

Recently interest has increased tremendously in the possible roles of adhesion molecules on inflammatory cells, vascular endothelium, and matrix proteins in the allergic inflammatory response. The authors have reviewed some of the recent findings and speculated about their implications, with the understanding that some of the conclusions will require confirmation by in vivo studies of allergic reactions.

A possible role for adhesion molecules in asthma

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The bridging of two or more IgE molecules by an allergen on the surface of basophils and mast cells results in degranulation and release of chemical mediators. The immediate consequences, when this reaction occurs in respiratory bronchi, are smooth muscle contraction and increased vascular permeability. The late-phase response, which occurs 4 to 8 hours later, is characterized by an inflammatory cellular infiltrate in the bronchial mucosa and submucosa.

Cell adhesion molecules are glycoproteins, identified within the last decade, expressed on all surfaces that allow cell-to-cell contact. They have a variety of functions. These include the following: (a) promoting adhesion of one cell to another or to a tissue matrix, (b) activating cells, and (c) promoting cellular migration and infiltration. Therefore the term "adhesion molecule" means more than only attachment; it includes a broad spectrum of cellular functions. These adhesion molecules may play an essential role in the pathogenesis of asthma.

ADHESION MOLECULES: FUNCTIONS

Adhesion molecules¹⁻³ (a) provide form through cell adhesion, (b) promote leukocyte-vascular endothelium and leukocyte-extracellular tissue matrix adhesion, (c) promote leukocyte migration from vascular compartments into extravascular tissues, (d) promote antigen-specific recognition by T lymphocytes, (e) are costimulatory signals for T-cell activation, (f) stimulate effector mechanisms of activated lymphocytes, and (g) stimulate cell proliferation and regulation of cell growth.

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TABLE I. Adhesion molecules: classification

Classification	
A. Immunoglobulin supergene family	
T cell receptor (CD3)	LFA-2 (CD2)
LFA-3 (CD58)	ICAM-1 (CD54)
ICAM-2	ICAM-3*
VCAM-1	MHC class I
MCH class II	CD4
CD8	
B. Integrin family	
1. Group β_1	
VLA-1 ($\alpha_1\beta_1$)	VLA-4 ($\alpha_4\beta_1$)
VLA-2 ($\alpha_2\beta_1$)	VLA-5 ($\alpha_5\beta_1$)
VLA-3 ($\alpha_3\beta_1$)	VLA-6 ($\alpha_6\beta_1$)
2. Group β_2	
LFA-1 (CD11a/CD18)	
Mac-1 (CD11b/CD18)	
p150,95 (CD11c/CD18)	
3. Group β_3	
VNR	
Platelet glyco IIb/IIIa	
C. Selectin family	
E-selectin (ELAM-1)	
L-selectin (LAM-1)	
P-selectin (GMP-140 or CD62)	

*Not yet classified.

ADHESION MOLECULES: CLASSIFICATION

On the basis of their chemical structural similarities, three major adhesion molecule groups have been described, each of which has many members (Table I).^{1, 4, 8}

Immunoglobulin supergene family

The immunoglobulin supergene family is a large group of membrane glycoprotein molecules characterized by the presence of one or more immunoglobulin domains. They share the immunoglobulin domain, composed of 90 to 100 amino acids, arranged in a circular structure of two sheets

Abbreviations used

β_2 or CD18:	One of the six groups of integrins that has three members	Mac-1:	Integrin corresponding to the β_2 group
CD:	Cluster of differentiation antigens of lymphocytes	MEL-14:	The murine adhesion molecule equivalent to the human L-selectin corresponding to the selectin family
ELAM:	Endothelial cell adhesion molecule	MHC:	Major histocompatibility complex
FMLP:	N-formyl-L-methionyl-L-leucyl-phenylalanine	NY:	Neuropeptide Y
GM-CSF:	Granulocyte-macrophage-colony stimulating factor	p150,95:	Integrin corresponding to the β_2 group
GMP:	Granule membrane protein	PAF:	Platelet-activating factor
HUVECs:	Human umbilical vein endothelial cells	Platelet glycoprotein IIb/IIIa:	Platelet glycoprotein integrin corresponding to the β_3 group
ICAM:	Intercellular adhesion molecule	PMNLs:	Polymorphonuclear leukocytes
IL:	Interleukin	RGD:	Adhesive tripeptide (L-arginine-L-glycyl-L-aspartyl) for many integrins
LAD:	Leukocyte adhesion deficiency	SP:	Substance P
LAK:	Lymphocyte activated killer cells	TNF:	Tumor necrosis factor
LAM:	Leukocyte adhesion molecule	VCAM:	Vascular cell adhesion molecule
LFA:	Lymphocyte function-associated antigen	VLA:	Very late activation integrin corresponding to the β_1 group
LT:	Leukotriene, eicosanoid derived from the 5-lipoxygenase pathway of the arachidonic acid metabolism	VNR:	Vitronectin receptor corresponding to the β_3 group of integrins
MAb:	Monoclonal antibody		

of β -strands, which are closed by a disulfide bond (Fig. 1). This family includes the CD3 and the antigen-independent receptor lymphocyte function-associated antigen (LFA)-2 (CD2) expressed by T cells. LFA-3 (CD58), which attaches to LFA-2, is a cell surface adhesive ligand with broad tissue distribution.³ The intercellular adhesion molecule (ICAM)-1 (CD54), which is expressed by mast cells, lymphocytes, eosinophils, and endothelial and bronchial epithelial cells, is a counter-receptor for LFA-1 as are Mac-1 and p150,95 (both integrins).⁹ Human sensitized pulmonary endothelial cells increase ICAM-1 gene expression after IgE receptor perturbation.¹⁰ ICAM-2, naturally expressed on endothelial cells, is not induced by inflammatory cytokines. It enables leukocytes to adhere to the endothelium and migrate from the bloodstream and may be important in leukocyte-resting endothelial cell interaction.^{4,11} ICAM-3 is expressed at high levels on resting lymphocytes, monocytes, and neutrophils and not on either resting or stimulated endothelial cells.¹² Since the adhesion of resting T lymphocytes to cells bearing LFA-1 is primarily via ICAM-3, and ICAM-3 is found in higher concentration on hematopoietic cells than are ICAM-1 or ICAM-2; ICAM-3 may be the most functionally important ICAM. Although ICAM-3 has not been yet classified, it can be transiently considered as a member of the

immunoglobulin supergene family. The vascular cell adhesion molecule (VCAM)-1 promotes adhesion of lymphocytes, monocytes, and some leukocytes to endothelium in vitro and may promote inflammation in vivo.¹³ Other molecules primarily involved in antigen recognition, such as the major histocompatibility complex (MHC) class I and class II antigens and their T-cell receptors, CD8 and CD4, also have adhesion properties.

