

Vascular Cell Adhesion Molecule-1 Expression and Signaling During Disease: Regulation by Reactive Oxygen Species and Antioxidants

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Abstract

The endothelium is immunoregulatory in that inhibiting the function of vascular adhesion molecules blocks leukocyte recruitment and thus tissue inflammation. The function of endothelial cells during leukocyte recruitment is regulated by reactive oxygen species (ROS) and antioxidants. In inflammatory sites and lymph nodes, the endothelium is stimulated to express adhesion molecules that mediate leukocyte binding. Upon leukocyte binding, these adhesion molecules activate endothelial cell signal transduction that then alters endothelial cell shape for the opening of passageways through which leukocytes can migrate. If the stimulation of this opening is blocked, inflammation is blocked. In this review, we focus on the endothelial cell adhesion molecule, vascular cell adhesion molecule-1 (VCAM-1). Expression of VCAM-1 is induced on endothelial cells during inflammatory diseases by several mediators, including ROS. Then, VCAM-1 on the endothelium functions as both a scaffold for leukocyte migration and a trigger of endothelial signaling through NADPH oxidase-generated ROS. These ROS induce signals for the opening of intercellular passageways through which leukocytes migrate. In several inflammatory diseases, inflammation is blocked by inhibition of leukocyte binding to VCAM-1 or by inhibition of VCAM-1 signal transduction. VCAM-1 signal transduction and VCAM-1-dependent inflammation are blocked by antioxidants. Thus, VCAM-1 signaling is a target for intervention by pharmacological agents and by antioxidants during inflammatory diseases. This review discusses ROS and antioxidant functions during activation of VCAM-1 expression and VCAM-1 signaling in inflammatory diseases. *Antioxid. Redox Signal.* 15, 1607–1638.

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I. Introduction to Leukocyte Recruitment

LEUKOCYTE RECRUITMENT IS REGULATED by reactive oxygen species (ROS) during inflammation. During inflammation, leukocytes are recruited into tissues by adhesion molecules and chemokines (Fig. 1). The specificity of leukocyte homing to tissues is regulated by the combination of chemokines in the microenvironment, adhesion molecules on the endothelium, and leukocyte receptors for these chemokines and adhesion molecules (159). Further, the combination of vascular adhesion molecules expressed by an endothelial cell is dependent on the stimulant(s) for endothelial activation (231). In peripheral lymph nodes, endothelial cells constitutively express adhesion molecules (270) as they are continuously activated (115). In contrast, endothelial cells at sites of inflammation require induction of adhesion molecule expression. Adhesion molecule expression is induced by several mediators, including cytokines produced in the tissue, high levels of ROS, turbulent blood flow at vessel bifurcations, or microbial stimulation of endothelial toll-like receptors (TLRs) (49, 59, 121, 124, 125, 152, 177, 182, 210, 212, 242). Thus, the microenvironment stimuli regulate the specificity of leukocyte recruitment.

Leukocyte binding to the adhesion molecules activates signals within the endothelial cells that allow opening of narrow vascular passageways as small intercellular gaps through which leukocytes migrate (Fig. 1) (64, 184, 201). Leukocyte movement through these passageways is stimulated by chemokines that are produced by the endothelium and the tissue (Fig. 1). The majority of leukocyte migrate through intercellular gaps, but under conditions of high levels of inflammation, a small percentage of leukocytes can also migrate through individual endothelial cells by transcellular migration (50, 180, 195). When there is inhibition of the en-

dothelial cell adhesion molecule signals, leukocytes bind to the endothelium but do not complete transendothelial migration (2). The cells that bind to the endothelium but do not complete transendothelial migration are often released from the endothelium and continue in the blood flow as demonstrated by intravital microscopy. Thus, the endothelial cell adhesion molecules and their intracellular signals are a source for intervention in leukocyte recruitment.

The vascular recruitment of leukocytes is a three-step process involving rolling of leukocytes on the endothelium followed by arrest of the leukocyte on the endothelium through high affinity adhesion, and then transmigration of the leukocyte through the endothelium (Fig. 1). The rolling of leukocytes on the luminal side of the endothelium is mediated by the low affinity receptors, selectins and addressins (188, 298). In lieu of the selectin interactions with addressin, rolling can also be mediated by leukocyte $\alpha_4\beta_1$ -integrin in its low affinity state interacting with vascular cell adhesion molecule-1 (VCAM-1/CD106) on the endothelium (15). Binding of selectins on leukocytes stimulates "outside-in" signals in leukocytes, increasing the affinity of the integrin family of receptors that then bind to the endothelial cell adhesion molecules intercellular adhesion molecule-1 (ICAM-1/CD54) or VCAM-1 (13, 45, 262). The high affinity integrin binding by blood leukocytes mediates arrest of the leukocytes on the endothelium. Then, the arrested leukocytes migrate into the tissue.

The affinity of leukocyte integrins for vascular adhesion molecules is also rapidly increased by "inside-out" signals from chemokine receptors on leukocytes (14, 48, 52, 131, 160, 161, 307). Chemokines have specificity for leukocyte cell types that express the chemokine receptors. This chemokine-specific activation results in increased integrin affinity on those leukocyte subsets that are responding to chemokines in the microenvironment. Thus, T cells, B cells, mast cells,

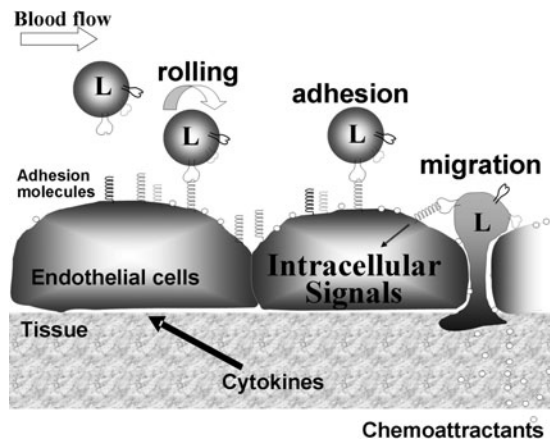


FIG. 1. Leukocyte transendothelial migration. During inflammation, cytokines produced in the tissue induce endothelial cell adhesion molecule expression. In addition, chemoattractants released by both the tissue and endothelial cells increase leukocyte adhesion molecule affinity as well as provide direction for leukocyte migration. This vascular recruitment of leukocytes is a three-step process involving low affinity rolling of leukocytes on the endothelium followed by arrest of the leukocyte on the endothelium through high affinity adhesion, and then transmigration of the leukocyte through the endothelium. L, leukocyte.

eosinophils, monocytes, and stem cells migrate on VCAM-1, but their activation for binding to VCAM-1 depends on cell-type-specific chemokines in the microenvironment (4, 5, 8, 10, 23, 36, 57, 107, 108, 123, 138, 218, 243, 250, 268). The chemokine-activated leukocytes are selected for migration by their integrin-mediated high affinity adhesion. The adherent leukocytes then migrate on chemokine gradients into the tissue (195). It has also been reported that $\alpha_4\beta_1$ -integrin binding affinity on CD34+ bone marrow-derived cells or eosinophils is enhanced by ligand binding to the coexpressed adhesion receptor platelet-endothelial cell adhesion molecule-1 (PECAM-1), implicating signals transmitted from PECAM-1 as determinants of $\alpha_4\beta_1$ -integrin affinity (56, 162). The binding of leukocytes to the endothelium and the specificity of these interactions have been discussed in previous reviews (52, 83, 131, 160, 161, 170, 181, 201, 269, 270). This review will focus on VCAM-1 expression and function during VCAM-1 regulation of leukocyte transendothelial migration as it is regulated by ROS and antioxidants. Also discussed are the important regulatory roles for VCAM-1 signals and antioxidants during VCAM-1-dependent inflammation *in vivo*.

II. VCAM-1 Regulation of Leukocyte Recruitment and Inflammation in Several Diseases

A. VCAM-1 expression and shedding

VCAM-1 functions in combination with other adhesion molecules to regulate immune surveillance and inflammation. VCAM-1 expression is induced by cytokines produced in the tissue, high levels of ROS, oxidized low density lipoprotein (oxLDL), 25-hydroxycholesterol, turbulent shear stress, high glucose, and microbial stimulation of endothelial cell TLRs (49, 121, 124, 167, 177, 179, 182, 208, 210, 212, 229, 230, 242, 310, 322). This activation of VCAM-1 gene expression is regulated by the transcription factors nuclear factor kappa B (NF κ B),

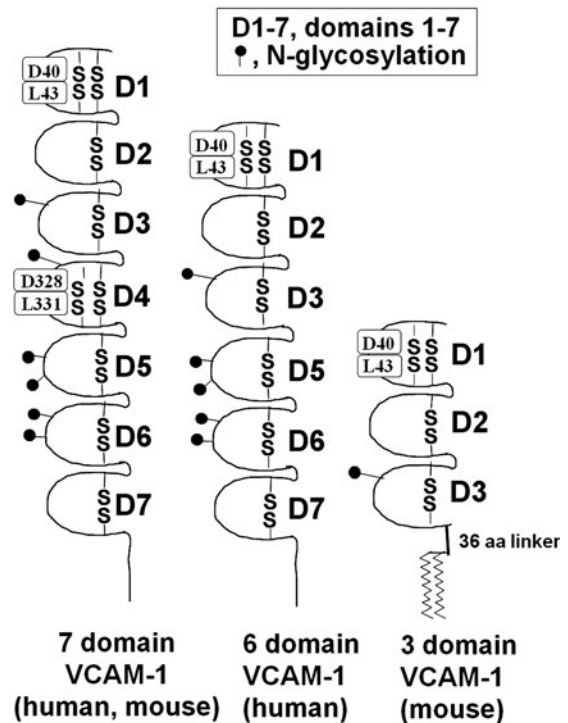


FIG. 2. VCAM-1 splice variants. Human VCAM-1 has two splice variants that contain either six or seven immunoglobulin-like domains with disulfide linkages. The six-domain form of human VCAM-1 lacks domain 4. Mouse VCAM-1 has the seven-domain form and unique three-domain form. The three-domain form is linked to glycoposphatidylinositol through a 36 amino acid glycoposphatidylinositol-linker. Within VCAM-1, domains 1 and 4 contain the binding sites for integrins. VCAM-1 is also N-glycosylated. VCAM-1, vascular cell adhesion molecule-1.

SP-1, Ap-1, and interferon regulatory factor-1 (68, 163, 167, 182, 230, 287). For example, VCAM-1 expression is induced by the cytokines tumor necrosis factor (TNF) α and interleukin (IL)-1 β , the adipokine Visfatin, the proatherogenic amino acid homocysteine, and proatherogenic hyperglycemia (46, 142, 144, 182, 196, 230). The mechanism of action of these stimulants is through induction of ROS generation for the stimulation of NF κ B (46, 142, 144, 182, 196). However, the concentrations of endothelial cell ROS generated in response to these stimulants are not known. It is reported that high concentrations of ROS (400 μ M hydrogen peroxide) can activate NF κ B and consequently VCAM-1 expression in aortic endothelial cells (166). TNF α -induced VCAM-1 expression is blocked by scavenging superoxide by overexpression of superoxide dismutase but not blocked by scavenging hydrogen peroxide by overexpression of catalase in endothelial cells (54). Consistent with this finding, the TNF α -induced expression of VCAM-1 by NF κ B binding to the VCAM-1 promoter is blocked by nitric oxide, which is known to react with superoxide (142). Conversely, the nitric oxide synthase inhibitor *N*-monomethyl-L-arginine augments TNF α -induced VCAM-1 expression (142). The IL-1 β activation of VCAM-1 is blocked by antioxidants, including pyrrolidine dithiocarbamate, *N*-acetylcysteine, and α -tocopherol (182, 322). oxLDL and 25-hydroxycholesterol induction of VCAM-1 expression, ICAM-1 expression, and monocyte adhesion to endothelium is blocked by α -tocopherol or tocotrienols *in vitro*

(208, 322). Shear stress through cyclic strain also induces ROS generation in endothelial cells, which then activate VCAM-1 expression (276).

VCAM-1 can also be released from the endothelial surface through cleavage by a disintegrin and metalloprotease 17 (ADAM17) (97) and, although less characterized, may be released by ADAM8 (185, 186) or ADAM9 (103, 222). Therefore, VCAM-1 is present in the plasma in a soluble form (sVCAM-1) and is used as predictive biomarker of disease (17, 127, 154, 297, 319). Levels of sVCAM-1 in plasma increase with activation of the endothelium in multiple diseases (44, 55, 89, 98, 133, 154, 206, 219, 227). This sVCAM-1 is thought to either limit leukocyte integrin binding to endothelial VCAM-1 by binding to leukocytes or stimulate leukocyte chemotaxis (147, 282, 288).

B. VCAM-1 function in the bone marrow and lymph nodes

VCAM-1 is expressed in lymph nodes and the bone marrow for the regulation of leukocyte homing (Table 1). The function of VCAM-1 in the bone marrow has been demonstrated in a mouse model with a conditional deletion of mu-

rine VCAM-1. In these mice, deletion of VCAM-1 results in reduced B cell homing to the bone marrow (243). In the bone marrow, it has also been reported that VCAM-1 regulates proplatelet formation in the osteoblastic niche (221). VCAM-1 expression has also been reported to be induced on mesenchymal stem cells by cytokine stimulation (313). This mesenchymal stem cell expression of VCAM-1 is reported to participate in immunosuppression of T cell responses (243). Further, VCAM-1 regulates hematopoietic stem cell recruitment to injured liver and melanoma metastasis to the liver (138, 255, 299). In lymph nodes and tonsils, VCAM-1 is expressed by postcapillary high endothelial venule cells and follicular dendritic cells (151, 187, 317). VCAM-1 on the lymph node follicular dendritic cells mediates B cell binding (21, 151). Thus, VCAM-1 has a role in the bone marrow, lymph nodes, and liver.

