellular compartments. Recently, it has been established that integrins are capable of bidirectional signaling, as they also transduce signals "inside out" (15). Thus, many integrins constitutively have minimal ability to bind their ligands. Upon activation of the cell, integrins undergo conformational changes that permit binding of divalent cations by the α-subunit and markedly increase their avidity for ligand. The presence of conformationally dissimilar forms has been confirmed by the description of monoclonal antibodies (mAb) specific for individual integrins at distinct states of activation.

As is true of other adhesion receptors, integrins exhibit pleiotropy and redundancy. Thus, most integrins are expressed on more than one cell type, and most cells express more than one integrin on their surface. Moreover, several molecules function as ligand for more than a single integrin, although their avidity for that ligand may be variable. Although many integrins have been shown to play some role in the pathophysiology of allergic diseases, two may be of particular importance: LFA-1 (CD11a/CD18) and VLA-4 (CD49d/CD29). These molecules have been shown to be central to the adhesion and transendothelial migration of T cells, eosinophils, and other leukocytes (1,2,6,8). Because antigen-driven T cells serve a key role in the orchestration of allergic diseases, and eosinophils play an important pathophysiologic role in these diseases, these adhesion receptors would be expected to be of particular importance. Moreover, LFA-1 and VLA-4 serve as accessory or co-stimulatory molecules for T cells (1,2,16,17). In conjunction with stimulation via the antigen-specific T cell receptor, these molecules are capable of providing a 'second signal' and thereby driving a productive immunologic response. As will be discussed subsequently, therapy directed at these adhesion molecules might therefore be expected not only to inhibit the accrual of T cells and other leukocytes at inflammatory sites, but also to attenuate the activation of cells that is integral to the immune response.

Table 2. Selectins

Receptor	Counter-Receptor	Distribution	MW (kD)	Regulation
E-Selectin° CD62-E (ELAM-1)	**sialyl Lewis X** (sLe^x, CD15s) (recognizes sLe^x presented on L-Selectin, CLA‡, LFA-1, CD66); **ESL-1✓**, PSGL-1†, sialyl Lewis A	activated endothelial cells (EC)	107 -115 (varied glycosyl-ation)	synthesized, expression ↑ by IL-1, TNF-α, LPS, IFN-γ; expression ↓ withhn 16-24 hrs.
L-Selectin• CD62-L (Leu-8, Mel-14Ag)	**PNAd*: GlyCAM-1√** (sgp50), **CD34** (sgp90), sgp200, **MAdCAM-1′**, E-Selectin, P-Selectin, other ligands at inflammatory sites	resting leukocytes	74 (lymph) 90-100 (PMN)	rapidly, proteolytically cleaved upon activation (between Lys^{321} and Ser^{322})
P-Selectin• CD62-P (GMP-140, PADGEM)	**PSGL-1†**, Sialyl Lewis X (as described above for E-selectin), Lewis X (CD15),	activated EC (from Weibel-Palade bodies); activated platelets (from α granules)	140	rapidly redistibuted to cell surface by PAF thrombin, histamine, LTC_4, LTB_4, H_20_2

° E-Selectin serves as a vascular addressin for skin-homing T cells (via CLA binding); it also mediates the adhesion of various leukocytes to endothelium during rolling. E-selectin appears to recognize extended chain sLe^x, such as sialyl-dimeric Lewis X.

‡ CLA = the Cutaneous Lymphocyte Antigen, a sialylated oligosaccharide that defines a subset of memory T cells that exhibit tropism to skin (present on ~ 7 - 20% of circulating T cells)

† PSGL-1 = P-Selectin glcoprotein ligand-1, a sialomucin glycoprotein expressed on myeloid cells and subsets of T cells; PSGL-1 is capable of binding both P-Selectin and E-Selectin.

• L-Selectin is a protein with homology to C-type lectins. It is capable of mediating: 1) adhesion of various leukocytes to endothelium during rolling, and 2) homing of lymphocytes to peripheral and mesenteric lymph nodes. Binding is via Ca^{++}-dependent recognition of specific carbohydrate residues (e.g. sialic acid, fucose, sulfate) which are expressed on certain endothelial glycoprotein ligands, and may be presented on capping groups such as sLe^x. Neutrophil (but not lymphocyte) L-Selectin may be modified by sLe^x, allowing interaction with P and E-Selectin. In addition, neutrophil L-Selectin tends to be concentrated at the tips of microvilli, thereby facilitating interactions with ligand under flow.

• P-Selectin mediates the adhesion of various leukocytes to endothelium during rolling. Results from experiments using gene-targeted (knockout) animals suggest that L- and then P-selectin may be particulary important in the intial phases of leukocyte-endothelial interactions (tethering and capture), whereas the synergistic actions of L-,P-, and E-selectin may subsequently be required for optimal leukocyte rolling.

✓ ESL-1 = E-Selectin ligand-1 (homologous to fibroblast growth factor receptor)

* PNAd = the peripheral node addressin. Referred initially to the binding specificity of the mAb MECA-79, which recognizes various mucin-like glycoprotein L-Selectin ligands present on peripheral lymph node HEV

√ GlyCAM-1 = glycosylation dependent cell adhesion molecule-1; a mucin like molecule, it is expressed on lymph node HEV and mammary gland epithelium; GlyCAM-1 has no transmembrane region, therefore may function as a soluble or circulating receptor. CD34 is widely expressed on vascular endothelium and hematopoietic stem cells; a subset of CD34 molecules is capable of binding L-Selectin. GlyCAM-1, CD34, sgp200 and PSGL-1 are sialomucins, containing serine and threonine rich areas capable of binding sulfate.

′MAdCAM-1 = Mucosal addressin cell adhesion molecule-1; L-Selectin presumably recognizes a carbohydrate motif expressed on the mucin-like domain on a subset of MAdCAM-1 molecules synthesized in mesenteric lymph nodes.

- There is data to suggest that the ability to utilize P and E-selectin may discriminate between subsets of T helper cells; thus, the recruitment of TH1 but not TH2 T cells may be mediated by these molecules (Nature 1997;285:81).

b. Selectins

The selectins (Table 2), so named because they are selectively expressed on cells related to the vasculature and contain a lectin binding domain, are composed of three extracellular domains: one of complement regulatory protein-like short consensus repeat units, an epidermal growth factor (EGF)-like domain, and the amino-terminal lectin domain that confers binding specificity. The EGF and complement-like domains may function primarily as a scaffold; optimally positioning the lectin domain above the cellular glycocalyx, thereby facilitating its interaction with ligand. Selectin counter-receptors are typically sialylated, fucosylated carbohydrate moieties, such as the sialyl Lewis blood group oligosaccharides (e.g. sialyl Lewis X [sLe^x]) (18). Glycosylation may be a key regulatory point for the selectins. For example, L-selectin is differentially glycosylated on lymphocytes and neutrophils, with resultant distinct binding profiles.