Integrin family

Integrins are noncovalent heterodimers composed of an α and a β polypeptide subunit¹ (Fig. 2). At least eight groups of integrins exist as determined by β chain diversity, that share distinct α subunits. The name integrin was coined to signify the presumed role of these proteins that link the intracellular cytoskeleton with the extracellular matrix.⁶ Integrins appear to be present on all human cells. They are essential in leukocyte transmigration, platelet aggregation, tissue repair, and tumor invasion. The β_1 , β_3 , and β_5 groups are receptors for peptides that contain the L-arginine-L-glycyl-L-aspartyl (RGD) sequence; in addition, these integrins can bind other ligands. The RGD sequence is present in the low-affinity IgE receptor (Fc ϵ RI) and in proteins such as laminin, fibronectin, type I and III collagen, and vitro-

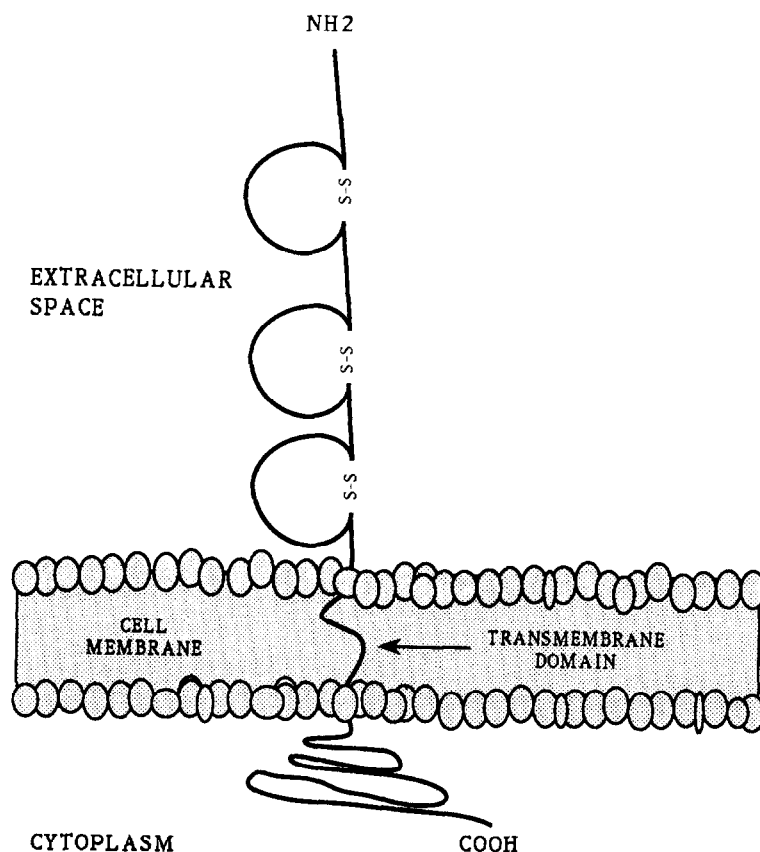


FIG. 1. Basic structure of an adhesion molecule of the immunoglobulin supergene family. Each circular structure is closed by a disulfide bond and represents a single immunoglobulin domain.

nectin, all of which are high in concentration in the bronchial epithelial basement membrane.⁶ Integrins of the β_1 group (i.e., $\alpha_4\beta_1$) are also receptors for L-leucine-L-aspartyl-L-valine (LDV), a different tripeptide sequence present on fibronectin.¹⁴ The β_2 (CD18) group has three members: LFA-1, Mac-1, and p150,95. LFA-1, expressed exclusively on leukocytes, when activated, is the receptor for ICAM-1, ICAM-2, and ICAM-3. ICAM-1 has the highest affinity for activated LFA-1, and ICAM-2 and ICAM-3 have similar but lower affinities. Mac-1 is present on the plasma membranes of neutrophils, monocytes, macrophages, large granular lymphocytes, and some B cells. p150,95 is expressed by macrophages, monocytes, granulocytes, and some T cells. Genetic mutations of the β_2 polypeptide causes the disease leukocyte adhesion deficiency (LAD). Leukocytes of patients with LAD have an intravascular-to-extravascular migration defect that results in an increased susceptibility to bacterial infections and poor wound healing. Integrins are essential for bronchial epithelium because they enable epithelial cells to bind to the basement membrane.

Selectin family

Selectins, on leukocyte and endothelial cell membranes, regulate leukocyte binding to vascular endothelium of inflamed tissues. They have an *N*-terminal domain of 117 to 120 amino acids with homology to calcium-dependent

lectins¹ (Fig. 3). L-selectin (leukocyte adhesion molecule [LAM]-1),¹⁵ expressed by granulocytes, monocytes, and lymphocytes, mediates lymphocyte adhesion to endothelium of lymph node vessels as well as neutrophil binding to vascular endothelial cells.⁴ E-selectin (endothelial cell adhesion molecule [ELAM]-1),¹⁵ expressed by cytokine-stimulated endothelial cells, promotes neutrophil adhesion and migration to inflammatory sites.¹⁶ The Sialyl-Lewis^x determinant, the carbohydrate receptor for E-selectin, is structurally present on monocytes and granulocytes and on cells of some types of lung and colon carcinomas.¹⁷ E-selectin plays an important role in the influx of neutrophils in IgE-mediated late-phase reaction in the lung and skin.^{18, 19} P-selectin (granule membrane protein [GMP]-140 or CD62),¹⁵ on activated platelet and endothelial cell surfaces, is primarily located in small veins and venules to which leukocytes migrate initially during inflammation.

EXPRESSION OF ADHESION MOLECULES INDUCED BY INFLAMMATORY MEDIATORS AND CELLULAR RESPONSES ASSOCIATED WITH THE PATHOGENESIS OF ASTHMA

Inflammatory mediators released by anti-IgE-dependent challenge *in vitro* promote or enhance the expression of adhesion molecules by cells involved in the pathogenesis of asthma (Table II).