C. VCAM-1 regulation of inflammatory diseases: treatment of clinical disease with natalizumab

VCAM-1 has a regulatory role in peripheral tissue inflammation in several diseases (Table 1). In these diseases, there are different leukocyte cell types that bind VCAM-1 *via* the

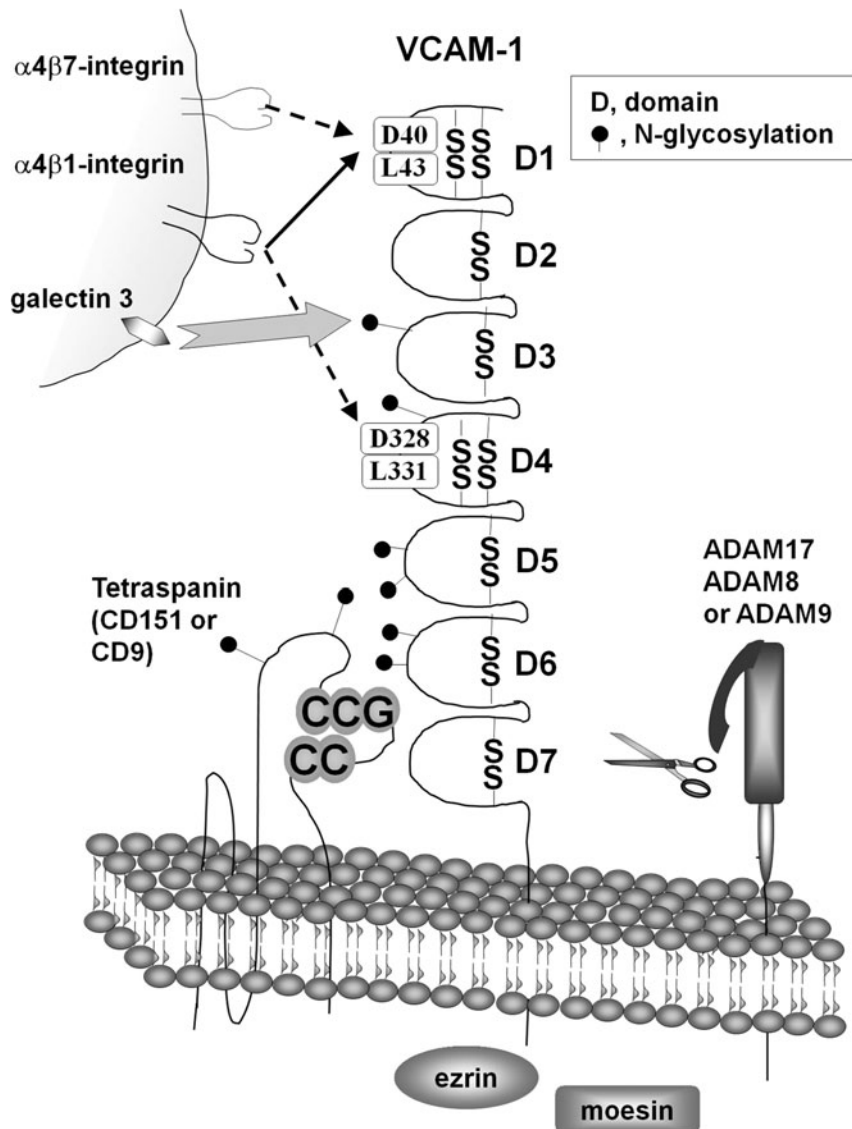


FIG. 3. Ligand binding to VCAM-1. Integrin binding to VCAM-1 is regulated by the integrin activation state. $\alpha 4 \beta 1$ -integrin binds readily to domain 1 but requires higher affinity activation for binding to domain 4. This integrin binding to domains 1 and 4 requires the amino acids D40 and L43, or D328 and L331, respectively. $\alpha 4 \beta 7$ -integrin also binds to VCAM-1 (dashed arrow) but with a lower affinity than its binding to another adhesion molecule, mucosal addressin cell adhesion molecule-1 (not shown). Galectin 3 binds to N-glycosylation sites on VCAM-1. VCAM-1 has six N-glycosylation sites that may participate in galectin 3 binding. VCAM-1 also coimmunoprecipitates with ezrin and moesin. VCAM-1 cell surface expression requires associated tetraspanins CD151 or CD9. The tetraspanin long extracellular loop (LEL) is necessary for its binding to immunoglobulin superfamily members. This LEL contains a CCG and CC motif. VCAM-1 can also be clipped from the cell surface by ADAM17, ADAM8, and ADAM9. Solid arrow, major ligand binding site. Dashed arrow, ligand binding requires higher integrin activation. Large filled arrow, galectin3 binds to N-glycosylation sites. ADAM, a disintegrin and metalloprotease.

TABLE 1. VASCULAR CELL ADHESION MOLECULE-1 FUNCTION IN DISEASE

VCAM-1 function in the bone marrow and lymph node

1. B cell homing to the bone marrow (243)
2. Proplatelet formation in the osteoblastic niche (221)
3. Hematopoietic stem cell recruitment to injured liver (138)
4. Melanoma metastasis to the liver (255, 299)
5. Mesenchymal stem cell immunosuppression of T cell responses (243, 313)

VCAM-1 regulation of inflammatory disease

1. Eosinophil recruitment in asthma models (57, 107, 250)
2. Mast cell precursor recruitment to lungs and intestine (4, 5, 8, 36, 108)
3. Severity and onset of atopic dermatitis (53)
4. T cell infiltration into the intestine in inflammatory bowel disease (268)
5. T cell infiltration into the brain in multiple sclerosis (10, 23)

VCAM-1 regulation of inflammation during infection

1. VCAM-1 expression is induced by microbial activation of toll-like receptors on endothelium, dendritic cells, and fibroblasts (82, 121, 295, 313).
2. CD8+ T cell infiltration into the brain during lymphocytic choriomeningitis virus infections (218)
3. Expansion of dendritic cells in experimental visceral leishmaniasis (271)

VCAM-1 function in cardiovascular diseases

1. Embryonic development of the heart (105)
2. VCAM-1 is the first adhesion molecule before atherosclerotic plaque development (125).
3. Neointimal formation in carotid artery (27, 236)
4. Monocyte adhesion to atherosclerotic carotid arteries (123)
5. Calcification of aortic stenosis in patients with coronary artery disease (173)
6. Leukocyte recruitment after ischemia-reperfusion of the liver (138)
7. Cardiac allograft rejection (41, 320)
8. VCAM-1 expression in aorta during HIV infection (106)

Treatment with Natalizumab (anti- α 4-integrin antibody)

1. Reduces disease severity in multiple sclerosis and Crohn's disease (61, 211)
2. Complications include the rare occurrence of progressive multifocal leukoencephalopathy (61, 211).

VCAM-1, vascular cell adhesion molecule-1.

leukocyte ligand α 4 β 1-integrin (Table 2). This is, at least in part, a result of leukocyte-specific chemokine activation of α 4 β 1-integrin into the integrin's high affinity conformation (52, 131, 160, 161). The cell types with high affinity integrin migrate on VCAM-1. In allergic disease, blocking VCAM-1 by intravenous injection of anti-VCAM-1 blocking antibodies inhibits eosinophil recruitment in asthma models in several species (57, 107, 250). Further, in allergic disease, blocking VCAM-1 or using VCAM-1 knockout mice inhibits mast cell precursor binding to endothelium and inhibits recruitment of mast cell precursors to antigen-stimulated lungs and intestine (4, 5, 8, 36, 108). In a mouse model of atopic dermatitis, VCAM-1 blockade reduces severity of inflammatory disease and delays the onset of disease (53). In inflammatory bowel disease, antibody inhibition of VCAM-1 blocks T cell infiltration into the intestine (268). In an experimental model of multiple sclerosis, blocking VCAM-1 inhibits T cell infiltration into the brain (23). Consistent with this, multiple sclerosis patients have elevated VCAM-1 but not mucosal addressin cell adhesion molecule-1 expression in brain tissue (10). In clinical trials, blocking the VCAM-1 ligand, α 4-integrin, with antibodies (natalizumab) reduces disease severity in multiple sclerosis and Crohn's disease (61, 211). Unfortunately, treatment of multiple sclerosis with natalizumab is complicated by the rare occurrence of progressive multifocal leukoencephalopathy (61, 211). Thus, due to the side effects of natalizumab, there is a need for alternative targets to limit VCAM-1-dependent inflammation. These alternative targets are VCAM-1 itself or VCAM-1 signaling intermediates that are discussed in this review.

D. VCAM-1 regulation of inflammation during infection

VCAM-1 also has a role in regulation of inflammation during infection (Table 1). During infections, microbial TLR ligands and the cytokines of the immune response likely stimulate VCAM-1 expression. VCAM-1 expression is induced by stimulation of TLRs on endothelium, dendritic cells, and fibroblasts (82, 121, 295, 313). During lymphocytic choriomeningitis virus infections, VCAM-1 expression by the endothelium mediates CD8+ T cell infiltration into the brain (218). Moreover, deletion of VCAM-1 blocks disease severity and blocks monocyte/dendritic cell migration into the brain during lymphocytic choriomeningitis virus infections (218). In experimental visceral leishmaniasis, VCAM-1 interaction with α 4 β 1 integrin regulates the production of dendritic cells since antibody inhibition of VCAM-1 or α 4 β 1-integrin blocks the dendritic cell response in the spleens in these mice (271). Thus, VCAM-1 has regulatory functions in infection-induced inflammation.

E. VCAM-1 function in cardiovascular diseases

It has been reported that VCAM-1 has an important role in cardiovascular diseases and in the embryonic development of the cardiovascular system (Table 1). VCAM-1 is required for development of the heart since the VCAM-1 knockout mouse is an embryonic lethal due to malformation of the heart (105). In atherosclerosis, VCAM-1 is the first adhesion molecule expressed before atherosclerotic plaque development (125). In the carotid artery, neointimal formation is reduced by VCAM-1 siRNA or by antibody blockade of α 4 β 1-integrin in rodents

TABLE 2. LEUKOCYTES THAT MIGRATE ON VASCULAR CELL ADHESION MOLECULE-1

Leukocyte	VCAM-1 site	References
B cell	Bone marrow	243
Eosinophil	Allergic lung	57, 107, 250
Mast cell precursor	Lungs and intestine	4, 5, 8, 36, 108
Monocyte	Atherosclerotic carotid arteries	123
T cell	Intestine in IBD	268
T cell	Brain in multiple sclerosis	10, 23
T cell	Brain in lymphocytic choriomeningitis virus infections	218

(27, 236). In advanced stages of atherosclerosis, VCAM-1 can be expressed by smooth muscle cells (38, 110). VCAM-1 is also linked to calcification of aortic stenosis in patients with coronary artery disease (173). In the atherosclerotic carotid arteries, VCAM-1 mediates monocyte adhesion as demonstrated with anti-VCAM-1 blocking antibodies (123). In cardiac allografts, lower levels of VCAM-1 expression are indicative of a reduction in rejection (41, 320). In ischemia-reperfusion of the liver, blocking VCAM-1 inhibits leukocyte recruitment and injury (138). Also of interest, VCAM-1 expression is induced in the aorta in HIV transgenic rats (106). Thus, VCAM-1 has an important regulatory role in cardiovascular diseases.

Thus, in VCAM-1-dependent inflammatory diseases, the specificity of cell types recruited in inflammatory diseases is dictated by the combination of adhesion molecules and by the specific chemokines for activation of integrins on leukocyte cell types. VCAM-1 binding and intracellular signaling are potential targets for intervention in several diseases. Therefore, it is important to understand the mechanisms for VCAM-1 functions so that approaches can be developed to modulate VCAM-1-dependent inflammation during disease. In addition, selective inhibition of VCAM-1 function would block excess inflammation in VCAM-1-mediated inflammatory disease while maintaining the beneficial antimicrobial immune responses that utilize the vascular adhesion molecules ICAM-1 or PECAM-1.

III. VCAM-1 Structure/Function

A. VCAM-1 structure

VCAM-1 is a member of the immunoglobulin (Ig) superfamily of proteins. VCAM-1 is comprised of several extracellular Ig-like domains that contain disulfide-linked loops, a single type I transmembrane domain, and a 19 amino acid carboxyl-terminus cytoplasmic domain (157, 215) (Fig. 2). Interestingly, the amino acid sequence of this cytoplasmic domain is 100% identical among several species, including rat, mouse, human, and rabbit (22, 100, 118, 224, 232, 233). The extracellular region of the full-length form of VCAM-1 contains seven Ig-like domains (Fig. 2). There is homology within these Ig-like domains, such that domains 1 and 4 have sequence homology, domains 2 and 5 have sequence homology, and domains 3 and 6 have sequence homology (69, 119, 233). In addition, there are splice variants of the full-length form of

VCAM-1. There are two human forms and two mouse forms of VCAM-1 (Fig. 2). Human VCAM-1's two splice variants result in a receptor with either a seven Ig-like domain protein or a six-domain VCAM-1 that lacks domain 4 (Fig. 2) (69, 70). Mouse VCAM-1 also has a full-length seven-domain form as well as a truncated form with only the first three domains (Fig. 2). This mouse three-domain form of VCAM-1 is linked to glycosylphosphatidylinositol (GPI) for insertion in the plasma membrane (146, 157, 200, 280). Moreover, this three-domain variant has a 36 amino acid GPI-linker that is a unique sequence not found in the six- or seven-domain VCAM-1 molecules or in other members of the Ig superfamily (Fig. 2) (200). It has been speculated that the GPI link might enable the three-domain VCAM-1 to move through the plasma membrane faster than the other VCAM-1 variants, thereby quickening the endothelial response to tethering a rolling leukocyte (146).