Some confusion concerning the selectins and their ligands originates historically from the various means by which this knowledge was derived. For example, ligands of the mouse homologue of L-selectin were named according to functional characteristics (e.g. the 'peripheral lymph node addressin') or by mAb binding specificity (Table 2). As ligand characteristics yield to molecular analyses the confusion surrounding these complex adhesion receptors may abate.

c. Immunoglobulin superfamily

Members of this family of adhesion receptors (Table 3) are composed of variable numbers of globular, immunoglobulin-like, extracellular domains. The immunoglobulin superfamily is phylogenetically ancient, having been described in insects where they have been shown to function as adhesion receptors in nervous system development. From an evolutionary standpoint, this implies that the adhesive functions of the immunoglobulin superfamily members antedate their antigen recognition capacity. Only those immunoglobulin family members associated with antigen recognition, namely immunoglobulin on B cells and the T cell receptor complex, undergo gene rearrangements and somatic mutation. Some adhesion molecules in the immunoglobulin superfamily, for example CD31 and NCAM, are capable of mediating homotypic adhesion. Others, such as ICAM-1 and VCAM-1, mediate adhesion via interactions with integrins (Table 3).

d. Other adhesion molecules

Several important adhesion molecules that can not be classified into one of the three families discussed above are shown in Table 4. Progress in the description of the characteristics of many of these molecules has recently proceeded apace, and their roles in immunologic inflammation may be expected to be revealed in the not too distant future.

e. Alternate forms

An interesting consideration that has received increased attention of late is the existence of alternative forms of certain adhesion molecules. For some adhesion molecules, such as CD44, different forms result from alternative gene splicing. Isoforms of CD44 have been found to be of substantial prognostic importance as regards the metastatic potential of several malignancies (19). Posttranslational modifications, particularly glycosylation, also exert significant effects upon binding specificities. This variability may allow a greater degree of specificity at the cellular or tissue level. For example, the binding specificity and molecular weight of L-selectin differ on lymphocytes and neutrophils, presumably due to variable glycosylation. This has implications for the differential binding capacity of the molecules on these distinct cells.

Table 3. Immunoglobulin Superfamily

Receptor	Counter-Receptor	Distribution	MW	Regulation
ICAM-1 (CD54) [5 Ig domains] [domain 1 binds CD11a/CD18; domain 3 binds CD11b/CD18] binds rhinovirus	**LFA-1** (CD11a/CD18), **Mac-1** (CD11b/CD18), CD43 (leukosialin, a mucin-like molecule)	endothelial cells, fibroblasts, epithelial cells, monocytes, lymphocytes, dendritic cells, chondrocytes; also on parenchymal cells (e.g. myocardiocytes, hepatocytes) after cytokine stimulation; present in variously glycosylated forms	75-110	constitutive; expression increased (~ 40X) by IL-1, TNF-α, IFN-γ, LPS, SP
ICAM-2 (CD102) [2 Ig domains] [domain 1 binds CD11a/CD18]	**LFA-1** (CD11b, via A domain)	endothelial cells (high expression); lymphocytes, monocytes, platelets (lower expression)	50	constitutive (resting endothelial cells ~ 2/3 ICAM-2, 1/3 ICAM-1
ICAM-3 (CD50) [5 Ig domains] [domain 1 binds CD11a/CD18; domains 3,4 bind α_D/CD18]	**LFA-1, α_D/CD18**	lymphocytes, monocytes, PMN, eosinophils, basophils - transduces signal for T cells; cytoplasmic portion associates with $p56^{lck}$ and $p59^{fyn}$	125	expression increased by activation
VCAM-1 (CD106) [6 or 7 Ig domains] [domains 1 and 4 bind VLA-4]	**$\alpha 4\beta 1$** (VLA-4, CD49d/CD29) **$\alpha 4\beta 7$** (lower affinity)	endothelial cells, monocytes, fibroblasts, dendritic cells, bone marrow stromal cells, myoblasts	90-110 splice	expression † by TNFα,LPS IL-1, 4, 13, oxidative stress
LFA-3 (CD58) [6 Ig domains]	**CD2**	endothelial cells, leukocytes, epithelial cells	50-75	
CD31 (PECAM-1,) [domains 1 and 2 mediate transendothelial migration, domain 6 mediates ECM migration]	**CD31** heparin, other	endothelial cells (at EC-EC junctions), T cell subsets, platelets, PMN, monocytes, smooth muscle cells, bone marrow stem cells	130	polymorphic forms exist (? functions as a minor HLA)
NCAM (NKH1 [CD56] homolog)	**NCAM** heparan SO_4, heparin	neural cells, glial cells, heart, muscle, kidney, NK cells, subset of activated T cells	120	unknown
MAdCAM-1 (4 domains [mice]: ICAM/VCAM like, VCAM-1 like, mucin-like, Cα2 [not human])	**$\alpha 4\beta 7$** (via Ig domains) **L-Selectin** (via mucin)	HEV of Peyer's patch and mesenteric lymph nodes; also, mucosal endothelial cells (gut lamina propria, lactating mammary gland, exocrine pancreas); spleen sinus lining cells		expression increased by TNF-α, IL-1, IFN-γ
CD2	**CD58**, CD59, CD48	T cells, NK cells	48	

† other immunoglobulin superfamily members include: immunoglobulin, the CD3/T cell receptor complex, CD4, CD8, MHC Class I and II antigens, B7-1 (CD80), B7-2 (CD86), CD28, and CTLA-4.

Table 4. Other Adhesion Molecules

Receptor	Counter-Receptor	Distribution	MW	Regulation
CD44 (cartilage link protein family)	**hyaluronate** gp600 (serglycin; a proteoglycan stored in intracellular granules of lymphoid and myeloid cells), collagen type VI, osteopontin	lymphocytes (role in lymph homing, rolling on endothelial cells), monocytes, endothelial cells, fibroblasts, epithelial cells	90†	†(alternatively spliced isoforms exist [11 exons] ; various isoforms correlate with tumor metastases, inflammatory bowel disease expression, etc)
VAP-1 (vascular adhesion protein-1)	unknown (mediates lymphocyte - HEV binding)	HEV of lymph nodes; endothelial cells at chronic inflammatory sites (e.g. synovium, skin); dendritic cells	90	expression increased at inflammatory sites
CD73; L-VAP-2 (lymphocyte vascular adhesion protein-2); ecto-5'-nucleotidase	unknown (may mediate homing of lymphs to inflamed skin)	endothelial cells; subsets of lymphocytes (70% B cells, 50% CD8+ /10% CD4+T cells)	70	constitutive
Cadherins • **N-Cadherin** • **E-Cadherin** • **P-Cadherin**	**homophilic binding** (also $\alpha^E\beta7$ is a couner-receptor for E-cadherin, via HAV motif)	• N; brain, muscle, lens • E; epithelium • P; placenta, epithelium	120-135	Ca^{++}-dependent (cadherins function in structural integrity)
CD36 (platelet gpIV, gpIIIa)	**thrombospondin** (also, receptor for *Plasmodium falciparum*-infected erythrocytes)	platelets, monocytes, endothelial cells	88	unknown
CD26 (dipeptidyl peptidase IV) adenosine deaminase binding protein	**fibronectin, collagen**	endothelial cells, lymphocytes	120	unknown
HAL 1/13 Ag (hypoxia sensitive receptor)	unknown receptor on leukocytes	endothelial cells	85	expression induced by hypoxia