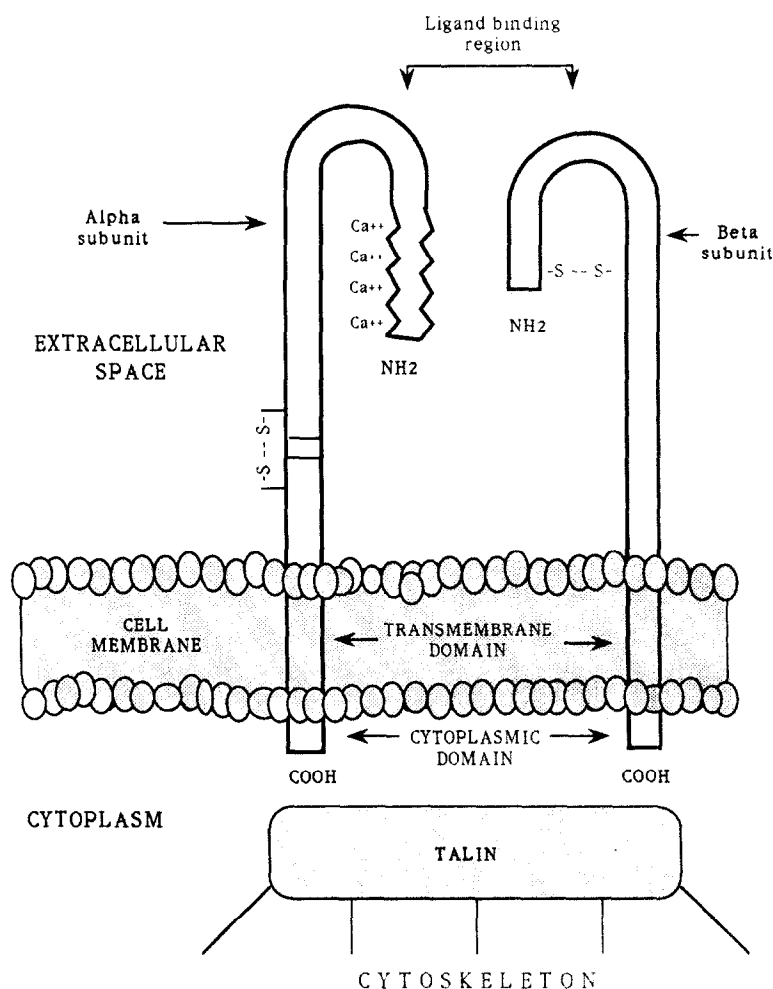


FIG. 2. Schematic structure of a typical integrin. At least eight groups of integrins, as determined by different β polypeptides, bind noncovalently to an α chain. Classification of integrins based on the different β chains.

SOLUBLE MEDIATORS

Histamine

Histamine (10^{-4} mol/L) stimulates human umbilical vein endothelial cells (HUVECs) to express P-selectin that induces a time-dependent neutrophil adhesion that maximizes in 5 to 10 minutes and then declines.²⁰ The inhibition of this histamine-induced neutrophil binding by the monoclonal antibody (MAb) G1 (anti-P-selectin) confirms that it is P-selectin that attenuates neutrophil adhesion to sites of acute inflammation. Stimulation with histamine (10^{-5} mol/L, which alters vascular permeability) causes a fourfold increase in expression of P-selectin in HUVECs. The concentration of P-selectin reaches near-maximal values within 3 minutes after histamine stimulation and declines to control levels in 20 minutes.²¹ Histamine (10^{-4} mol/L) also stimulates an increased concentration of P-selectin on the surfaces of megakaryocytes and platelets.²² Therefore during bronchial inflammation, as occurs in asthma, when basophils and mast cells release histamine, neutrophil adhesion may be enhanced by histamine-induced endothelial expression of P-selectin.

Platelet-activating factor (PAF)

PAF (10^{-9} mol/L, which alters vascular permeability) stimulates eosinophil adherence to cultured endothelial cells.²³ The MAb TS1/18 (anti-CD18) reduced the PAF-induced eosinophil adherence by about 92%, which indicates that CD18, a subunit of the integrin family, functions, in part, to increase adhesiveness of eosinophils to endothelial cells. PAF also induces expression of the CD11 subunit of the integrin family on the eosinophil surface membrane.²⁴ These two studies established that PAF induced, within 15 minutes, the expression of Mac-1, LFA-1, and p150.95 all of which play a major role in eosinophil adherence to endothelium. PAF synthesized by endothelial cells also provides a mechanism for adhesion to the endothelium by polymorphonuclear leukocytes (PMNLs) (neutrophils, eosinophils, basophils, and mast cells).²⁵ The PMNLs bind maximally at 5 minutes and return to unstimulated values at 60 minutes. Binding can be prevented by prior incubation of PAF receptors with PAF acetylhydrolase or by treatment with a competitive PAF receptor antagonist. Thus PAF, by increasing production of adhesion molecules, provides an

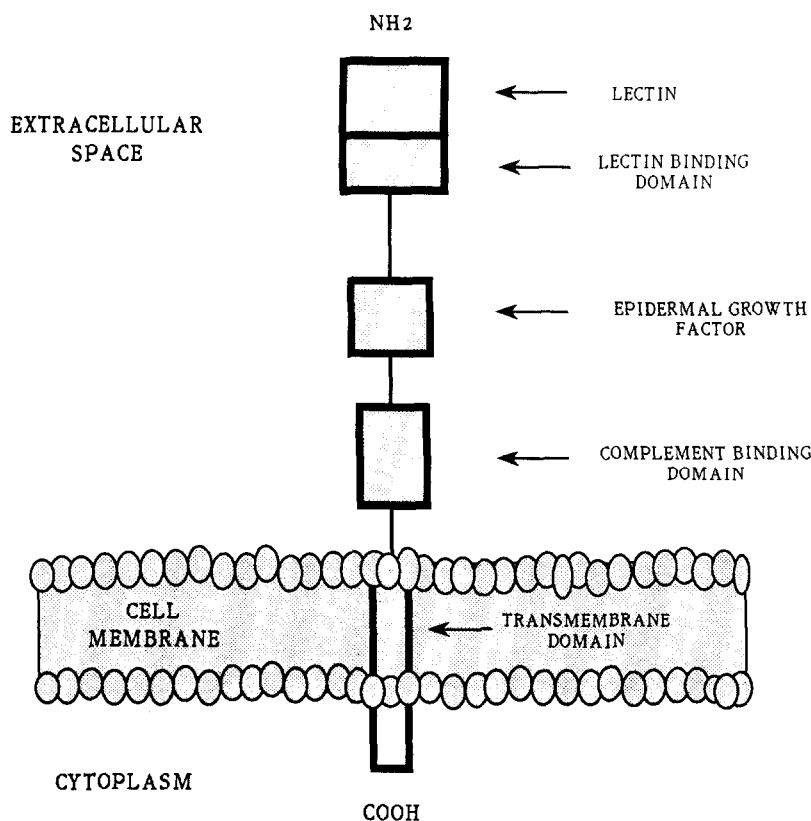


FIG. 3. Schematic structure of the selectin family which contains five different domains: lectin, lectin binding, epidermal growth factor, complement binding, and transmembrane. The complement binding domain can be expressed alone or in multiples in each of the three known selectins.

endothelium-dependent mechanism by which eosinophils and PMNLs adhere to endothelial cells. Adhesion molecules, along with other inflammatory mechanisms, may prolong recruitment of leukocytes at sites of bronchial inflammation, as in asthma.

Leukotriene (LT) B₄, C₄, and D₄

LTB₄, in physiologic concentrations, mobilizes latent intracellular pools of Mac-1 and p150,95 and increases the surface expression of these molecules on neutrophils and monocytes and thereby increases granulocyte and monocyte adherence to endothelial cells.^{26, 27} LTC₄ and LTD₄ also enhance binding of PMNLs to endothelial cells, but the identity of the adhesion molecule remains unknown.²⁸ PMNLs, specifically desensitized to PAF, show only 39% of the agonist-stimulated adherence as compared with controls. The fact that exogenous PAF increases adherence of PMNLs to unstimulated endothelium, and LTC₄ and LTD₄ induce PAF synthesis by endothelial cells, suggests that PAF could be the chemical mediator inducing the adhesion molecules that cause PMNLs adherence to endothelial cells.