B. Ligands and VCAM-1 binding regions

All of the variants of VCAM-1 have been shown to bind to $\alpha_4\beta_1$ integrin (VLA-4) (15, 85, 209, 215, 309) (Fig. 3). $\alpha_4\beta_1$ -integrin also binds to fibronectin, heparin, and junction adhesion molecule-B in endothelial junctions (176, 235, 258). In addition to $\alpha_4\beta_1$ -integrin, VCAM-1 can bind to other integrins such as $\alpha_4\beta_7$ integrin and $\alpha_d\beta_2$ integrin (51, 102, 247). These integrins are expressed by eosinophils, basophils, lymphocytes, mast cells, and monocytes (5, 33, 36, 174, 197, 307). The binding domains of VCAM-1 have been identified using a combination of domain truncations, substitutions with ICAM-1 sequences, and amino acid mutations. It has been shown that $\alpha_4\beta_1$ integrin binds to Ig-like domains 1 and 4 (216, 223, 244, 301). Antibody inhibition of either domain 1 or 4 partially blocks Ramos cell $\alpha_4\beta_1$ integrin binding to VCAM-1 (216) (Fig. 3). However, inhibition of both domains 1 and 4 completely blocks binding (216). In both domains 1 and 4, a mutation of either an aspartate (domain 1 amino acid 40 or domain 4 amino acid 328) or a leucine (domain 1 amino acid 43 or domain 4 amino acid 331) to an alanine results in a significant reduction of Ramos cell binding to these VCAM-1 mutants expressed in COS cells (Fig. 3) (244, 301). Mutating several other amino acids in domain 1, including R36, Q38, I39, P42, L70, or T72, reduces binding of $\alpha_4\beta_1$ integrin and $\alpha_4\beta_7$ integrin (58). However, mutations in domain 1 at N44 or E66 specifically reduce binding of $\alpha_4\beta_7$ integrin but not $\alpha_4\beta_1$ integrin (58). Of these mutations, D40 and L70 mutations are reported to inhibit binding while not perturbing the gross structure of VCAM-1 (58, 244). In addition, domain 2 is necessary for the binding function of domain 1 (244). Domain 1 of VCAM-1 also binds $\alpha_4\beta_7$ integrin as demonstrated using anti-VCAM-1 domain 1 blocking antibodies or domain 1 blocking peptides (216, 247, 321).

The binding to VCAM-1 is regulated by the activation state of the integrins (51, 143). Integrins at low affinity roll on VCAM-1, whereas the high affinity conformation of the integrins mediates firm adhesion to the endothelium that can withstand the force of the blood flow (15, 99, 307). In addition, integrin binding to domains 1 versus domain 4 of VCAM-1 is modulated by the degree of activation of the $\alpha_4\beta_1$ integrin. The $\alpha_4\beta_1$ integrin binding to domain 4 has a higher requirement for activation than for binding to domain 1 (Fig. 3) (143). Moreover, $\alpha_4\beta_1$ integrin versus $\alpha_4\beta_7$ integrin differ in their activation

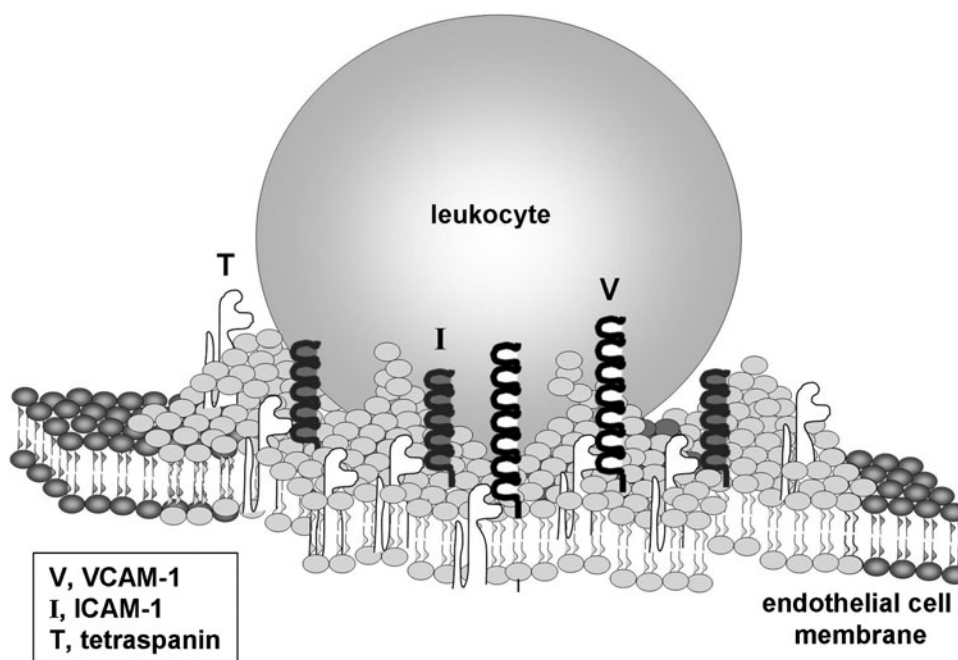
requirements for binding to domains 1 and 4 of VCAM-1 (143). For half maximal binding to domain 4 of VCAM-1, $\alpha_4\beta_1$ integrin requires significantly higher activating concentrations of divalent cations than $\alpha_4\beta_7$ integrin (Fig. 3) (143). The binding activity of $\alpha_4\beta_1$ integrin to domain 1 of VCAM-1 is also regulated by CD24 expression (143). Moreover, $\alpha_4\beta_7$ integrin binding to VCAM-1 requires a higher activation state than for its binding to the mucosal addressin cell adhesion molecule-1, an endothelial cell adhesion molecule (Fig. 3) (31). Thus, the α_4 integrins bind to two domains of VCAM-1 and this binding to VCAM-1 domains is regulated by the activation state of the integrins.

In addition to integrins, VCAM-1 can bind galectin-3 (Fig. 3). It has been reported that recombinant galectin 3 columns bind several proteins from BALB/3T3 cells, and the major band, at 100 kD, was identified as VCAM-1 by mass spectrometry (278). Moreover, VCAM-1 is immunoprecipitated from proteins bound to a recombinant galectin-3 column (278). This galectin-3 binding to VCAM-1 is lost by treatment of VCAM-1 with *N*-glycanase, indicating that VCAM-1's *N*-glycans bind to galectin-3 (278). There are six *N*-glycosylation sites on VCAM-1 and these are located in domains 3–6 (Fig. 3) (198). Galectin-3 has been implicated in eosinophil binding to VCAM-1 (239). Eosinophil binding to endothelial cells expressing VCAM-1 is blocked to an equal extent with anti- $\alpha_4\beta_1$ -integrin or anti-galectin-3 antibodies (239). Treatment with these two antibodies together does not exhibit further inhibition of adhesion. Moreover, the interactions with galectin-3 are complicated since it has been demonstrated by ELISA that $\alpha_4\beta_1$ -integrin binds directly to galectin-3, that galectin-3 can bind to VCAM-1, and that endothelial cells express galectin-3 in addition to VCAM-1 (239). In this study of eosinophil binding to endothelial cells, it was not demonstrated whether galectin-3 on eosinophils directly binds VCAM-1 (239). Thus, during eosinophil interactions with endothelium, there are several galectin-3 ligands expressing *N*-glycans, including VCAM-1.

C. VCAM-1 is a part of the tetraspanin-enriched microdomains

Several studies have examined the role of VCAM-1 in cell adhesion and migration. In activated endothelial cells, VCAM-1 is found in a lipid-raft-like platform containing ICAM-1 and the tetraspanins CD9, CD81, and CD151, known as the tetraspanin-enriched microdomain (Fig. 4) (24, 26, 113). Fluorescent microscopy shows that when T cells adhere to activated human umbilical vein endothelial cell (HUVEC) monolayers, VCAM-1, ICAM-1, CD9, CD81, and CD151 all colocalize to rings surrounding the lymphocyte (24). The specific interactions between ICAM-1, VCAM-1, and the tetraspanins within the tetraspanin-enriched microdomain were demonstrated using coimmunoprecipitation and fluorescence resonance energy transfer (FRET) analysis studies. Coimmunoprecipitation studies demonstrate that VCAM-1 associates with CD151 and CD9 and that ICAM-1 associates with CD9 (25). FRET-fluorescence lifetime imaging microscopic analysis in resting HUVECs reveal that VCAM-1 does not homodimerize nor does it form a heterodimer with ICAM-1. However, there is a low incidence of ICAM-1 homodimerization (26). The FRET-fluorescence lifetime imaging microscopic analysis also confirmed that VCAM-1 interacts with CD151 and that ICAM-1 interacts with CD9 [19, 22]. When CD151 and CD9 expression is reduced by siRNAs, the surface expression of VCAM-1 and ICAM-1 is similarly reduced, thereby suggesting a role for tetraspanins in structurally supporting the surface expression of VCAM-1 and ICAM-1. Under static binding conditions, the siRNA reduction of CD9 or CD151 in HUVECs does not alter the level of lymphocyte adhesion as compared to scrambled siRNA. In addition, the siRNA does not alter paracellular permeability (24). In contrast, under physiological vascular shear stress of 5–15 dyn/cm², siRNA reduction of CD9 or CD151 significantly decreases lymphocyte adhesion and lymphocyte transmigration across HUVECs (24). Therefore, tetraspanins

FIG. 4. VCAM-1 is located in tetraspanin-enriched microdomains. VCAM-1 is found in a lipid-raft-like platform containing ICAM-1 and the tetraspanins CD9, CD81, and CD151, known as the tetraspanin-enriched microdomain. Upon ligand binding to VCAM-1 or ICAM-1, the membrane forms apical projections toward the leukocyte. ICAM-1, intercellular adhesion molecule-1.



CD9 and CD151 are important for VCAM-1 expression and function.

D. VCAM-1 in apical cup-like structures

When a leukocyte binds to the endothelium, the plasma membrane of the endothelial cell forms an apical cup-like structure (also referred to as the endothelial adhesive platform) to surround the rolling leukocyte (Fig. 4) (24, 26, 47). Fluorescent microscopy shows that these cup-like structures contain VCAM-1 and ICAM-1 but not ICAM-2, vascular endothelial-cadherin, or PECAM-1 (47). It is likely that the cup-like structure is important in mediating firm adhesion between the leukocyte and endothelial cell and enabling transmigration. Confocal microscopy indicates that this apical cup-like structure is surrounded by polymerized actin that is associated with vinculin and VASP but is not connected to basal stress fibers or tubulin (25). VCAM-1's cytoplasmic domain is not required for the formation of the apical cups. In resting HUVECs transfected with a cytoplasmic tail-truncated VCAM-1, T lymphocyte binding to the endothelial cells still leads to the formation of an apical cup containing both the transfected VCAM-1 and endogenously expressed ICAM-1 (26). This finding suggests that proteins within the apical cup-like structure are recruited through extracellular interactions and not through their cytoplasmic domains (26).

E. Cytoplasmic domain of VCAM-1

The amino acid sequence of the cytoplasmic domain of VCAM-1 is 100% identical among many mammalian species, including the human, mouse, rat, rabbit, Sumatran orangutan, chimpanzee, common shrew, and microbat (22, 100, 118, 224, 232, 233) (NCBI NP001126200.1, NCBI XP001135527.1, Ensemble ENSSARP00000011070, Ensemble ENSMLUP00000011488). The protein sequence for the cytoplasmic domain of VCAM-1 in guinea pig and dolphin differs from the above species by only one conserved amino acid substitution (Ensemble ENSTTRP0000001370, Ensemble ENSCPOP00000006062). This high degree of identity suggests that the cytoplasmic domain is important for VCAM-1 expression or function. VCAM-1 has been shown to coimmunoprecipitate with ezrin and moesin, two structural proteins in the cytosol that are known to bind to actin (Fig. 3) (25). This was supported by confocal microscopy showing the colocalization of VCAM-1 with ezrin and moesin (25). The structure and function of the cytoplasmic domain of VCAM-1 during VCAM-1 signaling are currently under investigation.

IV. Overview of a Model for VCAM-1 Signaling

A. Model of VCAM-1 signaling through ROS

During inflammation, VCAM-1 expression is induced on endothelial cells by cytokines or turbulent shear stress. The cytokines and turbulent shear stress signal through high levels of short-lived ROS to induce NF κ B-dependent activation of VCAM-1 expression in endothelial cells (46, 54, 142, 144, 166, 182, 196, 276). This VCAM-1 protein synthesis requires several hours. Then, ligand binding to VCAM-1 induces rapid transient signaling through low levels of ROS that induce signals for the support of leukocyte transendothelial migration. The activated endothelial cells in lymph nodes and inflammatory sites express VCAM-1 on their luminal surface and their lateral surface but not on their basal surface (Fig. 5B).