Variability in the glycosylation of ICAM-1, which is also illustrated by different molecular weights, may underlie its inconstant affinity for ligand that has been demonstrated on a tissue level. This has also been illustrated *in vivo*. For example, in models of ischemia-reperfusion injury, binding of ICAM-1 to CD11b/CD18 (Mac-1) seems to be of greater importance to the ultimate damage sustained by particular tissues than ICAM-1 binding to CD11a/CD18 (LFA-1).

4. Concepts Related to Adhesion Molecule Utilization

Perhaps of greater clinical relevance than the progress in the delineation of the characteristics of individual adhesion molecules has been the appreciation of the integrated utilization of these molecules. Indeed, it is probably most appropriate that adhesion molecules be considered a cascade, much like the complement and coagulation systems. Thus, although cells possess a large repertoire of adhesion molecules on their surface, they are not utilized randomly or *en masse*. Rather, they function in an hierarchal, sequential manner (Table 5 and figure 1). In the initial phases of an inflammatory response, perturbations in the endothelium allow predominantly selectin-based interactions to slow the velocity of the circulating cells. Consequent to activation mediated by chemokines and adhesion molecule interactions, some of these rolling cells become flatter and more tightly adherent. Subsequently, these cells may exit the vessel by migrating between endothelial cells, a process mediated to a large extent by activated integrins and immunoglobulin-family receptors. Migration of the cell through the ECM and further activation of cells within the inflammatory site are mediated by integrins as well as chemokines and cytokines.

Early in the investigation of adhesion molecules it had been hypothesized that the intricacies of tissue localization of circulating cells might be entirely explained by specific adhesion receptors. Although there may be preferential expression of certain molecules at defined tissue sites (e.g. addressins and homing receptors), adhesion molecules exhibit substantial redundancy. Rather than depending upon unique tissue expression, it appears that the specific function of adhesion molecules may be explained by other factors, such as tissue specific posttranslational modification. Perhaps more importantly, the synergistic and sequential use of combinations of adhesion molecules may impart specificity, with certain combinations functioning like telephone 'area codes' or postal 'zip codes' (9). Specificity may also be engendered by other components of the immune response such as the chemokines. This diverse and expanding group of inflammatory mediators molecules play a central role in allergic and other immunologic reactions by mediating chemoattractant and activating functions (20). By activating certain adhesion receptors and upregulating their avidity for ligand, chemokines liberated in a particular inflammatory milieu may help impart a degree of specificity. Moreover, because they can exhibit selectivity for target cells (e.g. RANTES is a specific chemoattractant for memory T cells and eosinophils, as is eotaxin for eosinophils), the repertoire of chemokines in the local milieu may contribute significantly to the specific composition of cells at that site. In addition, other factors that upregulate the avidity of various adhesion receptors for their ligands, such as pro-inflammatory cytokines, peptide and lipid mediators, may also contribute to specificity at inflammatory sites.

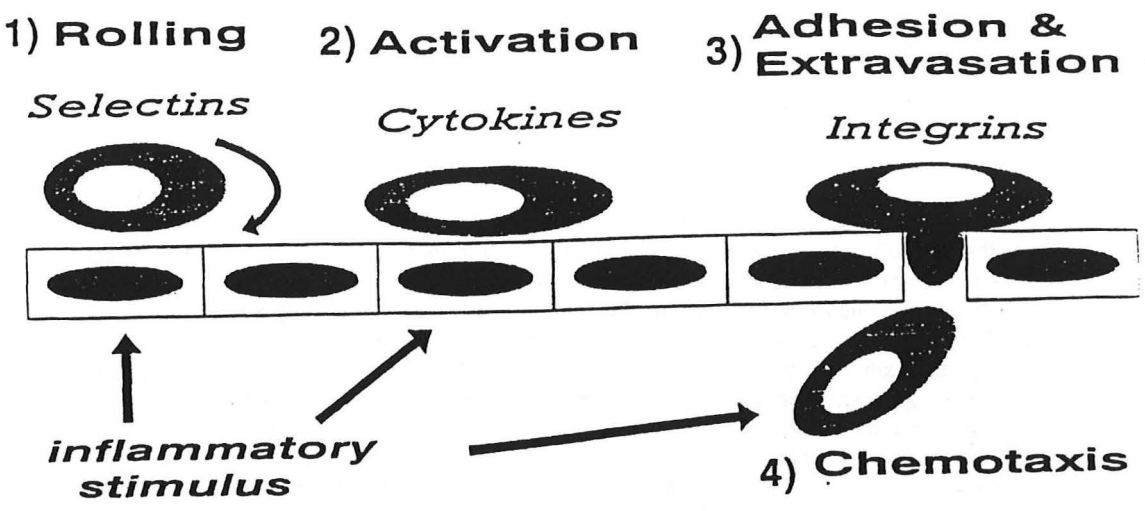


Figure 1 and Table 5. The Adhesion Cascade

	Rolling	Activation	Firm Adhesion & Transmigration	Haptotaxis / Chemotaxis; Tissue Retention
Leukocyte Component	sLe^x, L-Selectin, PSGL-1, VLA-4, CD44	Cytokine and Chemokine Receptors; CD31	$\beta 2$ integrins (also $\beta 1$ and $\beta 7$ integrins, CD31)	Chemokine receptors; $\beta 1$ integrins
Endothelial / Tissue Component	P-Selectin, E-Selectin, GlyCAM-1, MAdCAM-1, PAF; VCAM-1	Chemokines, Cytokines, CD31, PAF, C5a, fMLP	ICAM-1, 2; VCAM-1, CD31	Chemokines (form a directional gradient); lipid mediators; ECM molecules