Adhesiveness promoted by LTs (eicosanoids derived from the 5-lipoxygenase pathway of the arachidonic acid metabolism) contributes to the inflammatory response of bronchial epithelium in asthma. LTB₄ is produced by cells present in bronchial inflammation in asthma, that is, eosinophils,

monocytes, alveolar macrophages, and neutrophils. LTC₄ and LTD₄, produced by mast cells, basophils, and eosinophils, also have chemotactic properties that similarly contribute to the pathogenesis of asthma.

Hydrogen peroxide (H₂O₂)

Exposure of endothelial cells for 2 hours or less to sub-millimolar concentrations of oxygen radicals (H₂O₂, t-butylhydroperoxide or menadione) induces a prolonged expression of P-selectin on the endothelial cell surface resulting in enhanced PMNL adherence.²⁹ This binding does not require de novo protein synthesis and is inhibited by antioxidants or by MAb anti-P-selectin. H₂O₂ facilitates adhesion of leukocytes to cultured endothelial cells.³⁰ Mac-1 mediates neutrophil adherence-dependent production of H₂O₂. H₂O₂ production by LAD neutrophils and normal neutrophils was compared with use of monoclonal antibodies 904 and R15.7, which react with both chains, CD11b and CD18, of Mac-1. Normal neutrophils adherent to endothelial cells secreted larger amounts of H₂O₂ than adherent LAD neutrophils. The H₂O₂ production by normal neutrophils, stimulated with *N*-formyl-L-methionyl-L-leucyl-phenylalanine (FMLP), was blocked by these monoclonal antibodies, which further suggests that Mac-1 is the necessary signal for adherence-dependent potentiation of H₂O₂ production. Oxygen radicals, produced by activated mast cells, basophils, eosinophils,

TABLE II. Adhesion molecules induced by inflammatory mediators known to be present in asthma

Inflammatory mediator	Adhesion molecule expressed or activated	Cells	Ref
Histamine	P-selectin	Endothelial cell and platelet	20-22
PAF	CD18	Eosinophil	23
Hydrogen peroxide	Mac-1	Neutrophil	30
LTB ₄	Mac-1 and p150,95	Mononuclear and PMNLs	26,27
LTC ₄ and D ₄	Unknown	Neutrophil	28
Substance P	E-selectin	Endothelial cell	88
Cytokines			
IL-1 and TNF- α	VCAM-1	Endothelial cell	34
	L-selectin	Neutrophil	35
	ICAM-1	Endothelial cell	9
	E-selectin	Endothelial cell	16,19
IL-2	ICAM-1	Lymphocyte	39
IL-3	CD18	Basophil and monocyte	43,44
IL-4	ICAM-1	Mast cell	49
	VCAM-1	Endothelial cell	45
	Unknown	Endothelial cell	46
IL-5	CD11/18	Eosinophil	23,24
IL-6	CD18, CD11b	Promonocytic cell	51
	CD11c and ICAM-1		
IL-7	CD3	T lymphocyte	57
	ICAM-1	Lymphocyte	56
IL-8	Mac-1 and p150,95	PMNLs	61
IFN- γ	ICAM-1 and E-selectin	Endothelial cell	9,65
GM-CSF	CD11b	Neutrophil	67
	CD11b	Granulocyte	68
	CD18	Endothelial cell	43

neutrophils, and alveolar macrophages, cause bronchial tissue damage during the asthmatic response.

Neuropeptide Y (NY)

Neuropeptide Y induces adhesion of HUVECs to human neutrophils or to human promonocytic U937 cells in vitro.³¹ This was not associated with expression of ICAM-1 by HUVECs and was not inhibited by cycloheximide (inhibitor of protein synthesis). This suggests that E-selectin or VCAM-1 are not involved in NY stimulation.³¹ NY induces histamine release from mast cells, reduces bronchial blood flow and modulates cholinergic neurotransmission in the airways.^{32,33} Therefore NY may exacerbate asthma because of its proinflammatory functions, including its induction of adhesion of leukocytes to endothelial cells.

Cytokines

Interleukin-1 (IL-1) and tumor necrosis factor (TNF)- α . IL-1 and TNF- α increase the expression of a variety of adhesion molecules on endothelial cells and neutrophils (Table II):

1. VCAM-1 is expressed by endothelial cells within hours of stimulation by IL-1 or TNF- α and remains at high levels for at least 72 hours thereafter.³⁴ VCAM-1 mRNA is present in endothelial cells 2 hours after stimulation,

and the lymphocyte binding activity to endothelial cells parallels the mRNA induction. Therefore VCAM-1 participates to recruit mononuclear leukocytes into inflamed bronchi where these cytokines are present.

2. Unstimulated circulating neutrophils adhere to IL-1-stimulated endothelial cells by a mechanism that is dependent on the lectin adhesion molecule 1 (L-selectin or LECAM-1), the human homologue of the murine MEL-14.³⁵ MAb DREG-56 (anti-L-selectin) is effective in reducing adhesion of neutrophils indicating that L-selectin, on the surface of neutrophils, plays a significant role in circulating neutrophil-endothelial adherence. L-selectin is critical for the initial binding in the circulation between unstimulated neutrophils and IL-1-stimulated endothelial cells.
3. Endothelium exposed to IL-1 or TNF- α synthesizes E-selectin, which is expressed on the surface.¹⁶ Biopsies of allergen-induced late-phase responses in the skin from patients with respiratory allergies, in the presence of IL-1 and TNF- α (secreted locally), show that the E-selectin antigen expression by endothelial cells occurs concurrently with the inflammatory cell infiltration (2 to 4 hours).¹⁹ Anti-TNF- α and anti-IL-1 blocked the expression of E-selectin.
4. IL-1 β or TNF- α , in a concentration-dependent manner,

enhances ICAM-1 expression on a monolayer of cultured monkey bronchial epithelial cells.⁹ The distribution of ICAM-1 correlated topographically with areas of inflammatory damage observed by histologic study.

5. PMNLs adhesion and transmigration across recombinant (r) IL-1 β -activated endothelium requires the coordinate expression of E-selectin and ICAM-1.³⁶ Even in the absence of an exogenous chemoattractant gradient, adhesion of PMNLs increases markedly at 4 hours and transmigration is 3 to 4 times greater than the basal level. Both circulating monocytes and cultured lung tissue have been found capable of secreting IL-1 β and TNF- α in an IgE-dependent fashion. The stimulation by allergens of an IgE-dependent secretion of these cytokines by monocytes and cultured lung tissue is a critical amplification mechanism,^{37, 38} which may promote the expression of complementary inflammatory elements, such as adhesion molecules, in allergic asthma.