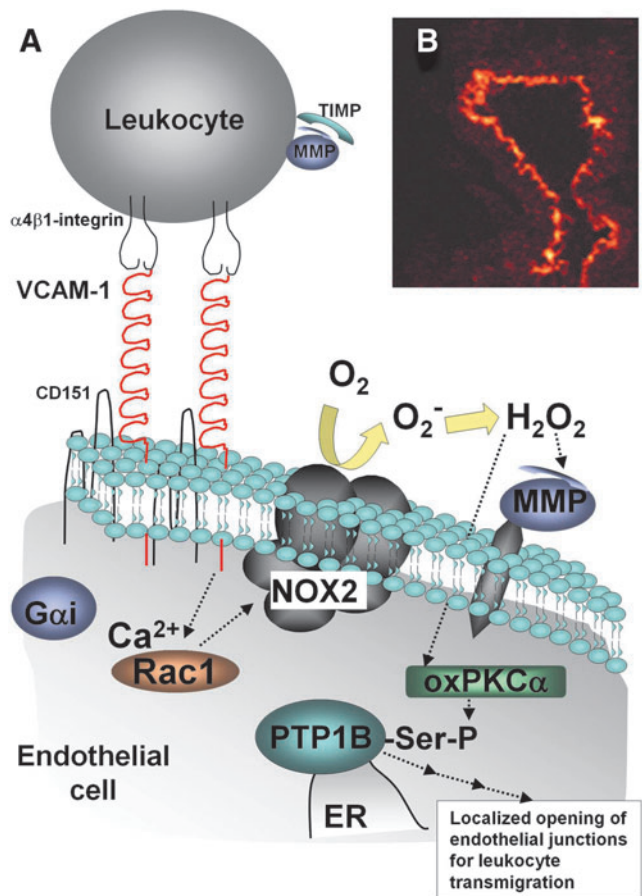


FIG. 5. VCAM-1 signal transduction. (A) Model for VCAM-1 signaling. Crosslinking of VCAM-1 activates calcium fluxes and Rac-1, which then activates endothelial cell NOX2. Nox2 catalyzes the production of superoxide that then dismutates to H₂O₂. VCAM-1 induces the production of only 1 μ M H₂O₂. H₂O₂ activates endothelial cell-associated MMPs that degrade extracellular matrix and endothelial cell surface receptors in cell junctions. The endothelial cell-derived H₂O₂ also mediates a 2–5 h delayed activation of lymphocyte-associated MMPs by inducing the degradation of leukocyte TIMPs. H₂O₂ diffuses through membranes at 100 μ m/s to activate p38MAPK. H₂O₂ also oxidizes and transiently activates endothelial cell PKC α . PKC α phosphorylates and activates PTP1B on the endoplasmic reticulum. PTP1B is not oxidized. These signals through ROS, MMPs, PKC α , and PTP1B are required for VCAM-1-dependent leukocyte transendothelial migration. The G protein Gxi is also involved in VCAM-1 signaling. (B) Mouse lung tissue section from antigen-challenged lungs was labeled with anti-VCAM-1 and a TRITC-conjugated secondary antibody. VCAM-1 labels the luminal and lateral, but not the basal surface of vascular endothelial cells *in vivo*. ER, endoplasmic reticulum; H₂O₂, hydrogen peroxide; MMP, matrix metalloproteinase; oxPKC α , oxidized protein kinase C α ; PTP1B, protein tyrosine phosphatase 1B; ROS, reactive oxygen species; TIMP, tissue inhibitor of metalloproteinase.

Therefore, at these endothelial surfaces, VCAM-1 activates intracellular signals through ROS (Fig. 5A). Localized VCAM-1 signals that induce changes in endothelial cell shape during leukocyte transendothelial migration are important since endothelial cell shape changes are confined to the site of leukocyte binding to the endothelium during leukocyte rolling and

transendothelial migration. The signals in this pathway are transient and occur within minutes, consistent with the transient, rapid nature of leukocyte transendothelial migration.

An overview of VCAM-1 signals is introduced here before specifically discussing each of these signals. It has been reported that activation of VCAM-1 stimulates calcium channels, intracellular calcium release, the G protein *Gxi2*, and the low-molecular-weight G protein *Rac1* (65, 184, 225). The calcium flux and *Rac1* activate the NADPH oxidase NOX2 (65, 184). VCAM-1 does not activate other enzymes that generate ROS (184). The activated NOX2 generates superoxide that then dismutates to hydrogen peroxide (H_2O_2), generating $1 \mu M H_2O_2$ during VCAM-1 signaling (65, 286). This $1 \mu M H_2O_2$ is relatively low as compared to the 50–200 $\mu M H_2O_2$ produced by macrophages or neutrophils (66, 74). It is also much lower than the exogenous 100–1000 $\mu M H_2O_2$ added to endothelial cells in studies on oxidative damage of endothelium or the exogenous 400 $\mu M H_2O_2$ added to endothelial cells in studies for ROS induction of VCAM-1 expression (20, 116, 120, 165, 289). These differences in H_2O_2 levels are important in understanding functions of oxidation, as we and others reported that $1 \mu M H_2O_2$ and $>50 \mu M H_2O_2$ have opposing effects on signal transduction (1, 73, 90, 238).

During VCAM-1 signaling, the $1 \mu M H_2O_2$ oxidizes the pro-domain of matrix metalloproteinases (MMPs), causing autocatalytic cleavage of the pro-domain and activation of the MMPs. The $1 \mu M H_2O_2$ also diffuses through cell membranes at $100 \mu m/s$ (183). In contrast, superoxide remains primarily extracellular as it has a relatively low diffusion rate across membranes. These intracellular ROS activate endothelial cell p38MAPK for the regulation of endothelial cell gap formation (294). The $1 \mu M H_2O_2$ also directly oxidizes and transiently activates intracellular protein kinase *C α* (PKC α) in endothelial cells (1). This activated PKC α induces phosphorylation and activation of protein tyrosine phosphatase 1B (PTP1B) (72). Interestingly, the PTP1B that has an oxidizable cysteine in its catalytic domain is not oxidized during VCAM-1 signaling in endothelial cells (72), indicating specificity of targets for oxidation by the low concentrations of ROS generated during VCAM-1 signaling. The signals downstream of the PTP1B that regulate endothelial cell junctions are currently under further investigation. Most importantly for this signaling pathway, the signals in Figure 4A have been demonstrated to function in regulation of VCAM-1-dependent leukocyte transendothelial migration *in vitro* and *in vivo* (1, 2, 30, 65, 72, 73, 140, 184, 225). Thus, VCAM-1 is not simply a scaffold for leukocyte adhesion, since it also activates “outside-in” signal transduction in endothelial cells. Several of these VCAM-1 signals are also activated by other adhesion molecules. For example, VCAM-1 and ICAM-1 both activate calcium fluxes, PKC, p38MAPK, and cytoskeletal changes in endothelial cells (88, 305, 306).

B. Cell models for VCAM-1 signals

To examine VCAM-1 signaling, both cytokine-activated primary cultures of endothelial cells and endothelial cell lines are necessary, since there are distinct advantages to each of these approaches. Primary cultures of cytokine-activated endothelial cells have the advantage of being primary cells that can be specifically stimulated by crosslinking VCAM-1 with anti-VCAM-1 antibody-coated beads (Fig. 6). However, activated primary cultures of endothelial cells express multiple

adhesion receptors for leukocytes and thus are difficult to use to examine functions specific to VCAM-1 during leukocyte transendothelial migration (Fig. 6). The advantage of the murine endothelial cell line lymph node-derived high endothelial venule-like (mHEV) cells is that the mHEV cells express VCAM-1 but not multiple other receptors for leukocyte migration (Fig. 6, Table 3) (285). Thus, leukocytes migrate specifically on VCAM-1 on the mHEV cells, without complications due to leukocyte binding to many other vascular adhesion molecules (285). The leukocyte adhesion to the mHEV cells is blocked by function blocking antibodies to VCAM-1 and its ligand $\alpha 4$ -integrin but not other adhesion molecules (Fig. 6, Table 3) (184, 285). Further, VCAM-1 is constitutively expressed by the mHEV cells; therefore, analysis of VCAM-1 signaling is not complicated by signals from cytokine induction of VCAM-1 expression (64, 285). Thus, the mHEV cells provide a model to test the functional outcome of VCAM-1 signals on VCAM-1-dependent leukocyte migration. In addition, the migration of leukocytes across the mHEV cells is induced by the chemokine monocyte chemoattractant protein-1, which is constitutively expressed by the mHEV cells (Fig. 6) (237). When examining the migration of spleen cells, the cells that migrate across the mHEV cells are $>90\%$ lymphocytes (286). Eosinophils also migrate on VCAM-1 in this mHEV model (unpublished data). Moreover, antibody crosslinking of VCAM-1 on mHEV cells or activated primary cultures of endothelial cells generate the same time course and magnitude of signals. Thus, the mHEV cell lines and primary cultures of activated endothelial cells provide models with unique assets to examine VCAM-1 signals during VCAM-1-dependent leukocyte migration (Fig. 6).

For activation of VCAM-1 by antibody crosslinking, anti-VCAM-1 antibodies are either used to coat $10 \mu m$ beads that are the size of leukocytes (Fig. 6) or are used in antibody complexes composed of anti-VCAM-1 and a secondary antibody (1, 65, 72, 73). VCAM-1 signals are not activated by primary anti-VCAM-1 antibodies alone (184), indicating that crosslinking is necessary. Leukocyte binding to the mHEV cell lines or antibody crosslinking of VCAM-1 on mHEV cell lines activates signals with the same magnitude and time course (65, 184). Further, antibody crosslinking of VCAM-1 on cytokine-activated primary cultures of human endothelial cells (HUVECs or human microvascular endothelial cells from lung) stimulates VCAM-1 signaling with the same magnitude and time course as the mHEV cell lines, indicating that the VCAM-1 signals are consistent for these endothelial cells. In addition to activation by VCAM-1 directly, the VCAM-1 signaling intermediate $1 \mu M H_2O_2$ (Fig. 6) is sufficient to activate the downstream signals with the same time course as crosslinking VCAM-1 (65, 184). Therefore, for the study of VCAM-1 function, a combination of cell approaches is used to identify VCAM-1 “outside-in” signals and, importantly, used to define whether the VCAM-1 signals have a functional role in VCAM-1-dependent leukocyte migration. Approaches for examining the *in vivo* function VCAM-1 signals are included later in this review in section VII discussing the *in vivo* role of VCAM-1 signals.

V. VCAM-1 Signals Through ROS During Leukocyte Transmigration

A. VCAM-1 activates calcium fluxes, *Rac1*, and *Gxi*

Ligand binding to VCAM-1 activates rapid signals in endothelial cells (Fig. 5). Lymphocyte binding to VCAM-1 or

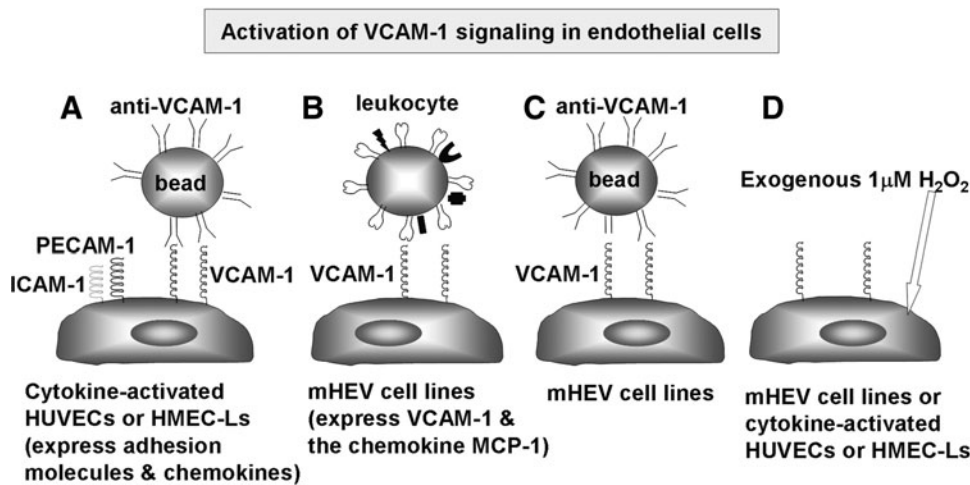


FIG. 6. Activation of VCAM-1 signals. Cells: (A, D) Cytokine-activated primary cultures of endothelial cells express multiple receptors for leukocyte adhesion. (B–D) Immortalized endothelial cell lines (mHEV) constitutively express VCAM-1 but not other ligands for leukocytes. The mHEV cells also express MCP-1 that induces leukocyte transmigration. Stimulation: (A, C) Anti-VCAM-1-coated beads crosslink VCAM-1 and activate VCAM-1 signaling. In contrast, soluble anti-VCAM-1 antibodies do not activate VCAM-1 signaling. (B) Leukocyte binding to VCAM-1 on mHEV cells crosslinks VCAM-1 and activates VCAM-1 signaling. (D) Exogenous 1 μ M H₂O₂ activates VCAM-1 signals downstream of NOX2 to determine whether H₂O₂ is sufficient for the signaling. MCP-1, monocyte chemoattractant protein; mHEV, lymph node-derived high endothelial venule-like cells.

anti-VCAM-1-coated 10 μ m beads stimulates a calcium flux in 30 s in endothelial cells. This calcium flux is mediated by verapamil-sensitive calcium channels and the release of intracellular calcium, which are required for the production of 1 μ M H₂O₂ in mHEV cells (65). A calcium flux is also induced by VCAM-1-dependent monocyte adhesion or antibody cross-linking of VCAM-1 on lipopolysaccharide-activated HUVECs (175). In addition, anti-VCAM-1-coated 10 μ m bead stimulation of endothelial cells activates Rac1 that is required for the production of the 1 μ M H₂O₂ by the endothelial cells (Fig. 5) (65). Transfection with dominant negative Rac1 prevents the anti-VCAM-1-stimulated generation of H₂O₂ (65). Dominant negative Rac-1 also blocks VCAM-1-dependent migration of lymphocytes across mHEV cell monolayers (65) and blocks migration of U-937 cells across cytokine-activated HUVECs (294). Thus, since Rac1 is involved in the assembly of the active NOX2 complex and, as discussed below, VCAM-1 stimulation of H₂O₂ generation occurs through NOX2, endothelial Rac1 likely promotes assembly of NOX2 complex formation during VCAM-1 signaling (Fig. 5) (34, 77, 168). In addition to VCAM-1 signaling through the low-molecular-weight G protein Rac1, VCAM-1 also signals through G α i. In *in vitro* assays for VCAM-1-dependent transmigration, leukocyte transmigration across monolayers of endothelial cells was blocked by the G α i inhibitor pertussis toxin without altering the VCAM-1-dependent binding to the apical surface of the endothelial cells (225). Thus, VCAM-1 activates calcium fluxes and the G proteins Rac1 and G α i. The mechanisms for VCAM-1 activation of these G proteins are under investigation.