- The adhesion cascade is initiated by an inflammatory stimulus acting directly or indirectly upon the endothelium. Such a stimulus may be delivered by various molecules such as histamine, bradykinin, thrombin, pro-inflammatory cytokines (e.g. TNF-α, IL-1), neuropeptides (e.g. substance P, VIP), and pro-inflammatory lipids (PAF, leukotrienes, prostaglandins); these molecules may derive from the circulation or cells in the local milieu (e.g. mast cells). The pro-inflammatory stimulus may also derive from shear or oxidative stress on the endothelium.
- Chemokines subserve multiple functions in the adhesive cascade, including: 1) activation of circulating leukocytes, qualitative and/or quantitative upregulation of adhesion molecules (e.g. chemokines upregulate integrin mediated adhesion via guanine nucleotide binding protein [G protein]-linked receptors of the rhodopsin-related [Rho] seven transmembrane family, 2) inducing redistribution of adhesion receptors (e.g. concentration of adhesion molecules at the leading edge or 'uropod' of the migrating cell) to facilitate their usage, 3) providing a directional gradient for cell migration through the ECM (via chemokine binding to glycosaminoglycans and other residues in the ECM). Chemoattractant induced adhesive and effector functions (e.g. chemotaxis) may be independently regulated. Because of their discriminatory potential for the selective recruitment of target cells (e.g. eotaxin for eosinophils, RANTES for memory T cells and eosinophils, DC-CK1 for naive T cells), chemokines may also determine the specific nature of an inflammatory response.

II. Inhibition of Adhesion Molecules; Rheumatoid Arthritis (RA) as a paradigm

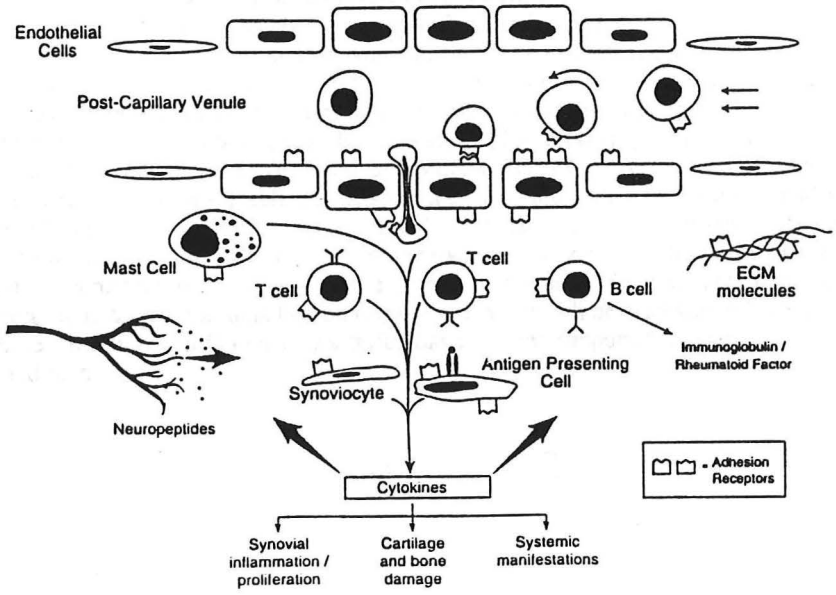
The topic of greatest interest to the clinician is the potential utility of these molecules as therapeutic targets. In order to explore this in more detail, I will focus on Rheumatoid Arthritis. However, the breadth of potential applicability of anti-adhesion therapies includes myriad areas of Medicine. Therefore, the use of such therapy in several other conditions will be mentioned.

1. Immunopathogenesis of RA

The potential utility of adhesion molecules as therapeutic targets in RA derives from the significant role these molecules play in the initiation and propagation of this disease. Adhesion molecules mediate diverse interactions central to the pathogenesis of RA via interactions with specific receptors expressed on the surface of other cells or on ECM molecules (Figure 2) (1-8).

Much information concerning the pathogenesis of RA has been derived from histopathological analysis of synovial cell populations. An early observation, and one that has gathered considerable renewed interest as a result of the progress in our understanding of adhesion receptors, is that there are characteristic alterations in the vasculature of the rheumatoid synovium (9-11). Angiogenesis, which depends upon the regulated action of adhesion receptors, is critical to the growth of the synovium and may play a prominent role in cartilage damage (10). Moreover, post capillary venules in the inflamed rheumatoid synovium assume the specialized phenotypic and functional characteristics of vessels found in lymphoid tissue (9,11,12). These so-called 'high endothelial venules' (HEVs) support high levels of lymphocyte extravasation form the blood, and thereby potentiate the initiation and sustenance of rheumatoid inflammation (12). From a therapeutic standpoint, a vital characteristic of HEVs is that maintenance of their phentoype is dynamic. Without persistent exposure to cellular and soluble elements in its afferent vessels, HEVs revert, assuming the characterisitcs of normal endothelium. Thus, even in chronic conditions such as RA, interruption of this active process, for example by inhibiting adhesion receptors, might reasonably be hypothesized to achieve a beneficial effect. Adding support to this concept is the observation that the synovial tissue lymphocytes and synovial fluid graulocytes in patients with RA patients are both derived predominantly from pools of circulating cells (13).

Figure 2
Immunopathogenesis of Rheumatoid Arthritis



In addition to vascular changes, another histopathologic characteristic of RA is the accumulation of a dense mononuclear cell infiltrate within the synovium (9,14). Evidence from a variety of sources has demonstrated that CD4+ T lymphocytes, the predominant cell type within these infiltrates, subserve a critical role in the orchestraton of rheumatoid inflammation (9,14,15). Therefore, antiadhesion therapy in RA might best be directed against those adhesion receptors most relevant to T cell interactions (*vide infra*). Further analysis demonstrated that the T cells most prominently involved in RA primarily express a 'memory' phenotype, indicative of previous antigenic exposure (9,14). As compared to naive cells, these T cells express higher concentrations of several adhesion receptors and other activation markers (15,16). Of note, the longterm survival of CD4+ T memory cells has been suggested to depend on continuous exposure to antigen (17). Therefore, although RA is a chronic disease, immunomodulatory interventions such as antiadhesion therapy may have the potential to modify the disease course. The accrual of memory T cells within the rheumatoid synovium is presumbly related to an enhanced transendothelial migratory capacity and a distinct pattern of recirculation. Synovial memory T cells would be expected to recirculate from the blood, to the inflamed synovium, through the lymphatics, and back to the blood (16). Consequently, appropriate antiadhesion therapy might be anticipated to be efficacious, even in established disease. In addition to T cells, other cell types play an important role in the pathogenesis of RA. For example, B cells that produce rheumatoid factor not only may participate in the disease process, but have also been demonstrated to recirculate to the joint (18). Other cells that contribute to the pathogenesis of RA, and may also serve as the ultimate targets of antiadhesion therapy include macrophages, dendritic cells, mast cells, and synoviocytes.