IL-2. IL-2 increases the expression of ICAM-1 on human memory T lymphocytes.³⁹ The blocking effect achieved by the MAb RR1/1 (anti-ICAM-1) suggests a role for ICAM-1 in memory T cells. That study demonstrated the capacity to induce ICAM-1 expression by activated T cells, since they have the capacity to produce IL-2. Endothelial cells of patients with metastatic cancer treated with IL-2 increased production of both E-selectin and ICAM-1.⁴⁰ The mechanism of these increases is not clear (because IL-2 can induce the production of IL-1, TNF α , IFN- γ , and other cytokines that have the ability to induce the endothelial expression of E-selectin and ICAM-1). The latter study, however, revealed that IL-2 stimulated the expression of E-selectin by postcapillary venular endothelium and ICAM-1 by stromal cells and mononuclear cells. The role for IL-2 in asthma is not clearly defined, but IL-2 stimulates the production of several cytokines and activates eosinophils to their hypodense state.⁴¹ Moreover, the serum concentration of the soluble IL-2 receptor (IL-2R) and the expression of IL-2R by CD4 cells are elevated in patients with severe acute asthma as compared with mild asthma, chronic obstructive pulmonary disease, and healthy control groups and its concentration decreases with clinical improvement.⁴²

IL-3. IL-3 induces basophil and monocyte adhesion to endothelial cells,^{43, 44} and rIL-3 treatment led to rapid and sustained increases in basophil surface expression of CD11, as determined by indirect immunofluorescence and flow cytometric analysis. Monoclonal antibody anti-CD11 and anti-CD18 decreased the basophil adherence induced by rIL-3, suggesting important roles for the α and β chains of the integrin family.⁴⁴ Human (h)rIL-3 also stimulates human monocyte adhesion to endothelial cells.⁴³ Monocyte adhesion was partially inhibited by a MAb to CD18, but not by a MAb to CD11b, suggesting that CD18 is important for monocyte adhesion to endothelial cells. Therefore the local production of IL-3, a regulatory cytokine of basophil function, at sites of ongoing allergic asthma, increases histamine release by basophils and the expression of the α and β chains of the integrin family. This activates and recruits circulating basophils and monocytes and promotes their adhesion to the vascular endothelium adjacent to inflammatory sites.

IL-4. IL-4 induces the adhesion of lymphocytes to microvascular endothelial cells and IL-1 β enhances this action.⁴⁵ MAb 6G10 (anti-VCAM-1) blocks the lymphocyte adhesion to IL-4-induced microvascular endothelial cells suggesting that VCAM-1 is the adhesion molecule that mediates lymphocyte binding to microvascular endothelium.⁴⁵ MAb anti-LFA-1 did not diminish endothelial cell adhesiveness for IL-4 augmented T cells. The IL-4 induced T-cell adhesion molecule remains unknown.⁴⁶ IL-4, by inducing endothelial cell expression of VCAM-1, stimulates adhesion of eosinophils and basophils in 3 hours but not neutrophils.⁴⁷ Eosinophils and basophils also bind to plates coated with rVCAM-1. MAb anti-VCAM-1 or anti-VLA-4 inhibits the binding in both conditions. IL-4 decreases the expression of ICAM-1 on the surface of unstimulated endothelial cells as well as partially inhibits the expression of ICAM-1 induced by IL-1, TNF, and IFN γ and of E-selectin induced by IL-1 and TNF on the same cells.⁴⁸ The inhibitory effect of IL-4 on the expression of ICAM-1 and E-selectin, two adhesion molecules commonly involved in PMNLs adhesion to endothelial cells, suggests that IL-4 does not act on endothelium facilitating the adhesion of PMNLs. IL-4 promotes expression of ICAM-1 and ICAM-1 mRNA by human mast cells and macrophages.⁴⁹ This observation (that IL-4 regulates adhesiveness of endothelial cells, mast cells, and macrophages) supports the concept that this interleukin, which is known to promote mast cell differentiation and IgE synthesis, also plays a central regulatory role in recruitment, distribution and regulation of leukocytes in asthma.

IL-5. IL-5 enhances human eosinophil adhesion to human microvascular endothelial cells, which is dependent, in part, on the CD11/CD18 integrin.²⁴ IL-5, and to a lesser extent, IL-3, is a potent stimulant for eosinophil but not neutrophil adhesion to endothelial cells. IL-5 also promotes terminal differentiation of eosinophil precursors, enhances the effector capacity of mature eosinophils, prolongs the survival of eosinophils, and is present in the bronchial mucosa of asthmatic individuals.⁵⁰ This partially explains the eosinophil infiltration present in asthma.

IL-6. IL-6, produced by epithelial cells, T cells, mast cells, and alveolar macrophages, induces the expression of CD18, CD11b, CD11c, and ICAM-1 on cultured U937 cells (human promonocytic cells).⁵¹⁻⁵³ Recombinant hIL-6 induced both a dose-dependent growth inhibition and expression of adhesion molecules. Anti-hrIL-6 antiserum completely inhibited the IL-6 effect. IL-6 may have an adverse effect on asthma, because it potentiates the IL-4-dependent IgE synthesis and plays a role in the in vitro survival of eosinophils.^{54, 55}

IL-7. IL-7 provides a weak costimulatory signal for T-cell proliferation induced by MAb anti-CD3 but does not effect ICAM-1 expression after stimulation for 2 hours.⁵⁶ IL-7 increased the IL-2-induced proliferation of T cells by MAb 64.1 (anti-CD3) compared with IL-2 or IL-7 alone. IL-2, IL-6, or IL-7 alone or in combination did not augment ICAM-1 expression in T cells stimulated with suboptimal concentrations of MAb 64.1. Stimulation of CD3 by MAb 64.1 increased the ICAM-1 expression more than 85%. However, another study revealed the opposed effect; IL-7

induced a high degree of expression of ICAM-1 on lymphokine-activated killer (LAK) cells. This parallels enhanced expression of IL-2R. No effect was observed for LFA-1 and LFA-3.⁵⁷ Although IL-7 has not been implicated in the pathogenesis of asthma, it may have an adverse effect because of its lymphopoietic properties. Lymphocytes appear to have a critical role because of their immunoregulatory functions. IL-7 is a major growth factor for human lymphocyte progenitors and provides a potent stimulus for maturation and proliferation for T cells.^{58, 59}

IL-8. A leukocyte adhesion inhibitor secreted by endothelial cells is apparently identical to IL-8 and acts on neutrophils to inhibit binding to E-selectin.⁶⁰ This suggests that IL-8 may limit the extent of leukocyte-vessel wall adhesion. However, another study revealed the contrary, that is, that IL-8 increases the expression of Mac-1 and p150,95 in PMNLs promoting adherence to endothelial cells. The expression reached a plateau in 15 minutes and remained at that level for at least 90 minutes.⁶¹ When bronchial epithelial and endothelial cells, alveolar macrophages, monocytes, and T lymphocytes are stimulated by IL-1 and TNF, they produce IL-8. It is chemotactic for human basophils, stimulates basophils from normal and atopic subjects to release histamine, and stimulates neutrophils to produce LTB₄.⁶²⁻⁶⁴ Because of these properties, IL-8 may play a role in the inflammation of asthma.⁶²

Interferon (IFN)- γ . IFN- γ stimulates lymphocyte adhesion and migration through endothelial cell monolayers.⁶⁵ IFN- γ , with IL-1, recruits lymphocytes in vivo. IFN- γ , which is more potent than IL-1 β and TNF- α , enhances ICAM-1 expression by monkey bronchial epithelial cells in vitro in a concentration dependent manner.⁹ In vivo, ICAM-1 contributes to the eosinophil infiltration and increase in airway responsiveness. IFN- γ also augments and prolongs the expression of E-selectin by human endothelial cells.⁶⁶ E-selectin is normally not expressed by endothelial cells, but its expression is induced by IL-1 or TNF. Whereas IFN- γ , per se, did not induce expression of E-selectin, it enhanced and prolonged the expression of E-selectin. Viral infections stimulate inflammatory cells to increase the production of IFN- γ , partially explaining the bronchial hyperresponsiveness caused by viral infections of asthmatic patients.