B. VCAM-1 activation of endothelial cell NOX2 during leukocyte transmigration

1. VCAM-1 activates NOX2. The VCAM-1 stimulation of calcium fluxes and Rac-1 activates the membrane complex NADPH oxidase in endothelial cells for the production of ROS (Fig. 5) (65, 294). NADPH oxidase catalyzes the production of superoxide from oxygen using the cofactor NADPH. Then, superoxide dismutates to H₂O₂. NADPH

oxidases consist of two transmembrane subunits and three cytoplasmic subunits that are recruited to the membrane to form the active NADPH oxidase complex (7, 192). There are several forms of NADPH oxidase that differ in their catalytic subunit and their cell-specific expression (65, 74, 132, 169, 192, 290). Endothelial cells express the NADPH oxidase subunits gp91 phox, p22 phox, p47 phox, and p67 phox (132, 192). Lymphocyte binding to VCAM-1 or anti-VCAM-1-coated beads activate the NOX2 form of NADPH oxidase, which utilizes the gp91phox catalytic subunit (2, 63, 65, 73, 140, 184). The VCAM-1 activation of NOX2 has been demonstrated in

TABLE 3. LEUKOCYTE BINDING TO LYMPH NODE-DERIVED HIGH ENDOTHELIAL VENULE-LIKE CELLS

<i>Adhesion Molecule</i>	<i>mAbs that inhibit adhesion</i>
α 4-integrin	R1-2 +9C10 PS/2
VCAM-1	M/k-2 MVCAM.A
	<i>mAbs that do not inhibit adhesion</i>
LFA-1	M17/4
ICAM-1	3E2
ICAM-2	3C4
L-selectin	MEL-14
activated β 1-integrin	9EG7
α 4 β 7-integrin	DATK32
<i>Adhesion molecules not expressed by lymph node-derived high endothelial venule-like cells (as determined by cDNA microarray and immunolabeling)</i>	
Platelet-endothelial cell adhesion molecule-1	
ICAM-1	
P-selectin	
E-selectin	
Mucosal addressin cell adhesion molecule-1	
MECA antigens	

ICAM-1, intercellular adhesion molecule-1.

several studies using antisense for the catalytic subunit of NOX2 (gp91phox), pharmacological inhibitors, extracellular scavengers of superoxide and H₂O₂, and chimeric gp91phox deficient (CYBB) mice (2, 63, 65, 73, 140, 184). Antisense for gp91 phox blocks expression of gp91 phox in endothelial cell lines and blocks VCAM-1-induced H₂O₂ production in endothelial cells (65). The pharmacological inhibitor of NADPH oxidase apocynin blocks VCAM-1-stimulated H₂O₂ generation in endothelial cell lines and primary cultures of endothelial cells (184). Exogenous addition of the scavenger of superoxide, superoxide dismutase, or the scavenger of hydrogen, catalase, scavenge these species of extracellular ROS and block VCAM-1-dependent leukocyte transendothelial migration *in vitro* (184). Importantly, anti-VCAM-1-coated beads stimulate ROS generation in primary cultures of endothelial cells and in mHEV cells with the same time course and magnitude, indicating that these signals are consistent for endothelial cells (184, 294). In contrast to VCAM-1 activation of NADPH oxidase, it has been reported that antibody cross-linking of the endothelial cell adhesion molecules ICAM-1 and PECAM-1 does not activate endothelial cell NADPH oxidase (184, 291). In support of NOX2 activation by VCAM-1 but not ICAM-1 or PECAM-1, mice deficient in nonhematopoietic NOX2 exhibit a reduction in VCAM-1-dependent recruitment of leukocytes but no effect on ICAM-1-dependent or PECAM-1-dependent recruitment of leukocytes (2).

2. VCAM-1 signals through NOX2 mediate VCAM-1-dependent leukocyte transmigration. VCAM-1-dependent lymphocyte transmigration requires NOX2-generated ROS. It is reported that VCAM-1-dependent lymphocyte transendothelial migration *in vitro* is blocked by pharmacological inhibition of NADPH oxidase with apocynin, blocked by inhibition of endothelial cell flavoproteins with diphenyliodonium, and blocked by extracellular scavenging of ROS with superoxide dismutase or catalase (2, 184). These inhibitors block lymphocyte transmigration without affecting VCAM-1-dependent adhesion of leukocytes to the endothelial cells (2, 184). In contrast, VCAM-1 does not activate other ROS generating enzymes for VCAM-1-dependent lymphocyte migration because VCAM-1-dependent migration is not affected by pharmacological inhibition of xanthine oxidase, nitric oxide synthase, or cytochrome P450 (184). In addition, inhibition of endothelial cell PI3 kinase and tyrosine kinases does not block VCAM-1-dependent leukocyte transendothelial migration (184). In contrast to the function of endothelial cell NADPH oxidase, lymphocyte flavoproteins, including NADPH oxidase, are not required for VCAM-1-dependent lymphocyte transendothelial migration (184). Thus, NADPH oxidase in endothelial cells but not lymphocytes is required for VCAM-1-dependent lymphocyte migration. Moreover, pharmacologic and antisense inhibition of NADPH oxidase or scavenging of ROS in endothelial cells blocks VCAM-1-dependent ROS generation and VCAM-1-dependent leukocyte migration.

3. VCAM-1-induced NOX2 generates low concentrations of ROS with specific signals that are distinct from signals by high levels of ROS. The level of VCAM-1-stimulated ROS production is much lower than the level of ROS that cause oxidative damage in tissues. To measure these low levels of VCAM-1-stimulated ROS, the ROS-sensitive probe dihy-

drodrhodamine 123 has been used (65, 184). This probe becomes fluorescent when oxidized by H₂O₂, but not by superoxide, in the presence of cellular peroxidases (114). In addition, although xanthine oxidase-generated ROS can oxidize dihydrorhodamine 123, VCAM-1 does not activate xanthine oxidase signaling (184). Importantly, only 1 μ M H₂O₂ is produced by the endothelial cells when lymphocytes bind to VCAM-1 or when VCAM-1 is crosslinked by anti-VCAM-1-coated beads (63, 65, 73, 184, 286). This is in contrast to the 50–200 μ M H₂O₂ released by neutrophils and macrophages for the destruction of pathogens (66, 74) or released in disease states for oxidative damage such as atherosclerosis, pulmonary fibrosis, ischemia-reperfusion syndrome, and neurodegenerative diseases (241, 281). The oxidative damage to endothelial cell functions and junctions by large amounts of H₂O₂ (200–1000 μ M) (28, 93, 116, 120, 141, 165, 193) are not consistent with the signals that occur during VCAM-1 signaling and leukocyte transendothelial migration. During VCAM-1 signaling, 1 μ M H₂O₂ directly activates MMPs (73, 238), directly activates PKC α (1), and indirectly stimulates an increase in PTP1B activity (1). In contrast, high levels of H₂O₂ (>50 μ M) directly inhibit MMPs (73, 238), inhibit PKC α (1), and inhibit tyrosine phosphatases (90). The function of the low levels of ROS for the generation of rapid, transient, and reversible signals is important because once a leukocyte reaches an endothelial cell junction, the process of transmigration occurs within a couple of minutes. The mechanisms for VCAM-1/ROS-induced activation of MMPs, PKC α , and changes in endothelial cell shape are discussed below.

C. VCAM-1 signals through NOX2 modify endothelial cell actin polymerization and intercellular gap formation

VCAM-1 signals mediate changes in endothelial cell actin structure. At the site of VCAM-1 binding, the endothelial cell actin coalesces at the endothelial cell surface, forming a cup-like structure in endothelial cell lines and cytokine-activated primary endothelial cells (25, 26, 47, 184). This VCAM-1-stimulated change in actin structure is mediated by endothelial cell NADPH oxidase (184, 294). VCAM-1-stimulated Rac1 and ROS also induce intercellular gap formation and loss of β -catenin at the gaps in IL-1 β -activated HUVECs (294). The VCAM-1-activated intercellular gaps require VCAM-1 activation of Rac1 and ROS since the VCAM-1-induced gap formation is blocked by a dominant negative Rac1 or the ROS scavengers *N*-acetyl-L-cysteine and catalase (294). VCAM-1-stimulated Rac1 and ROS activate p38MAPK and the inhibition of p38MAPK with the pharmacological inhibitor SB-203580 blocks the VCAM-1-induced intercellular gap formation in monolayers of endothelial cells (294). Thus, VCAM-1 induces cell shape changes in endothelial cell structure. Endothelial cell actin restructuring is important for endothelial cell shape changes during transendothelial migration of leukocytes.

D. VCAM-1-induced ROS activate MMPs

1. Rapid activation of endothelial cell-associated MMPs. ROS production by VCAM-1-stimulated-endothelial cells activates MMPs (Fig. 5) (73). MMPs are held at the cell surface by membrane type-MMPs (MT-MMPs) and adhesion molecules. MMP-2 binds to transmembrane MT1-MMP (MMP14) (329). MMP-9 and MMP-7 bind to cell surface CD44 (3, 205, 324),

and pro-MMP-9 can bind to ICAM-1 (92). It is also reported that MMP-1, MMP-2, and MMP-9 bind to $\alpha_2\beta_1$ integrin on keratinocytes, $\alpha_v\beta_3$ integrin on endothelial cells, and α_2 integrin on epithelial cells, respectively (260). The MMPs bound to the endothelial cell surface can have local functions, whereas MMPs released by endothelial cells are washed away by the flow of blood. Endothelial cell-associated MMP2 and MMP9 are activated by lymphocyte binding to VCAM-1 or antibody crosslinking of VCAM-1 as determined by gelatin zymography (73). This occurs without altering levels of cell-associated MMPs or tissue inhibitors of MMPs (TIMPs) as determined by western blot (73). The time course and magnitude of this MMP activation is the same for lymphocyte binding to VCAM-1 and for antibody crosslinking of VCAM-1 on either endothelial cell lines or IL-4-activated primary cultures of endothelial cells (73). Moreover, this activation of endothelial cell-associated MMPs occurs within minutes, which is consistent with the 2 min leukocyte transendothelial migration process once a leukocyte reaches a site for migration. The anti-VCAM-1-coated bead activation of the endothelial cell MMPs is not altered by laminar flow, at the rate found in postcapillary venules (2 dynes/cm²) (73). Thus, this force of "tugging" on VCAM-1 does not influence the signaling for activation of MMPs, whereas signaling by other adhesion molecules is influenced by the force on the receptor (12). The VCAM-1-stimulated activation of endothelial cell-associated MMP2 and MMP9 is mediated through ROS because the MMP activation is blocked by pretreatment of endothelial cells with antisense against the NADPH catalytic subunit gp91 phox, pharmacologic inhibitors of NADPH oxidase or scavenging of H₂O₂ with exogenous catalase (73). Interestingly, the same magnitude and rapid time course for activation of endothelial-associated MMPs also occurs when VCAM-1 is bypassed by addition of the VCAM-1 signaling intermediate, exogenous 1 μ M H₂O₂ (73).

In contrast to the VCAM-1-mediated activation of MMPs by 1 μ M H₂O₂, high concentrations of exogenous H₂O₂ (>50 μ M H₂O₂) induce oxidative damage and inhibit basal endothelial cell-associated MMP activity (73). This is consistent with a report by Rajagopalan *et al.* (238), indicating that purified MMPs are inhibited by H₂O₂ at concentrations >50 μ M, whereas 1 μ M H₂O₂ activates purified MMPs (238). Thus, low concentrations of H₂O₂ activate MMPs, whereas high levels of H₂O₂ inhibit MMP enzymatic activities and induce oxidative damage (20, 165, 289). The data in these reports emphasize the opposing regulatory functions of low *versus* high levels of ROS.