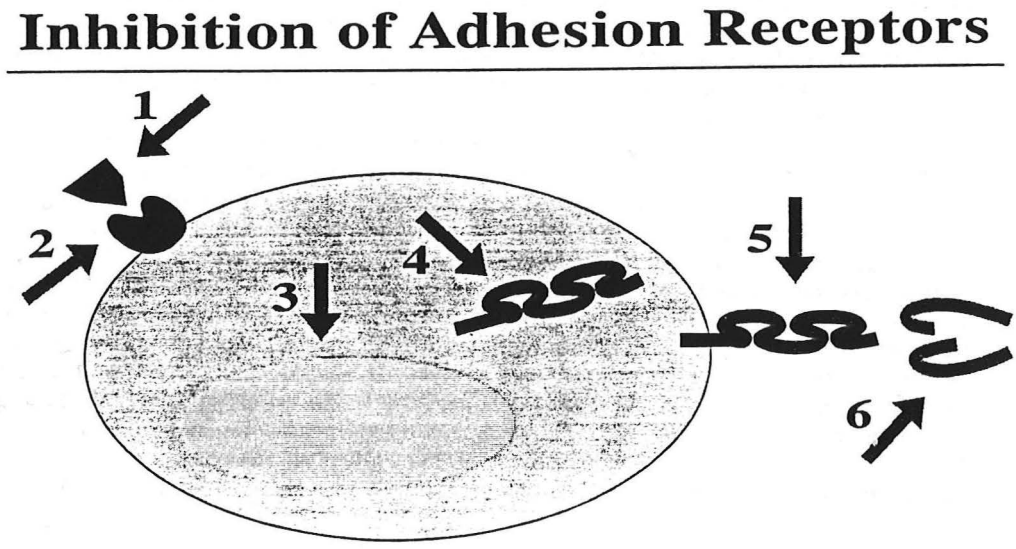
2. Adhesion Receptors as Targets

There is evidence supporting some role for multiple adhesion receptors in the pathogenesis of RA (19-41). Supporting data includes immunohistochemical analyses revealing adhesion receptors expressed on cells within the synovium, demonstration of quantitative and/or qualitative upregulation of adhesion receptors on cells in the synovium, and the detection of increased levels of soluble forms of adhesion receptors in the serum and synovial fluid of RA patients (19-28). It had previously been conjectured that the HEVs, T cells, or other cells within the rheumatoid synovium might uniquely express specific adhesion receptors. Although adhesion receptors unique to the inflamed rheumatoid synovium would be ideal therapeutic targets, it now appears that the adhesion receptors expressed by these cells are also expressed at other sites (12). While there may, therefore, be multiple potential targets of antiadhesion therapy, there are two adhesion receptor/counter receptor pairs that appear to play especially critical roles in the pathogenesis of RA; namely LFA-1/ICAM-1 and VLA-4/VCAM-1. These pairs of adhesion receptors are critical to various T cell interactions, including the ability of T cells to undergo transendothelial migration. Moreover, LFA-1 and VLA-4 can serve as accessory molecules, providing costimulatory signals to T cells. As these processes are central to the ability of T cells to orchestrate rheumatoid inflammation, these pairs of adhesion receptors are particularly attractive therapeutic targets for antiadhesion therapy in RA. As will be discussed subsequently, a role for these molecules in inflammatory arthritis has also been supported by therapeutic trials in animals and man.

3. Approaches to Antiadhesion Therapy

There are several methods by which adhesion receptor function might be inhibited. The most direct strategy involves specific inhibition of the adhesion receptor (Figure 3; arrow #5) or its counter-receptor (arrow #6). This has been the method utilized most widely in both animal models of RA as well as in human studies (4). Most studies have utilized monoclonal antibodies (mAb) as the therapeutic agent. MAb's possess several desirable characteristics, including exquisite target specificity, availabity in large quantities, and the ability to execute various effector functions via their Fc receptors. However, as most mAb produced to date have been generated in mice, there are potential limitations to their therapeutic use for human disease. Because they are foreign, murine mAb will elicit human anti-mouse antibody (HAMA) responses. On repeated administration, these HAMA may not only decrease the serum half life and thereby the therapeutic utility of the mAb, but may also cause potentially serious adverse effects. To circumvent these problems, several methods to reduce the immunogenicity of therapeutic mAb have been developed. Utilizing the techniques of molecular biology, parts of human antibodies can be substituted for the murine, yielding 'chimeric' and 'humanized' mAbs (3,4). The most eagerly awaited development is the ability to produce human mAbs directed against targets such as adhesion receptors. The function of adhesion receptors can be directly inhibited by agents other than mAb. For example, several adhesion receptors have been detected in soluble form in the serum and synovial fluid of patients with various diseases, including RA (25-27). Increases in the concentrations of several soluble adhesion receptors have correlated with inflammatory activity in RA (25,27). In various *in vitro* assays, however, soluble adhesion receptors have been effectively used as competetive inhibitors of adhesion receptor interactions (42). Moreover, therapeutically administered forms of soluble adhesion receptors have effectively abrogated inflammation in animal models (43). Interestingly, soluble forms of E-Selectin and VCAM-1 have recently been shown to modulate angiogenesis (44). Some constructs of soluble adhesion receptors might ultimately find utility as therapeutic agents in RA.

Figure 3



Traditionally, desirable characteristics of pharmacologic compounds have included low molecular weight, oral availability, and relatively low production cost. In the near future, agents with these characteristics that are also capable of direct inhibition of specific adhesion receptor interactions may become available. Perhaps the most notable progress in this field has been for adhesion receptors whose binding is mediated via the RGD (arginine-glycine-aspartic acid) motif. For example, several peptides and analogues capable of inhibiting the binding of the platelet integrin gpIIb/IIIa to the RGD sequence on fibrinogen have been discovered or synthesized (45,46). Several such agents are currently under investigation as inhibitors of platelet adhesion in human studies. There is a tremendous effort within the pharmaceutical industry to develop such inhibitors for other adhesion receptors, including those relevant to inflammatory diseases such as RA. Interestingly, some RGD-based inhibitors have been found to inhibit the adhesive interactions of ligands previously thought not to be mediated by the RGD domain. For example, adhesive interactions of VLA-4 with its ligands VCAM-1 and fibronectin have been shown to be inhibited by a cyclic RGD peptide (47). As noted, VLA-4/VCAM-1 interactions play an central role in RA, and such inhibitors might be expected to be of value in this disease. Fibronectin, another ligand for VLA-4, may also be important for leukocyte recirculation to the rheumatoid synovium. Peptide fragments of fibronectin have been used to attenuate inflammation in a rat model of arthritis (48,49). Potential small soluble inhibitors of interactions between integrins and immunoglobulin superfamily adhesion receptors, such as those mediated by CD11a/CD18, CD11b/CD18, ICAM-1, and ICAM-2, are also an area of intense development (50,51). Interestingly, some investigators have turned to nature, uncovering prokaryotic and eukaryotic products capable of inhibiting specific adhesion receptor interactions (46,52).