Granulocyte-macrophage-colony-stimulating factor (GM-CSF). GM-CSF induces the expression of CD11b and diminishes the expression of L-selectin by neutrophils.⁶⁷ Neutrophils, monocytes and their precursor cells, exposed to GM-CSF, lost L-selectin, but GM-CSF did not effect L-selectin expressed by lymphocytes. hrGM-CSF enhances the adhesion of human monocytes to HUVECs.⁴³ MAb 60.3 (anti-CD18) inhibited this binding by 50%, whereas MAb 60.1 (anti-Mac-1) did not have any effect, suggesting that CD18 may be essential for monocyte adhesion. In vivo rGM-CSF upregulates the expression of CD11b but not CD11a or CD11c on human granulocytes.⁶⁸ The intravenous infusion of rGM-CSF, 32 or 64 μ g/kg/d, to nine patients with sarcoma caused a transient leukopenia within 15 minutes and was associated with a significant increase in CD11b. The increased expression of CD11b was highest at

60 minutes and was present 24 hours later. GM-CSF, produced by T cells, alveolar macrophages, endothelial cells, eosinophils, and other cells, is produced by mast cells in response to IgE receptor activation and increases the eosinophil survival in tissues undergoing an allergic response.⁶⁶ GM-CSF may participate in the pathogenesis of asthma.

CELLULAR RESPONSES

Eosinophils

The eosinophil and B-lymphocyte C terminal extracellular domain of the low-affinity IgE receptor (Fc ϵ RII) contains the tripeptide RGD.⁷⁰ Preincubation of eosinophils with MAb BB10 (referred to as Fc ϵ RIIb), or MAb 135 (referred to as Fc ϵ RIIa), or MAb anti-*Leishmania* gp63 inhibits the IgE-dependent cytotoxicity against parasite targets. A cross-reactivity between MAb BB10 and MAb 135 and MAb gp63, which contains RGD, indicates the presence of such a tripeptide is the common structure present on eosinophil and B-cell Fc ϵ RII. It is possible that Fc ϵ RII plays a similar role in leishmaniasis and asthma since the same proinflammatory cells that participate in immune defense against parasites are also involved in bronchial asthma. RGD, and its receptors (i.e., β_1 group of integrins), constitute a system for cell signaling and is the essential structure for recognition of many extracellular molecules.⁷¹ The recognition site for many of the integrins that bind to the extracellular matrix is the tripeptide RGD.⁷ Endobronchial biopsies of asthmatic subjects show increased presence of collagens type III and V, fibronectin, and of glycoproteins rich in RGD.⁷²⁻⁷⁴ Therefore Fc ϵ RII contains RGD, and it may play an essential role for eosinophil and B-cell adhesion and transmigration.^{75, 76}

ICAM-1, which mediates eosinophil adhesion to endothelium in vitro, is up regulated on inflamed bronchial epithelium and endothelium in vivo.⁹ In vitro, the adhesion of eosinophils to monkey airway epithelial cells is inhibited by monoclonal antibodies RR1/1 or R15.7 (anti-ICAM-1). Treatment of monkeys intravenously with MAb R6.5 (anti-ICAM-1) attenuates the eosinophil infiltration and hyperresponsiveness. The eosinophilic infiltration, which characterizes asthmatic inflammation, is blocked by MAb R6.5. ICAM-1, abundant in inflamed airway epithelium, may contribute to eosinophil adhesion and migration into the bronchi in vivo.⁹ Therapeutic blocking of the endothelium was shown in the monkey model of asthma in which both the eosinophilia and hyperresponsiveness were attenuated. The adhesion of eosinophils and neutrophils to endothelial cells, which is stimulated by TNF and IL-1, is also mediated by E-selectin and ICAM-1.⁷⁷ The kinetics and dose-responses of adhesion to IL-1- and TNF-stimulated endothelial cells is similar for eosinophils and neutrophils. However, a tendency exists for eosinophil adhesion to peak later and to exceed and to persist longer than neutrophil adhesion. This may account, in part, for the eosinophilic infiltration in the bronchial tissue of asthmatic patients. Expression of LFA-1, p150,95 and ICAM-1 by cultured human eosinophils derived from umbilical cord blood is greater than that observed for peripheral blood eosinophils from patients with allergic diseases and an associated peripheral eosinophilia

TABLE III. Cells involved in bronchial asthma and in vitro time period for expression of adhesion molecules

Adhesion molecule	Cells involved in expression	Onset	Time to: maximal expression	Total duration	Inducing agent	References
VCAM-1	Endothelial cell	2 hours	5-6 hours	72 hours	IL-1, TNF- α , LPS	34, 82
VCAM-1	Endothelial cell	3 hours	24 hours	ND	IL-4	47
ICAM-1	Endothelial cell	6 hours	24 hours	48 hours	IL-1, TNF- α , LPS	98
ICAM-1	T lymphocyte	ND	24-48 hours	ND	IL-2, IFN- γ	39
ICAM-1	Mast cell/macrophage	ND	4 days	9-14 days	IL-4	49
P-selectin	Endothelial cell	3-5 min	5-10 min	30 min	Histamine, thrombine, phorbol myristate acetate	20, 21
	Endothelial cell	30 min	1-2 hours	4 hours	Oxygen radicals	29
L-selectin	Neutrophil	1 min	3-10 min	ND	fMLP	35
E-selectin	Endothelial cell*	3-4 hours	6 hours	24 hours	IL-1, TNF- α	19
	Endothelial cell	ND	6 hours	ND	Substance P	88
CD18/ CD11b	Basophil	10 min	30 min	2 hours	IL-3	44
	Monocyte	2 min	30 min	2 hours	fMLP, TNF	26
	Granulocyte	2 min	30 min	2 hours	C5a, LTB ₄	27
	Neutrophil	ND	15 min	90 min	IL-8	61
	Eosinophil	15 min	60 min	2 hours	IL-3, IL-5, GM-CSF	24
	Eosinophil	ND	15 min	2 hours	PAF	24
CD18	Monocyte	ND	10 min	9 hours	IL-3, GM-CSF	43
	Eosinophil	15 min	30 min	90 min	PAF, fMLP	23
CD11b	Granulocyte*	30 min	1 hour	24 hours	GM-CSF	68

*In vivo study.