The mechanism for ROS activation of MMPs is conserved among the MMPs. Briefly, MMPs are synthesized in a non-active form, containing a conserved propeptide cysteine that is bound to the conserved zinc atom in the active site of the MMPs. ROS oxidize the cysteine in the propeptide domain that opens the propeptide arm and exposes the MMP active site (204). This opening of the propeptide arm stimulates autocatalytic removal of the arm, forming an active MMP (293). Thus, H₂O₂ does not have specificity for MMP isozymes given the conserved cysteine-zinc bond in pro-MMPs (204) and the rate of diffusion of H₂O₂ at 100 μ m/s (183). Therefore, H₂O₂ activates those MMP isozymes expressed at the sites of VCAM-1-stimulated ROS generation.

The MMPs that are activated during VCAM-1 signaling degrade extracellular matrix and may cleave endothelial cell

junction molecules. It has been reported that MMPs can cleave the endothelial cell junction molecule vascular endothelial-cadherin (117). This degradation by MMPs likely participates in opening endothelial cell-cell adhesions for the formation of passageways through which leukocytes can migrate. Consistent with this, it is reported that the VCAM-1-stimulated ROS-activation of endothelial cell-associated MMPs regulates VCAM-1-dependent leukocyte transmigration. In these studies, endothelial cells on transwells were pretreated with the MMP inhibitors GM6001 or BB3103 and washed before the migration assay (73). MMP inhibitor pretreatment of endothelial cell lines blocked VCAM-1-dependent lymphocyte migration in a dose-dependent manner without affecting cell viability (73). The last wash, from cells that had been pretreated with inhibitor, did not affect migration of untreated cells, indicating that the inhibitor-treated cells were sufficiently washed and that the effect of the inhibitor was on the endothelial cells (73). In addition, the MMP inhibitor blocked anti-VCAM-1 stimulated endothelial cell-associated MMP activity (73). Romanic *et al.* (245) also demonstrated that TIMP-2-mediated inhibition of MMP activity blocks T cell transmigration (245), although they did not determine whether inhibition with TIMP-2 was mediated by blocking lymphocyte or endothelial cell MMPs. Thus, endothelial cell-associated MMP activity is necessary for VCAM-1-dependent lymphocyte transendothelial migration. Further, the requirement for VCAM-1-stimulated endothelial cell ROS generation and endothelial cell-associated MMP activity during lymphocyte migration indicate that the endothelial cell has an active role in VCAM-1-dependent lymphocyte migration (Fig. 5).

2. Delayed activation of lymphocyte-associated MMPs. Since H₂O₂ diffuses rapidly (183), endothelial cell H₂O₂ that is generated during VCAM-1 signaling has the potential to also very rapidly activate MMPs on the surface of leukocytes when leukocytes are bound to the endothelium. However, several reports indicate that upon binding to VCAM-1, lymphocyte MMPs are activated but only after prolonged periods of 2–12 h (73, 87, 245, 318). This delay in lymphocyte MMP activation is a consequence of the high levels of TIMPs expressed by the leukocytes. Deem *et al.* reported that endothelial cell-derived ROS generated during lymphocyte binding to VCAM-1 activates lymphocyte MMPs at 2 h (73). In these studies, lymphocytes were incubated with monolayers of endothelial cells, nonbound lymphocytes were removed by washing, and bound lymphocytes were released from the monolayers by reversing lymphocyte binding with soluble anti-VCAM-1. Lymphocyte MMP9 was activated at 2–5 h (73). This activation of lymphocyte MMPs is mediated by endothelial cell-derived ROS, as the activation is blocked by pharmacological inhibition of endothelial cell ROS generation with diphenyliodonium or apocynin but not by pharmacologic inhibition of ROS-generating enzymes in lymphocytes (73). Moreover, exogenous addition of 1 μ M H₂O₂ to purified lymphocytes induces activation of lymphocyte-associated MMPs that is also delayed for 2 h, indicating that 1 μ M H₂O₂ activates the lymphocyte MMPs with the same time course and magnitude as ligand binding to VCAM-1 (73). Interestingly, after a 5 h treatment of purified lymphocytes with 1 μ M H₂O₂, the expression of lymphocyte MMP9 is not altered, but the expression of tissue inhibitor of MMP (TIMP)-1 and

TIMP2 by lymphocytes is reduced by 60%–80% as determined by western blot (73). Thus, H_2O_2 activates lymphocyte MMPs by oxidation and loss of TIMPs, the endogenous inhibitors of MMPs. The mechanism for this VCAM-1/ROS-induced loss of TIMPs is, at least, through proteasome degradation of the TIMPs because the $1 \mu M$ H_2O_2 -induced loss of TIMP expression is blocked by the proteasome inhibitor MG132 (Fig. 7). Thus, the ROS-induced reduction in TIMPs on lymphocytes results in a threefold increase in the MMP9/TIMP ratio, reflecting a net increased MMP activity at 2–5 h. Thus, H_2O_2 activation of the lymphocyte-associated MMPs is mediated by the downregulation of the expression of the relatively high levels of TIMPs on lymphocytes without altering expression of lymphocyte MMPs (73). Thus, the mechanism for the activation of lymphocyte MMPs is through ROS inactivation of the TIMPs. This is in contrast to a direct effect of H_2O_2 on endothelial cell-associated MMPs for the rapid activation of endothelial cell MMPs within minutes. The activation of lymphocyte-associated MMPs is too late for lymphocyte MMP function during transmigration because transmigration occurs within a few minutes. Moreover, pretreatment of lymphocytes with the MMP inhibitors GM6001 or BB3103, followed by a wash, does not alter VCAM-1-dependent transmigration (73).

3. The antioxidant bilirubin inhibits VCAM-1-induced MMP activation. The *in vitro* studies on VCAM-1 activation of ROS demonstrate that endothelial cell production of ROS rapidly activates endothelial cell-associated MMPs that are required for VCAM-1-dependent lymphocyte migration. The antioxidant bilirubin blocks this VCAM-1-dependent lymphocyte migration *in vitro* and blocks VCAM-1 activation of MMPs (140). Bilirubin is generated from heme by hemoxygenase-1 (140, 249). After generation of bilirubin, it can undergo redox cycling such that oxidation of bilirubin converts it to biliverdin (249, 259, 272, 273). Bilirubin and biliverdin are membrane permeable (217, 273). Biliverdin is recycled back to bilirubin by biliverdin reductase and the cofactor NADPH (140). Hemoxygenase-1 and biliverdin reductase are expressed by endothelial cells and endothelial cell lines (140). Further, bilirubin is taken up by endothelial cells, and thus bilirubin in endothelial cells can function as an antioxidant (140). Bilirubin acts as an antioxidant in that it reduces oxidized phospholipids with the approximate rate of antioxidant vitamins (274). Concentrations of bilirubin in the upper physiological range block anti-VCAM-1 activation of endothelial cell-associated MMP2 and MMP9 without affecting cell viability (140). Further, bilirubin blocks VCAM-1-dependent migration of lymphocytes across endothelial cells *in vitro* without affecting cell viability (140). Consistent with an antioxidant function for bilirubin, VCAM-1-dependent lymphocyte migration is not blocked by the stable bilirubin conjugate ditaurobilirubin that cannot scavenge ROS (140). The bilirubin inhibition of lymphocyte migration results from an inhibition of migration rather than inhibition of lymphocytes available for migration as the number of lymphocytes bound to the endothelial cell monolayer is unaffected by bilirubin (140). Therefore, the antioxidant bilirubin blocks VCAM-1-dependent lymphocyte migration across endothelial cells, at least by, blocking the ROS-mediated activation of MMPs.

To summarize VCAM-1 activation of MMPs, lymphocyte binding to VCAM-1 activates endothelial cell generation of

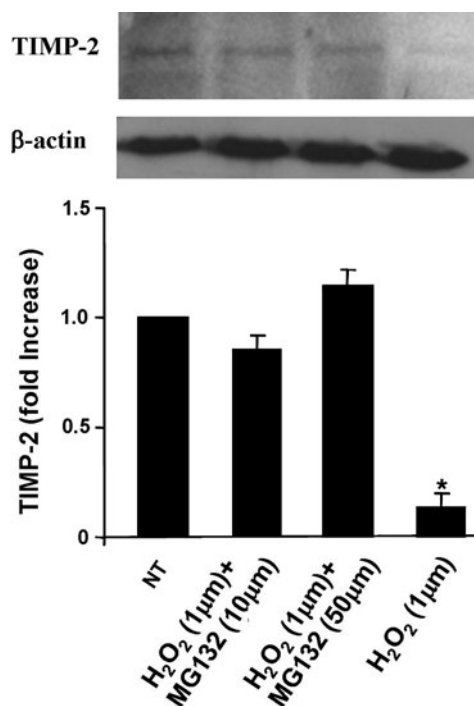


FIG. 7. The level of hydrogen peroxide produced during VCAM-1 signaling induces degradation of TIMP-2 on leukocytes. BALB/c mouse spleens were collected and red blood cells were lysed by hypotonic shock. The leukocytes were nontreated (NT) or treated with the proteasome inhibitor MG132, and then $1 \mu M$ H_2O_2 was added. This is the level of H_2O_2 generated during VCAM-1 signaling. After 5 h, the cells were lysed and TIMP-2 expression was examined by western blot. H_2O_2 induced the loss of TIMP-2 on leukocytes, and this loss was blocked by the proteasome inhibitor. The treatments had no effect on cell viability (data not shown). The western blots are representative from three independent experiments. The data are presented as mean \pm SEM. * $p < 0.05$ as compared to the NT control.

ROS, which induces a delayed activation of lymphocyte MMPs. This delay in ROS-induced MMP activity in lymphocytes is a consequence of the time required for a reduction in TIMP expression. The 2–5 h delay in activation of the lymphocyte-associated MMPs is too late for transendothelial migration but likely regulates migration of lymphocytes through extravascular tissues. In contrast, pretreatment of endothelial cells with these MMP inhibitors blocks leukocyte transendothelial migration. Thus, the delayed activation of lymphocyte MMPs is consistent with the requirement for endothelial cell MMPs, but not lymphocyte MMPs, during VCAM-1-dependent lymphocyte migration.

E. VCAM-1 signals oxidize and activate PKC α

VCAM-1-induced H_2O_2 , which can diffuse through membranes at $100 \mu m/s$ (183), stimulates intracellular signals through PKC α (Fig. 5) (1). Ligand binding to VCAM-1 activates autophosphorylation of PKC α Thr638 in endothelial cell lines or primary cultures of endothelial cells (1). This activation of PKC α is blocked by inhibition of NADPH oxidase or

scavenging of ROS (1). Moreover, this VCAM-1 activation of PKC α is induced by direct oxidation of PKC α cysteines (1). Bypassing VCAM-1 by exogenous addition of the VCAM-1 signaling intermediate, 1 μ M H₂O₂, also stimulates activation of PKC α with the same time course as that for ligand binding to VCAM-1, indicating that 1 μ M H₂O₂ is sufficient for activation of endothelial cell PKC α (1). Importantly, VCAM-1 activation of PKC α is required for VCAM-1-dependent leukocyte transmigration because VCAM-1-dependent leukocyte transendothelial migration is blocked by dominant negative PKC α or selective pharmacological inhibitors of PKC α without affecting leukocyte binding to the endothelial cells (1). Thus, VCAM-1 stimulates oxidative activation of endothelial cell PKC α , which is required for VCAM-1-dependent leukocyte transendothelial migration.

F. VCAM-induced PKC activates PTP1B

VCAM-1 signaling *via* ROS and PKC α activates downstream signals in endothelial cells through PTP1B (Fig. 5) (72). Ligand binding to VCAM-1 increases PTP1B serine phosphorylation and the phosphatase activity of PTP1B in endothelial cells (72). This activation of PTP1B is blocked by inhibition of endothelial cell NADPH oxidase or inhibition with the PTP1B inhibitor, CinnGEL-2ME (72). CinnGEL-2ME specifically inhibits PTP1B because it has a side chain that binds to a site on PTP1B specific for PTP1B and it also binds to and inhibits the active site of PTP1B (199, 326). Bypassing VCAM-1 by exogenous addition of 1 μ M H₂O₂ to the endothelial cells increases endothelial cell PTP1B activity that is similar in magnitude and time course as that observed with ligand binding to VCAM-1. Further, VCAM-1 activation of PTP1B is downstream of PKC α during VCAM-1 signaling (72) since anti-VCAM-1-stimulated serine phosphorylation of PTP1B, the active form of PTP1B (40), is blocked when endothelial cells are transfected with a plasmid containing dominant negative PKC α or treated with the PKC α inhibitor Gö-6976 (72).