Substantial progress in the development of small soluble adhesion receptor inhibitors has also been made for the selectins. An intriguing early observation, indeed one that antedated the field of adhesion receptors per se, was that infusion of simple sugars was able to alter leukocyte recirculation (53). With the discovery that the adhesive interactions of the selectins are mediated via specific carbohydrate moieties, the potential utility of oligosachharides as inhibitors of adhesion resurfaced. In both *in vitro* experiments as well as an *in vivo* animal model of arthritis, soluble carbohydrates have been effective inhibitors (53,54). One such carbohydrate inhibitor has been administered to RA patients (55). These glycomimetics are another area of intense development in the pharmaceutical industry. As with the integrins, investigators have also uncovered bacterial products capable of inhibiting selectins (56).

In addition to direct inhibition of adhesion receptors at the cell surface, there are other means by which these molecules can be inhibited (Figure 3). Both the cell surface expression as well as the avidity for ligand of many adhesion receptors may be modulated by the effects of cytokines (5-8). Pro-inflammatory cytokines such as IL-1 and TNF-α have been suggested to play a central role in the immunopathogenesis of RA, at least part of which may relate to their ability to upregulate various adhesion recptors (57-59). Therefore, inhibition of proinflammatory cytokines, as can be accomplished by diverse methods (Figure 3; arrow #1 inhibition of the cytokine; arrow #2, inhibition of the cytokine receptor), may ultimately exert an immunomodulatory effect by inhibiting adhesion receptor function. There is support for this concept. For example, the primary immunomodulatory mechanism of action of corticosteroids has been demonstrated to be the inhibition of the production of various cytokines, including IL-1 and IL-6 (60). However, treatment with steroids has been shown to inhibit adhesion receptor

expression *in vitro* and *in vivo*, and to attenuate the adhesion receptor-mediated accumulation of leukocytes at inflammatory sites (61,62). Also, therapy with cyclosporin, which acts predominantly via inhibition of IL-2, has been shown to decrease expression of ICAM-1 in psoriatic skin lesions (63). Recently, it has been shown that treatment of RA patients with a mAb directed against TNF-α resulted in decreases in serum levels of soluble E-selectin and ICAM-1. The decreases correlated with alterations in circulating lymphocyte counts and clinical response, suggesting that part of the mechanism of action of this agent relates to modulation of adhesion receptor function in the synovial endothelium (64).

Another novel approach to antiadhesion therapy involves direct inhibition of adhesion receptor synthesis. Inhibition of the transcription or translation (Figure 3, arrows #3 &4) of the DNA or RNA encoding adhesion receptor proteins can be achieved with specific 'antisense' oligonucleotides (65,66). This approach has been tested in animals (66). It may also be possible to block adhesion receptors synthesis by utilizing specific protease inhibitors, which could offer pharmacologic benefits such as oral availability (67). These elegant molecular biologic approaches will no doubt emerge as potential antiadhesion therapies for human disease in the near future.

4. Mechanisms of Action / Goals of Antiadhesion Therapy

Consideration of the possible utility of antiadhesion therapy in RA should include the potential mechanisms of action of such therapy. Because they play an important role at various stages in the immunpathogenesis of RA, targetting adhesion receptors might be expected to yield heterogeneous effects. Interestingly, in both animal models and human studies, it appears that several mechanisms of actions might indeed be operative.

The most straightforward mechanism of action of antiadhesion therapy would be alterations in cellular traffic. Inhibiting an adhesion receptor critical to the entry of a particular cell into an inflammatory site should block that cell's accrual at the site. While there is substantial evidence supporting this mechanism of action, it appears that the *in vivo* situation is even more complex. Thus, it has been shown that adhesion receptors function in a cascade fashion, much like the clotting and fibrinolytic systems. Inhibition of a given adhesion receptor/counter-receptor pair may ultimately affect the utilization of other adhesion receptors. Thus, it might be possible to block the function of a given adhesion receptor or a part of the immune response by targetting an interaction more proximal in the cascade. For example, the selectins have their primary role in the intial interactions between circulating leukocytes and the endothelium. However, inhibition of E-selectin function has been shown to attenuate the late phase airway response in an animal model of asthma (68). Another indirect mechanism by which blocking of adhesion receptors might produce clinical benefit is by mollifying the local damage secondary to leukocyte infiltration. During the course of transendothelial migration, circulating leukocytes release a host of products such as proteases and oxidants that are capable of injuring the endothelium, altering endothelial function, and degrading the extracellular matrix constituents. The impact of such injury, for example increased vascular permeability, may have a profound potentiating effect on the underlying inflammatory response. This impairment may be attenuated by antiadhesion therapy (69).

It has been shown that certain adhesion receptors are capable of providing activation signals to immunocompetent cells. Thus, inhibition of the activation of cells may be another important mechanism of antiadhesion therapy. Indeed, in some animal models it appears that decreased activation of cells in the inflammatory site may have been the primary mechanism of action of antiadhesive therapy. For example, treatment with an anti-VLA-4 antibody has been shown to exert significant effects on airway hyperresponsiveness, an indication of decreased eosinophil activity, without significantly altering the number of eosinophils recovered in bronchoalveolar lavage fluid (70). For immunologically driven disease such as RA, the preeminent goal of antiadhesion receptor therapy may be modulation of cellular activation. LFA-1 and VLA-4 have both been shown to function as co-stimulatory molecules on T cells (71,72). Inhibition of co-stimulatory molecules in the context of antigen presentation may facilitate the establishment of immunologic tolerance. Indeed, in animal models, antigen specific tolerance of solid organ allografts has been achieved utilizing antiadhesion receptor therapies (66,73). This method of tolerance induction has particular appeal in RA. Although RA is presumably caused by the exposure of susceptible hosts to relevant etiologic antigen(s), the failure to identify the etiologic agent precludes the use of other, antigen-specific methods of tolerance induction (4).