of 7% to 16%.⁷⁸ Sputum eosinophils, but not blood eosinophils, express high levels of CD11b and CD11c but not CD18.⁷⁹ Sputum eosinophils also express ICAM-1 and HLA-DR (in nine and eight of 11 asthmatic patients, respectively).⁷⁹ The presence of HLA-DR on eosinophils may permit antigen presentation and direct interaction with T helper cells. The expression of CD18 by human eosinophils of normal density is significantly reduced by eosinophils by low density.⁸⁰ Endothelial cells express CD18 after 4 hours of stimulation with IL-1 and TNF, which increases their adhesiveness to eosinophils.⁸¹ In addition, TNF, FMLP or PAF directly activates the adhesion of eosinophils to endothelial cells. MAb 60.3 (anti-CD18) inhibits eosinophilic binding to endothelial cells indicating that CD18 adhesion molecules are required to induce adherence. A specific adhesion pathway of human eosinophils for endothelial cells is dependent on VLA-4. Endothelial cells stimulated by IL-1, TNF, or IL-4, within 2 or 3 hours, express VCAM-1, which increases eosinophil but not neutrophil binding. VCAM-1 is the ligand for VLA-4, and eosinophils normally express VLA-4. MAb anti-VLA-4 almost completely inhibited the enhancement of eosinophil adhesion. VLA-4 appears to be the preferential eosinophil recruitment pathway for adhesion to endothelial cells.^{47, 82}

Mast cells and basophils

Murine mast cells possess receptors for laminin (the most abundant protein in the bronchial basement membrane containing RGD). Tissue mast cells, when exposed to a laminin substratum, exhibit adhesion and spreading and redistribution of histamine-containing granules.⁸³ This adhesion is enhanced by IL-3 and inhibited by MABs to laminin and to laminin receptors. The presence of these receptors explains, in part, the tissue distribution and accumulation of inflammatory products at sites of epithelial injury. Exposure of laminin by shedding epithelium facilitates adhesion of mast cells and explains the increased presence of mast cells associated with bronchial inflammation.

Human basophils express ICAM-1, but lung mast cells express it to a much lesser degree. Basophils also express VLA-4 α , whereas endothelial cells express the ligand, VCAM-1.⁸⁴ Four-hour treatment of endothelial cells with IL-1 induces a fourfold to eightfold increase in adhesion. Treatment with anti-VCAM-1 inhibits basophil adhesion to cytokine-activated endothelium. Basophils, eosinophils, lymphocytes, and monocytes, unlike neutrophils, are able to bind VCAM-1 via VLA-4. This represents a novel mechanism by which the endothelium may directly promote the recruitment of these leukocytes without neutrophil activa-

tion. Mast cells express very low numbers of ICAM-1 and/or may only express distinct epitopes of the ICAM-1.⁸⁵ Endothelial cells express E-selectin 2 hours after degranulation of human mast cells.⁸⁶ E-selectin expression in this experimental model was blocked by anti-TNF- α . This study shows that anti-IgE-mediated degranulation in the perivascular space induces a cytokine-dependent induction of E-selectin by mast cells in the adjacent microvessels. A complementary study suggests that human dermal mast cell degranulation *in vivo* and *in vitro* is associated with the release of preformed TNF- α , which induces E-selectin production by dermal endothelium.⁸⁷ Both studies establish a role for human mast cells as "gatekeepers" of the dermal endothelium and indicate that mast cell mediators other than vasoactive amines influence endothelium in the inflammatory process.

Finally, dermal mast cell degranulation by substance P (SP) increased expression of E-selectin in human foreskin cultures after exposure to SP for 45 minutes.⁸⁸ SP also induced E-selectin in venules after incubation for 6 hours, suggesting that SP, endogenously released by bronchial nerve fibers, may help to regulate endothelial-leukocyte interactions in asthma.

To summarize, mast cell adhesion molecules have a significant effect on mast cell-airway epithelial cell interaction. Airway function of asthmatic patients may thus be affected by intraluminal mast cells more than is currently appreciated. Considerable evidence exists that intraluminal mast cells have a more important role in asthma than do mast cells adherent to airway epithelium and submucosal glands. The inflammatory mediators released from activated intraluminal mast cells may reach high local concentrations and thus greatly affect airway function.

ADHESION MOLECULES: POSSIBLE MECHANISM PROMOTING LEUKOCYTE INFILTRATION OF BRONCHIAL TISSUE IN ASTHMATIC PATIENTS

The early- and late-phase IgE-mediated reactions in sensitized bronchial tissue can be triggered by specific allergens or by anti-IgE antibodies. This reaction causes release of chemical mediators and cytokines. They induce the local synthesis and expression of adhesion molecules over a given time period (Table III). This is followed by a sequence of adhesion, activation, and transmigration of inactivated circulating leukocytes into the bronchial tissue. Stimulation is provided by compounds secreted by bronchial epithelial cells, mast cells, and macrophages resulting in the production of adhesion molecules by themselves as well as by vascular endothelial cells. Adhesion molecules on the endothelial cell surface provide circulating leukocytes a place to localize. In addition, leukocytes constitutively express adhesion molecules, which participate in the binding process. Histamine, released during the allergic early response, stimulates endothelial cells to release vasodilators that relax vascular smooth muscle thereby increasing blood flow and delivery of leukocytes to the area. Under sheer stress con-

ditions, leukocytes start to roll on the endothelial surface. Histamine also induces the expression of P-selectin by epithelial cells. P-selectin, with a short life, binds neutrophils and monocytes for a brief period of time. The leukocyte-endothelial cell interaction is maintained by E-selectin, which has a longer life. Smith et al.³⁵ have demonstrated that, under shear forces, L-selectin expressed by neutrophils is the basic adhesion molecule to bind cytokine-activated endothelial cells. L-selectin on neutrophils contain 5% of the Sialyl-Lewis^x found on neutrophil membranes. Sialyl-Lewis^x on neutrophil L-selectin serves as counter-receptor for endothelial E-selectin and, probably, P-selectin.⁸⁹

Intracellular signals (cyclic adenosine monophosphate, Ca²⁺, arachidonic acid) and enzymes, such as protein kinase C, then activate cytosol metabolic pathways leading to structural and functional changes permitting leukocytes to transmigrate into the tissue causing inflammation. Transmigration is mediated by adhesion molecules different from those that cause adhesion. A spherical shape is adequate for circulating leukocytes, but transmigration through the bronchial tissues requires that the leukocytes become fluid and tractable. This is accomplished by adhesion and de-adhesion processes caused by adhesion molecules capable of cytoskeleton activation and cellular deformation.