Interestingly, during VCAM-1 signaling, PTP1B is activated and not inhibited by oxidation (72). Although it has been reported that PTP activity can be inhibited by high levels of oxidants (50–200 μ M H₂O₂) (94, 267, 281) through oxidation of the conserved cysteine in the PTP1B catalytic site (39, 90, 91, 94, 111, 234, 267, 281), VCAM-1 stimulates the production of only 1 μ M H₂O₂ (65, 73), which activates PTP1B rather than inhibits it (72). Moreover, analysis of PTP1B for oxidation of cysteines revealed that PTP1B in endothelial cells is not oxidized after VCAM-1 signaling or after exogenous addition of 1 μ M H₂O₂ (72). However, purified PTP1B is susceptible to oxidation upon addition of 1 μ M H₂O₂ (72). Thus, within VCAM-1-stimulated endothelial cells, there is compartmentalization of targets for oxidation by low levels of NOX2-generated ROS because PKC α is oxidized, but PTP1B is not oxidized (72). This occurs even though H₂O₂ diffuses through membranes at 100 μ m/s (183). Therefore, it is possible that the low concentrations of H₂O₂ are readily consumed as they oxidize targets, diffusion lowers the H₂O₂ below a threshold for oxidation of PTP1B, or local antioxidant mechanisms protect PTP1B. Thus, compartmentalization of ROS may limit the proteins that are modified by ROS. Forman *et al.* (94) proposes that low levels of ROS function as signaling molecules because (i) they have a restricted location of action, (ii)

their signals are transient, and (iii) their oxidation reactions are reversible. ROS modify thiolate anions (-S⁻) to form sulfenate (-SO⁻) as well as react with disulfide linkages (281). These can be reduced back to their native state by intracellular thiols in the cell such as thioredoxin, peroxiredoxins, and glutathione (94). Thus, PTP1B may be protected from oxidation by antioxidants or its compartmentalization to the endoplasmic reticulum (ER).

PTP1B is located on the ER membrane with its catalytic domain external to the ER (Fig. 5). An important question regarding compartmentalization is how PTP1B, which is localized to the ER, mediates VCAM-1 signaling. Studies have demonstrated that the ER membranes containing PTP1B reach the plasma membrane and that the PTP1B in this ER membrane can dephosphorylate receptors in the plasma membrane without receptor internalization (16, 263). Thus, during VCAM-1 signaling, endothelial cell PKC α phosphorylates and activates PTP1B, which then modulates localized signals in the endothelial cells. These signals need to have localized functions because the endothelial cell changes are limited to the site of leukocyte binding without retraction of the rest of the endothelial cell.

Most importantly, PTP1B participates in the active function of the endothelial cell during VCAM-1-dependent leukocyte transmigration because inhibition of PTP1B blocks VCAM-1-dependent lymphocyte transmigration without altering adhesion (72). Interestingly, when both the extracellular signals through MMPs and the intracellular signals through PTP1B are blocked, there is a greater inhibition of VCAM-1-dependent leukocyte transendothelial migration.

PTP1B is interesting as it has been a target for drug development. PTP1B is a potential target because PTP1B-deficient mice (without foreign antigen challenge) are physiologically normal and have normal body weight, making PTP1B a potentially promising drug target (29, 71, 84). PTP1B is most studied in diabetes because inhibitors of PTP1B block PTP1B dephosphorylation of the insulin receptor and block the development of diabetes in animal models (29, 79, 84, 104, 246, 253, 328). Moreover, PTP1B-deficient mice do not develop tumors, and do not develop diabetes in response to high fat diet, although there is a small effect on the immune system (29, 79, 84, 104, 112, 246, 253, 328). This has led to PTP1B inhibitors that have been in phase II clinical trials for diabetes (42, 79). Thus, whether clinical PTP1B inhibitors alter leukocyte recruitment during inflammation has clinical implications.

VI. VCAM-1 Signals in *In Vivo* Models

A. *Gxi2* regulation of VCAM-1-dependent leukocyte recruitment *in vivo*

Gxi2 functions in VCAM-1-dependent leukocyte recruitment *in vitro* and *in vivo* (Fig. 5). In *in vivo* studies using *Gxi2*^{-/-} mice, it was demonstrated that a signaling event in a nonlymphohematopoietic compartment of the lung is required for the recruitment of leukocytes during inflammation (225). This was examined in a model of allergic inflammation in which airway challenge with chicken egg ovalbumin (OVA) induces VCAM-1-dependent recruitment of eosinophils. In OVA-challenged *Gxi2*^{-/-} mice, VCAM-1-dependent eosinophil recruitment is inhibited (225). In addition, the inhibition of leukocyte recruitment was specific to the G protein *Gxi2*^{-/-} since mice deficient in *Gxi3* do not have altered leu-

kocyte recruitment in response to OVA (225). *Gzi2* function in eosinophils was not required for eosinophil chemotaxis since *Gzi2*^{-/-} eosinophils responded to chemotactic factors (225). Consistent with a function for nonhematopoietic *Gzi2*, *Gzi2*^{-/-} eosinophils adoptively transferred into wild-type mice are able to be recruited during allergic responses. In contrast, there is a reduced recruitment of wild-type eosinophils in *Gzi2*^{-/-} mice, indicating that signaling in a resident cell of the lung is required for the accumulation of eosinophils (225). Moreover, in the OVA-challenged *Gzi2*^{-/-} mice, there are elevated blood leukocyte numbers and an accumulation of leukocytes on the luminal surface of the blood vessels (225). Thus, the blood leukocytes are available for migration and capable of chemotaxis, but their transmigration is blocked. Interestingly, this occurs without altering the lung levels of the Th2 cytokines IL-4 and IL-5, and without inducing the Th1 cytokine interferon γ , indicating that the *Gzi2*^{-/-} deficiency did not alter regulatory inflammatory cytokines during allergic responses (225). *In vitro*, inhibition of endothelial cell *Gzi* blocks VCAM-1-dependent leukocyte transendothelial migration (225). These reports are consistent with specific *Gzi2*-mediated signaling in endothelial cells for the extravasation of leukocytes and for tissue-specific leukocyte accumulation. The mechanism for *Gzi2* function in the VCAM-1 signaling pathway is under investigation. Nevertheless, since *Gzi2*-deficient mice are viable and *Gzi2* regulates leukocyte recruitment, it may be a potential target for clinical intervention in inflammatory diseases.

B. NOX2 regulation of VCAM-1-dependent leukocyte recruitment *in vivo*

VCAM-1 signals through NOX2 *in vivo*. The gene that encodes the catalytic subunit of NOX2, gp91phox, is CYBB. CYBB-deficient mice have been used to examine VCAM-1-dependent eosinophil recruitment in response to OVA, a model of allergic inflammation. To examine the function of NOX2 in nonhematopoietic cells and avoid effects of NOX2 deficiency in leukocytes, green fluorescent protein C57BL/6j mouse bone marrow cells, which express wild-type gp91phox, were transplanted into irradiated CYBB mice and into control irradiated C57BL/6 wild-type mice (2). To induce VCAM-1-dependent eosinophil infiltration into the lung (2), the chimeric CYBB mice and chimeric wild-type mice were sensitized intraperitoneally with the antigen OVA in the adjuvant alum and then the lungs were challenged by intranasal administration of OVA (Fig. 8) (2). It is well established that OVA-stimulated eosinophilia in the lung as well as OVA-stimulated eosinophilia in the skin requires adhesion to VCAM-1 as antibodies to VCAM-1 block the eosinophilia (Fig. 8) (57, 107, 250). Interestingly, eosinophil infiltration into the bronchoalveolar lavage is inhibited by 68% in the OVA-challenged chimeric CYBB mice, but the infiltration of other leukocytes, which migrate on other adhesion molecules, is not altered (2). This is consistent with reports that 70% of OVA-induced eosinophil infiltration is VCAM-1-dependent (57, 107, 250). Most interestingly, there is an accumulation of eosinophils on the luminal surface of the endothelial cells in lung tissue of OVA-challenged chimeric gp91phox-deficient mice, suggesting that the eosinophils bound to endothelium but that they could not undergo VCAM-1-dependent transmigration (Fig. 9) (2).

In the CYBB chimeric mice, there are sufficient mediators present for induction of eosinophilia since there was no difference between the OVA-challenged chimeric wild-type and OVA-challenged chimeric deficient mice for cytokines, chemokines, VCAM-1 expression, or blood eosinophil numbers (2). Further, in these mice, there is no effect on the initial sensitization from the intraperitoneal administration of OVA since there is no effect on OVA-specific IgE in the OVA-challenged mice (2). The chimeric CYBB mice also exhibit a 70% reduction in airway hyperresponsiveness (AHR) (2). Moreover, intratracheal administration of purified eosinophils into the chimeric CYBB mice recovers the AHR (2), suggesting (i) that bypassing the endothelium overcomes the reduced AHR in the chimeric CYBB mice and (ii) that gp91phox expression by other cells of the lung such as fibroblasts are not critical for the reduced AHR in the nonhematopoietic gp91phox deficient mice. These studies provide support for the *in vivo* relevance of VCAM-1 signals through ROS for eosinophil recruitment in experimental allergic asthma.

VII. Antioxidant Regulation of VCAM-1 Signals in *In Vivo* Models

A. The antioxidant bilirubin inhibits VCAM-1-dependent inflammation

Consistent with the inhibitory function of bilirubin on VCAM-1-dependent leukocyte transmigration *in vitro* (140), it

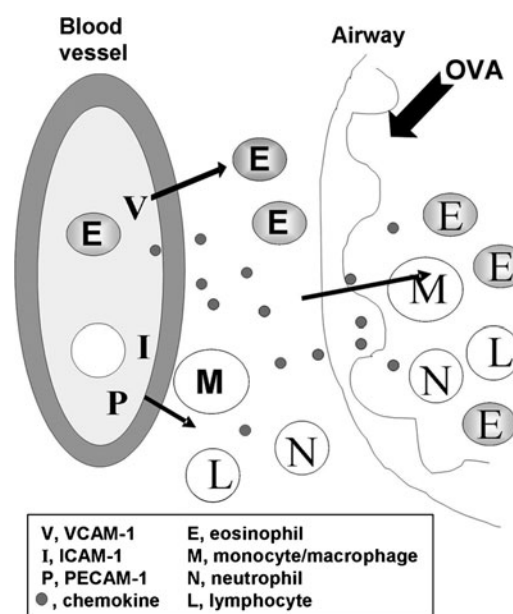


FIG. 8. VCAM-1-dependent eosinophil recruitment during allergic lung inflammation. After sensitization with the antigen OVA in the adjuvant alum, the lung is challenged with OVA. In this model, eosinophil recruitment from the blood is blocked with anti-VCAM-1 blocking antibodies. In contrast, lymphocytes, monocytes, and neutrophils migrate on ICAM-1 or PECAM-1. After the leukocytes undergo transendothelial migration, the leukocytes migrate through the tissue, across the epithelium and into the airway spaces. Chemokines in the tissue direct the leukocyte migration. OVA, chicken egg ovalbumin; PECAM-1, platelet-endothelial cell adhesion molecule-1.

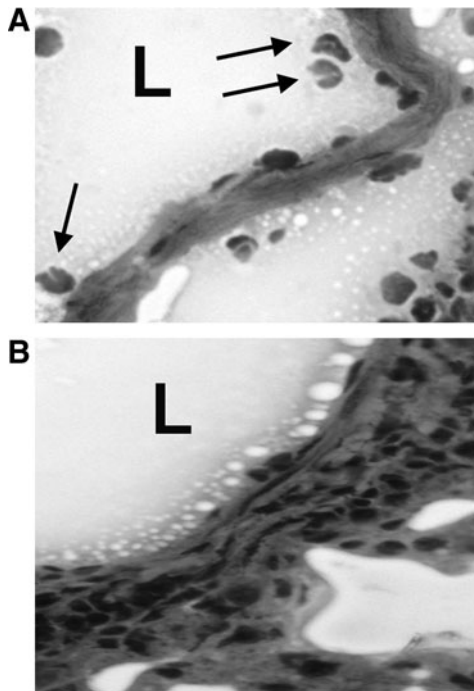


FIG. 9. VCAM-1-dependent eosinophil transendothelial migration in the lung is blocked in mice deficient in non-hematopoietic NOX2. Adapted from ref. (2). CYBB mice that lack NOX2 activity were irradiated and received a bone marrow transplant with wild-type bone marrow. Thus, the leukocytes expressed wild-type NOX2, but the non-hematopoietic cells, including endothelial cells, were NOX2 deficient. Control wild-type mice received wild-type bone marrow transplants. The mice were sensitized with OVA/alum intraperitoneally and challenged intranasally with OVA in saline. Lung tissue sections were collected and stained with hematoxylin and eosin. **(A)** Representative lung tissue section from OVA-challenged chimeric CYBB mice. Arrows indicate an accumulation of eosinophils bound to the luminal surface of the endothelium. **(B)** Representative lung tissue section from OVA-challenged chimeric wild-type control mice. Leukocytes are in the tissue and do not accumulate on the endothelium. L, vessel lumen.

has been reported that bilirubin blocks VCAM-1-dependent leukocyte migration *in vivo*. This was examined in a model of VCAM-1-dependent leukocyte infiltration into the lung in response to the antigen OVA. In studies examining bilirubin regulation of recruitment of eosinophils during allergic inflammation, mice were sensitized by intraperitoneal injection of OVA in alum and then challenged by intranasal inhalation of OVA (140). At the time of intranasal OVA challenge, mice also received either intraperitoneal injections of bilirubin at upper physiological concentrations or vehicle control (140). The treatment with bilirubin inhibits eosinophil infiltration into the bronchoalveolar lavage by >90% and inhibits lymphocyte infiltration by 60% (140). The migration of eosinophils into the tissue is also reduced by 90% as determined by immunohistochemistry for the eosinophil granule component, major basic protein (140). The reduction in eosinophil and lymphocyte infiltration is consistent with the VCAM-1 dependence of eosinophil migration and the partial VCAM-1 dependence of lymphocyte migration in this lung response to

OVA (57, 107, 250). As anticipated, there is no effect of bilirubin administration on the OVA-induced infiltration of monocytes or neutrophils (140), which is independent of binding to VCAM-1 in this model of allergic inflammation. Although there is reduced eosinophilia with the administration of bilirubin, there are sufficient numbers of eosinophils available for migration as there is not a reduction in blood eosinophils in the bilirubin-treated group compared to the nontreated group (140). In fact, there is a threefold increase in blood eosinophil numbers with bilirubin administration, which is consistent with inhibition of blood eosinophils transendothelial migration (140). VCAM-1 is available for eosinophil binding to the endothelium since bilirubin treatment does not alter the induction of endothelial VCAM-1 expression in OVA-treated mice (140). It is also reported that other antioxidants such as vitamin E also do not alter VCAM-1 expression but do block VCAM-1-dependent leukocyte recruitment (30). Therefore, although ROS can induce VCAM-1 expression, the lack of antioxidant effect on VCAM-1 expression is consistent with compensatory mechanisms for induction of VCAM-1 expression by the many pro-inflammatory mediators that induce VCAM-1 expression.