A relevant consideration in antiadhesion therapy is the potential for adverse effects, particularly increased susceptibility to infection. This possibility is supported by two human immunodeficiencies, leukocyte adhesion deficiency (LAD) types 1 and 2, that are characterized, respectively, by deficiencies of CD11/CD18 and selectin adhesion receptors. Moreover, in some animal studies, an increased proclivity for infection has been noted as a sequela of antiadhesion therapy (74). Other studies have not found an increase in infectious complications consequent to antiadhesion therapy. The risk of infection may be affected by the choice of target. For example, it might be expected that targetting ICAM-1, which is dramatically upregulated at inflammatory sites such as the rheumatoid synovium as compared to normal tisue, might be less immunosuppressive than targetting CD18, which is present on all leukocytes. Support for this comes from 'knockout' animals, where it has been shown that ICAM-1 mice do not appear excessively susceptible to infection. Similarly, targetting VLA-4, which is expressed on all leukocytes with the exception of neutrophils, might conceivable cause less immune suppression by leaving neutrophil function intact. Nevertheless, heightened vigilance for sequelae of immunosuppression is required for all studies utilizing antiadhesion therapies.

5. Antiadhesion Therapy in Animal Models of RA

A variety of animal models of arthritis that bear some semblance to human RA have been established (75). Various antiadhesion therapies have been tested and found efficacious in several of these models (Table 6) (49,76-82). While these models share certain histopathological characteristics with RA, they do not exactly replicate the disease. Therefore, extrapolation of the results of studies assessing antiadheison therapy in RA from animal models to human RA may be inexact. In particular, the temporal association between disease initiation and subsequent antiadhesion therapy is typically very close in animal studies, and certainly closer than might be expected for RA. This is noteworthy, because in several animal models, the ability of antiadhesion therapy to attenuate inflammation or to modulate immune responses tends to decrease as the disease becomes more established.

Table 6. Antiadhesion Therapy in Animal Models of RA

Agent	Model	Results
mAb to CD18	rabbit / antigen-induced arthritis	decreased development of acute arthritis and chronic arthritis
mAb to ICAM-1	rat / adjuvant arthritis	adoptive transfer experiment: treatment blocked both generation of effector cells and migration into joint
mAb to LFA-1 (CD11a/CD18) & ICAM-1	mouse / collagen-induced arthritis	treatment suppressed development of arthritis, but not antibody response
mAb to LFA-1 (CD11a/CD18)	rat / adjuvant arthritis	treatment inhibited neutrophil but not T cell migration into joints
mAb to CD11a/CD18 and CD11b/CD18	rat / adjuvant arthritis, cytokine-induced arthritis	treatment inhibited neutrophil migration into joint induced by IL-1 and TNF-α
mAb to VLA-4	rat / adjuvant arthritis	treatment decreased accumulation of lymphocytes in joints
mAb to VLA-4	rat / adjuvant arthritis	treatment prevented onset of synovitis
synthetic fibronectin peptides	rat / antigen-induced arthritis	treatment decreased leukocyte recruitment into joint and development of synovitis
oligosachharides / mannans	rat / adjuvant arthritis	treatment decreased development of synovitis

7. Antiadhesion Therapy in RA

As a prelude to the discussion of direct antiadhesion therapy in RA, it is noteworthy that inhibition of adhesion receptors may be a common mechanism for several traditional antirheumatic agents. For example, several disease modifying anti-rheumatic drugs (DMARDs) have recently been shown to be capable of altering adhesion receptor function and/or angiogenesis (Table 7) (83-90). Other drugs that have been suggested to have some ability to modulate leukocyte-endothelial adhesive interactions include nonsteroidal anti-inflammatory drugs (NSAIDs), colchicine, dapsone, leumedin, and tenidap (91-95).

Table 7. Effects of Antirheumatic Drugs on Adhesion Receptors

Agent	Effect on Adhesion Receptors / Endothelium
Methotrexate	- Inhibits CD11b/CD18 mediated adhesion via induction of adenosine release - Inhibits endothelial cell proliferation
Gold Salts	- Downregulate E-selectin, ICAM-1 and VCAM-1 expression cells - Inhibits endothelial cell proliferation and IFN-γ induced HLA-DR expression
Sulfasalazine	- Inhibits CD11b/CD18 mediated adhesion via induction of adenosine release - Inhibits endothelial cell proliferation
D-Penicillamine	- Inhibits endothelial cell proliferation and neovascularization *in vivo*
NSAIDs	- Inhibit neutrophil adherence mediated by CD11b/CD18 - Inhibit neutrophil-endothelial attachment via decrease in L-selectin expression

The greatest experience with direct antiadhesion therapy in RA has been with a murine anti-ICAM-1 mAb (96-100). The safety and efficacy of the anti-ICAM-1 mAb have been analyzed in heterogeneous populations of patients with active RA in several open trials (96,97). Initially, patients with longstanding, relatively refractory RA were assessed. Subsequently, patients with earlier disease were studied (Table 8). DMARDs were discontinued at least 1 month before treatment, but patients were allowed to remain on stable doses of NSAIDs and low-dose corticosteroids. To minimize the placebo response in these open trials, clinical responses were determined using composite criteria that required $\geq$ 20% improvements in at least 4 of 6 parameters of disease activity. The number of patients who experienced a response to 5 days of therapy is shown in Table 5. Of the 23 patients with refractory RA, 13 had a marked or moderate clinical response through 1 month of followup. The response was sustained through 2 months for 9/23 and through 3 months for 3/23. Of the 10 RA patients with less established disease, 7 experienced a clinical response through day 29 of followup, and 5/10 patients sustained their response through day 60. Three of the 10 patients treated had extended clinical benefit (Table 9). Thus, as had been noted in animal models, it appears that longstanding, chronic RA may be relatively less amenable to antiadhesion therapy than less established disease. An important caveat to the interpretation of these studies is that the trials were open in design. While the results seem encouraging, the ultimate clinical utility of this (or, for that matter, any therapy) would need to be definitively established via placebo-controlled studies. Another important footnote is that there did not appear to be a proclivity to infections due to therapy.

Table 8. Anti-ICAM-1 mAb in RA: Patient Demographics/Initial Evaluation Parameters

Parameter	Refractory RA Patients (n = 23)	Early RA Patients (n = 10)
age	48.3 ± 11.3	41.3 ± 9.8
sex	19 ♀, 4 ♂	9 ♀, 1 ♂
duration of RA (years)	15.2 ± 9.2	1.4 ± 2.1†
# of DMARDs failed	4.3 ± 1.5	0.3 ± 0.5
tender joint score	26 [11 - 83]	30 [19 - 96]
swollen joint score	28 [9 - 116]	15 [10 - 37]
a.m. stiffness (minutes)	180 [20 - 960]	180 [60 - 750]
ESR (mm/hr)	47 [10 - 120]	66 [26 - 104]
patient global assessment	3 [1 - 4]	2 [2 - 3]
physician global assessment	2 [1 - 4]	2 [1 - 3]

Data are reported as mean ± s.d., or as median and [range]
† 7 of the 10 patients had a disease duration of < 12 months at entry.