The cytoplasmic domain of both β_1 (VLA-4) and β_2 (Mac-1, and p150.95) integrin groups interact with the cytoskeleton via several intermediate molecules. Talin, a membrane protein, is a major interacting protein in this process.⁹⁰ Transmigration can also occur via a CD18-independent pathway, such as by E-selectin, ICAM-1 and L-selectin.^{91,92} A CD18-independent PMNL transmigration in the human lung capillaries has been reported.⁹² Such cytoskeletal activation, after the binding of the leukocyte to the endothelial cell, may prepare it for active transit from the vascular space to the bronchial lumen. De-adhesion, a basic step for transmigration, is a signal transduced from within the cell.⁹³ This process is basically regulated by the β_2 subunit of integrins by phosphorylation of the cytoplasmic domain or its interaction with a cytoskeletal protein. The α subunit does not play a role in de-adhesion. Leukocytes, facilitated by high concentrations of laminin, collagens, and fibronectin, pass through the endothelial layers and penetrate basement membranes. They can then migrate through the lumina of bronchi, directionally, across a gradient of an increasing concentration of chemotactic factors and cytokines, such as LTB₄, PAF, IL-1, and TNF, secreted by epithelial cells, macrophages, and mast cells. Bronchial epithelial stimulation alone is capable of initiating leukocyte-endothelium binding during an asthmatic response, even in the absence of stimulated circulating leukocytes. After the circulating cells have undergone specific adherence and leave the intravascular compartment, chemotactic agents complement their attraction to allergically stimulated tissue sites. IgE stimulation by specific allergens induces the expression of endothelial adhesion molecules and leukocyte binding to their ligands, which results in specific leukocyte adhesion, activation, enhanced motility, and bronchial infiltration (Fig. 4).

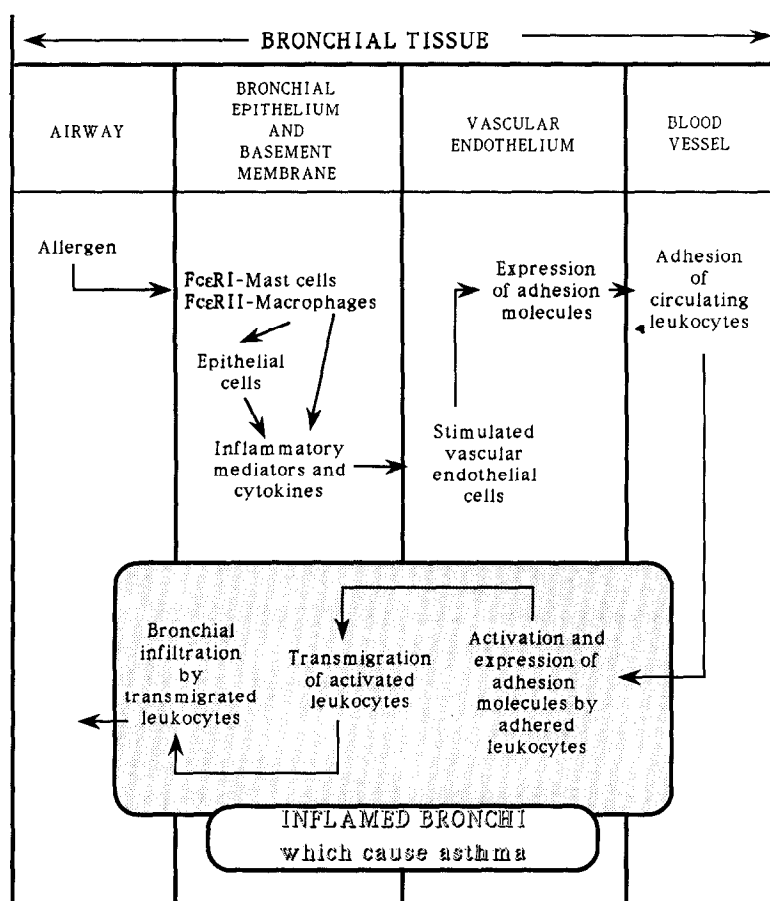


FIG. 4. Postulated mechanism for leukocyte bronchial infiltration in asthma. Allergens trigger IgE receptor-bearing cells (mast cell, basophil, lymphocyte, eosinophil, alveolar macrophage, and monocyte) inducing the release of inflammatory mediators and cytokines. These, in turn, perturbate epithelial cells causing the release of additional chemical mediators and cytokines. They stimulate vascular endothelial cells to express adhesion molecules causing circulating leukocytes to bind to endothelial surfaces. Adherent leukocytes are activated and express other adhesion molecules allowing PMNs to transmigrate into the bronchial tissue, causing the inflammatory infiltrate present in asthma. *FcεRI*, High-affinity receptor of IgE; *FcεRII*, low-affinity receptor of IgE.

ADHESION MOLECULES: SUMMARY AND CONCLUSIONS

Adhesion molecules may play a primary role in the pathogenesis of bronchial asthma. They are expressed by cells involved in the pathogenesis of asthma within a few minutes after IgE stimulation. Inactivated circulating leukocytes and/or platelets are recruited during the early phase, reach their maximal expression during the late phase, and can persist, causing chronic asthma that may coincide with the increased expression of adhesion molecules. Bronchial epithelial cells and epithelial basement membranes, previously thought to provide a physical barrier for the bronchi, are now recognized to play an active role in the stimulated IgE-biphasic response. Appropriately stimulated epithelial cells produce inflammatory mediators and cytokines that induce the expression of adhesion molecules and basement mem-

branes containing glycoproteins that are ligands for integrins on the surface of adherent leukocytes. Vascular endothelial and circulating cells, stimulated locally by cytokines and chemical mediators, then express a variety of adhesion molecules. These are important not only because of their specific adhesive properties but also because they regulate activation and transmigration of leukocytes from the blood vessel into the bronchial tissue.

Methods should be explored to prevent or reverse the expression of adhesion molecules by stimulated cells. Antiinflammatory drugs affect adhesion molecules differently: (a) cyclosporine inhibits ICAM-1 synthesis by endothelial cells,⁹⁴ (b) cromolyn sodium inhibits the ability of SP to induce E-selectin on endothelial cells,⁸³ (c) glucocorticoids inhibit IL-1-induced expression of ICAM-1 on SW-1353 cells (human chondrosarcoma) and on A-549 cells (human

lung adenocarcinoma) but do not inhibit E-selectin expression by endothelial cells,⁹⁵ (d) methotrexate also inhibits endothelial cell binding to PMNLs, suggesting that it affects an adhesion molecule not yet identified,⁹⁶ and (e) gold therapy decreases endothelial expression of E-selectin.⁹⁷ In addition, specific blocking of the adhesion molecules by monoclonal antibodies may complement the asthmatic antiinflammatory treatment. Therefore, antiadhesive molecule therapy for asthma appears to show great promise.

ADDENDUM

Adhesion properties of adhesion molecules have been emphasized. Recent findings demonstrate that during specific cell-to-cell contact promoted by adhesion molecules, intercellular communication also occurs. Adhesion molecules on intact plasmatic membranes, coupled to molecular pathways, allow signaling to reach the nucleus or cytoplasmic organella to produce effector responses. Since current studies describe these new functions and characteristics for "adhesion molecules," a better descriptive name for these molecules seems appropriate. We propose the term "receptors for intercellular signaling (RIS)."

The authors thank Dr. Samuel C. Bukantz for his help reviewing this manuscript.

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