Table 9. Response to Therapy

Refractory Patients (n = 23)

Response	Day 29	Day 60	Day 90
Marked	5	4	2
Moderate	8	5	1

Early Patients (n = 10)

Response	Day 29	Day 60	Day 90	Day 120	Day 150	Day 240	Day 310
Complete	0	0	0	1	1	1	1
Marked	4	3	3	1	2	1	0
Moderate	3	2	0	1	0	0	0

Several studies were performed to help delineate the mechanism of action of the anti-ICAM-1 mAb. Pharmacokinetic analysis revealed that all patients had detectable serum anti-ICAM-1 during treatment. Further, anti-ICAM-1 mAb could be measured in the synovial fluid. Anti-ICAM-1 mAb was detected on the surface of circulating leukocytes as well as on the vascular endothelium and perivascular leukocytes from skin biopsy specimens. As a correlate of this observation, serial DTH testing revealed that anti-ICAM-1 induced transient cutaneous anergy for a number of patients.

Analysis of the numbers of circulating leukocytes revealed a significant increase in the number of lymphocytes during therapy, without significant changes in neutrophils or monocytes. Phenotypic analysis revealed that the increase in lymphocytes consisted predominantly of CD3+CD4+ T cells. Of note, in this population there was an increase in the numbers of activated cells, as evidenced by the increased expression of HLA-DR and the IL-2 receptor (CD25). These results suggest that treatment with anti-ICAM-1 altered the recirculation of T cells, and may have caused a temporary redistribution of T cells out of the rheumatoid synovium. Further analysis revealed that there was an elevation of mRNA for IFN-γ from circulating mononuclear cells during therapy (98). This suggests that therapy altered the circulatory pattern of T cells with a Th1 like phenotype, the subset of T cells thought to play a central role in the pathogenesis of RA. Of note, the increase in IFN-γ correlated with clinical efficacy in treated patients, implying mechanistic relevance.

As noted above, interference with adhesion receptor function might facilitate the induction of immunologic tolerance. In these studies treatment with the anti-ICAM-1 mAb appeared to induce a state of T cell hyporesponsiveness. Thus, T cell proliferative responses to mitogens were impaired in some patients after therapy, whereas proliferative responses to recall antigens were preserved (99). Of note, there was a correlation between this T cell hyporesponsivenss and clinical outcome. Therefore, in this study a form of peripheral T cell anergy may have been induced, that might account for some of the clinical improvement noted. As noted, the ability to engender inmunologic tolerance decreases with increasing chronicity of disease. Thus, it may be quite difficult for any immunomodulatory agent to achieve a longstanding remission of disease activity when used as monotherapy. Therefore, the clinical results seen in the cohort of patients

with refractory RA who received anti-ICAM-1 mAb are quite encouraging. In addition, the extended clinical benefit noted for some patients with relatively early disease are promising.

Eight patients who had received an initial 5 day course of anti-ICAM-1 mAb also received a second course of mAb (100). Six patients developed serum sickness like symptoms several days following the second 5 day course of treatment. While the precise underlying mechanisms have not been defined, these symptoms are presumed to relate to the formation of immune complexes, possibly consisting of HAMA/anti-ICAM-1 mAb/circulating ICAM-1. In support of this, all patients developed detectable HAMA after the first treatment. In addition, there was evidence of transient depletion of complement proteins during the second course of therapy that was not observed in the initial course. Moreover, the clinical benefit associated with the second course of therapy was far inferior to that of the first course, both in the number of patients responding as well as the duration of response. In summary these observations indicate that although ICAM-1 appears to be an appropriate target for antiadhesion therapy in RA, a murine mAb is not a suitable agent on account of its immunogenicity.

Another agent with potential antiadhesion effects that has been used in patients with RA is the oligosaccharide dimeric Le^x (55). In a 6 month open study of 27 RA patients, 17 showed some improvement in clinical status related to intradermal therapy with this agent. As adverse effects were relatively minor, future therapies based on this approach would seem to be warranted. As noted above, data regarding the potential clinical efficacy of novel therapeutic agents in RA that is derived from open trials needs to be confirmed in placebo-controlled trials.

8. Future Directions in Antiadhesion Therapy in RA

There are great expectations that developments in antiadhesion therapy in the near future will yield important and useful therapeutic agents (3,4,5-8,101). Substantial progress in several facets of antiadhesion therapy may be forthcoming. Prominent among these may be novel types of agents. The ultimate goal is the development of agents that are not only effective, but also easy to administer, relatively inexpesive, and nonimmunogenic. This is particularly germane, as the costs and hence the cost efficacy of novel therapeutic agents impact substantially on their ultimate clinical utility (102). The combination of antiadhesion therapy with agents with distinct mechanisms of actions, for example cytokine directed therapies, might be shown to be complementary or synergistic. Alternatively, combinations of antiadhesion therapy with more traditional antirheumatic agents might produce increased efficacy for some patients. As it has been observed that there is substantial heterogeneity in patient response to immunomodulatory therapies, identification of the subsets of patients most likely to respond to antiadhesion therapy would be a significant advancement. Finally, the goals of antiadhesion therapy might be altered as our therapeutic armamentareium expands and our experience broadens. The ultimate goal would be true disease modification, as might be expected to result from the establishment of immunologic tolerance. Further advancements in this exciting discipline are eagerly awaited.

Table 10. Future Directions in Antiadhesion Therapy in RA

Goals (Disease Modification)

- induce immunologic tolerance (to etiologic and other relevant antigens)
- regulate angiogenesis
- attenuate tissue damage
- modulate apoptosis

Targets

- novel adhesion molecules
- cytokines / chemokines that potentiate adhesion molecules

Agents

- achieve better tolerance of available agents
  - less immunogenic preparations (e.g. PEG-treated mAb, soluble receptors)
  - optimize administration (e.g. oral absorption, Ig-constructs)
- human antibodies directed against adhesion receptors
  - repertoire cloning / phage libraries
  - human gene/chromosome transfer to animals / plants
- peptide and peptidomimetic or analogue adhesion molecule inhibitors
  - rationale drug design
  - high throughput screening
  - natural products (e.g. plant flavonoids)
- oligonucleotide and other molecular directed therapies
  - antisense
  - protease inhibitors
  - ribozymes
- oral availability
- cost considerations

Patients/trials

- define optimal therapeutic subsets of patients to receive antiadhesion therapy (e.g. those with early disease, or with specific cytokine profiles or disease activity, etc)
- combination therapy (e.g. antiadhesion therapy + cytokine directed therapy; combination of therapies directed against adhesion receptors and their counter-receptors)

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