The infiltration of eosinophils in response to OVA is regulated by cytokines and chemokines. However, bilirubin treatment does not alter the OVA-induced increase in Th2 cytokines (IL-4, IL-5, IL-6, or IL-10) in lung lavage fluid or in OVA-restimulated draining lymph node cells (140). Since IL-5 was not altered, this suggests that IL-5 was sufficient for bone marrow recruitment of eosinophils. In addition, bilirubin does not increase expression of Th1 cytokines (IL-2, IL-12, interferon γ , or TNF α), which are not expected to be upregulated by OVA stimulation (140). Bilirubin also does not alter the OVA-induced increase in the chemokines monocyte chemoattractant protein-1 or eotaxin (140). Thus, in this report, VCAM-1-dependent eosinophil and lymphocyte infiltration into the lung is reduced by the antioxidant bilirubin without altering the expression of the VCAM-1, cytokines, or chemokines that regulate eosinophil infiltration in response to OVA. These data are consistent with bilirubin scavenging of endothelial-cell derived ROS generated during VCAM-1 signaling. Moreover, it is reported that *in vitro*, bilirubin blocks VCAM-1 activation of MMPs in endothelial cells and blocks VCAM-1-dependent leukocyte transendothelial migration (140). Therefore, bilirubin, which blocks VCAM-1 signals through ROS *in vitro*, also inhibits VCAM-1-dependent eosinophilia in allergic responses in mice.

Bilirubin may also regulate VCAM-1-dependent inflammation in cardiovascular disease. It has been reported, in a patient population in China, that low bilirubin associates with increased cardiovascular disease risk factors, including older age, higher body mass and systolic blood pressure, increased glycated hemoglobin, fasting and 2 h insulin, triglyceride, very-low-density lipoprotein, apolipoprotein B concentrations, and lower high-density lipoprotein concentrations (149). They suggest in their report that abnormal intermediate bilirubin metabolism and antioxidant deficiency may be linking factors in cardiovascular disease (149). In another report on a prospective clinical study in Korea, low serum bilirubin is an independent predictor of stroke incidence, suggesting that bilirubin may have a protective function against stroke risk (145). Thus, bilirubin has a regulatory function in limiting leukocyte recruitment during inflamma-

tory diseases, since reports indicate that bilirubin protects against cardiovascular disease and asthmatic inflammation, that inflammation in these diseases is dependent on VCAM-1, and that bilirubin blocks VCAM-1-dependent inflammation by blocking VCAM-1 signaling.

B. Vitamin E regulation of VCAM-1-dependent inflammation

1. Introduction to vitamin E isoforms. Vitamin E is commonly used as an antioxidant to try to limit oxidative damage and inflammatory disease. Vitamin E has been used in diseases that involve VCAM-1-mediated leukocyte recruitment such as asthma, arthritis, and atherosclerosis. However, there are contradictory outcomes for vitamin E in clinical studies of asthma and atherosclerosis. In addition, there are contradictory outcomes for vitamin E supplementation in animal models of inflammation. These clinical and experimental studies have focused on analysis of one form of vitamin E, α -tocopherol, even though multiple forms of vitamin E are present in the studies. Our recent report on novel properties of tocopherol isoforms suggests that the reported contradictory outcomes of the previous studies are consistent with new expectations for the combination of isoforms of vitamin E that were present in these reported studies.

Vitamin E is an antioxidant lipid vitamin that consists of multiple natural and synthetic forms. The natural forms of vitamin E include α -tocopherol, β -tocopherol, γ -tocopherol, and δ -tocopherol as well as the tocotrienol forms of each of these. The α -tocopherol and γ -tocopherol isoforms (Fig. 10) are the most abundant in diets, supplements, and tissues. However, the α -tocopherol isoform in tissues is about 10-fold higher than γ -tocopherol since there is preferential transfer of the α -tocopherol isoform of vitamin E to lipid particles by liver α -tocopherol transfer protein (312). At equal molar concentrations, the α -tocopherol and γ -tocopherol isoforms have relatively similar capacity to scavenge ROS during lipid oxidation (18, 323). Thus, *in vivo*, there is likely more ROS scavenging by α -tocopherol than γ -tocopherol because it is at a 10-fold higher concentration in the tissues. However, γ -tocopherol, in contrast to α -tocopherol, also reacts with reactive nitrogen species such as peroxynitrite forming 5-nitro- γ -tocopherol (60, 311). When tocopherols are oxidized, they are recycled by reduction by vitamin C (43, 109, 122). Importantly, besides the antioxidant capacity of the tocopherols, it has been reported that tocopherols also have non-antioxidant functions (19, 30, 327).

2. Vitamin E isoforms regulate VCAM-1-dependent leukocyte transmigration through antioxidant and non-antioxidant mechanisms. *In vitro*, α -tocopherol blocks, whereas γ -tocopherol elevates, VCAM-1-dependent leukocyte transmigration at physiological concentrations (30, 322). Moreover, treatment with γ -tocopherol ablates the inhibition by α -tocopherol such that the leukocyte transmigration is the same as the vehicle-treated control (30). Interestingly, this occurs at physiological concentrations. Thus, γ -tocopherol ablates the effects of α -tocopherol even though it is at a concentration that is 1/10 that of α -tocopherol (30). These regulatory functions of the tocopherols on leukocyte transmigration are through a direct effect of the tocopherols on endothelial cells because pretreatment of the endothelial

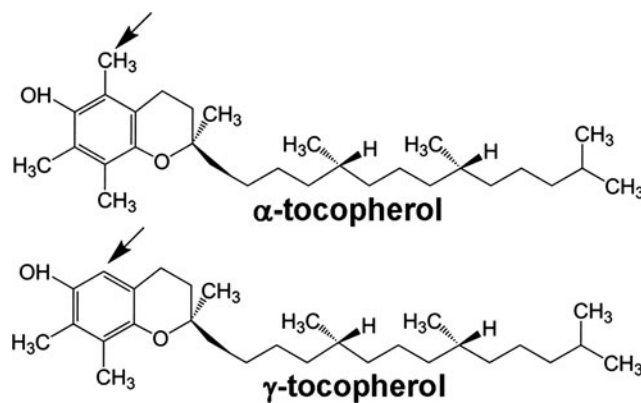


FIG. 10. Alpha and gamma-tocopherol. Tocopherols are lipids. α -Tocopherol differs from γ -tocopherol by one methyl group (arrows).

cells with α -tocopherol or γ -tocopherol overnight inhibits or elevates, respectively, leukocyte transmigration (30). In contrast, pretreatment of the leukocytes with physiological concentrations tocopherols has no effect on VCAM-1-dependent leukocyte transmigration (30). The γ -tocopherol elevation of transmigration is VCAM-1 dependent since anti-VCAM-1 blocking antibodies inhibit the leukocyte transmigration (30). The tocopherols do not modulate leukocyte-endothelial cell binding, because there is no effect of the tocopherols on VCAM-1-dependent adhesion of the leukocytes to the endothelium when either the endothelial cells or the leukocytes are pretreated with tocopherols (30). The tocopherols modulate endothelial function during VCAM-1-dependent transmigration by altering VCAM-1-induced oxidative activation of endothelial cell PKC α (Fig. 11) (30). Specifically, the VCAM-1-induced activation of PKC α is inhibited by α -tocopherol and the effect of α -tocopherol is ablated by γ -tocopherol.

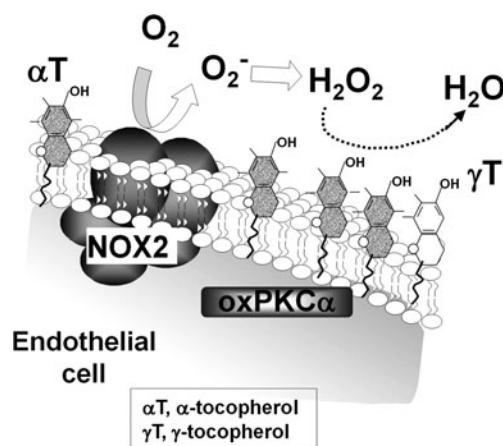


FIG. 11. Tocopherol regulation of VCAM-1-induced ROS. Tocopherols are lipids in the plasma membrane. The tocopherol head group is external to the membrane and is thus poised for scavenging of extracellular ROS. Tocopherols are also found in membranes of organelles and can scavenge intracellular ROS. There is ~10-fold more α -tocopherol than γ -tocopherol in membranes *in vivo*.

Therefore, the tocopherols have opposing regulatory functions on VCAM-1 signaling during leukocyte transmigration *in vitro*.

3. Vitamin E isoform-specific regulation of leukocyte recruitment *in vivo*. *In vivo*, α -tocopherol and γ -tocopherol also have opposing regulatory functions on leukocyte accumulation during VCAM-1-dependent allergic lung inflammation (30). The studies in this report focused on supplementation with tocopherols after OVA antigen sensitization to determine whether tocopherols modulate the OVA antigen challenge phase. This is important because patients are already sensitized. Supplementation with the tocopherols after OVA sensitization, such that the tissue tocopherols are raised 5–7-fold higher than mice consuming control rodent chow, does not affect body weight or lung weight (30, 191). Consistent with the *in vitro* studies with tocopherol regulation of leukocyte migration, d- γ -tocopherol elevates leukocyte accumulation in the bronchoalveolar lavage and lung tissue in response to OVA challenge. In contrast, d- α -tocopherol inhibits this inflammation. However, d- γ -tocopherol, at as little as 10% the concentration of d- α -tocopherol, ablates the anti-inflammatory benefit of the d- α -tocopherol isoform *in vivo* in response to OVA. Further, the levels of tocopherols in this study do not alter the blood eosinophil numbers, indicating that eosinophils were available for recruitment. It is also reported by Okamoto *et al.* (213) that mice fed with α -tocopherol starting 2 weeks before sensitization with OVA had reduced number of eosinophils in the bronchoalveolar lavage, even though the form or purity of α -tocopherol was not indicated. Therefore, α -tocopherol and γ -tocopherol have opposing functions *in vivo*.

The opposing functions of purified d- α -tocopherol or d- γ -tocopherol *in vivo* are not through modulation of expression of several cytokines, chemokines, prostaglandin E₂, or adhesion molecules that regulate leukocyte recruitment since these are not altered with tocopherol supplementation (30). The tocopherol modulation of leukocyte infiltration in allergic inflammation, without alteration of adhesion molecules, cytokines or chemokines, is similar to several previous reports of *in vivo* inhibition of intracellular signals in endothelial cells without alteration of expression of these immune modulators of leukocyte recruitment (2, 140, 225). Therefore, the tocopherol regulatory function in allergic responses is, at least in part, by regulation of endothelial cell VCAM-1 activation of PKC α and leukocyte transendothelial migration. Moreover, natural d- α -tocopherol and natural d- γ -tocopherol differ in structure by only one methyl group (Fig. 10) but at physiological concentrations have opposing regulatory functions in endothelial cells that modulate inflammation. The opposing functions of tocopherol isoforms have important implications for the interpretation of clinical reports and animal studies of vitamin E regulation of inflammation.

C. Reinterpretation of reports on vitamin E regulation of inflammation in experimental models

Our data on tocopherol isoform regulation of inflammation alter interpretations of animal studies with tocopherol modulation of VCAM-1-dependent inflammation. Many reports with animal studies indicate that vitamin E was administered to animals, but the form, source, and purity of tocopherols are often not reported. In addition, the tissue levels of tocopherol

isoforms after administration are sometimes not determined. Further, since tocopherols are lipids, there needs to be consideration for tocopherol isoforms that are present in the oils in animal and human diets or in the oil vehicles used for delivery of the tocopherols. We and others have determined the levels of α -tocopherol and γ -tocopherol in dietary oils (Fig. 12) (30, 130, 304). In rodent studies, rodent chow contains α -tocopherol but low to no γ -tocopherol. However, in some reports for allergic inflammation, α -tocopherol is administered in oil vehicles that contain other tocopherol isoforms. In a report by Suchankova *et al.* (275), purified α -tocopherol was administered in soy oil by gavage and they found no major effect of α -tocopherol on immune parameters or lung airway responsiveness in mice challenged with OVA. However, the soy oil vehicle used in this study contains an abundance of γ -tocopherol (Fig. 12) and they did not measure tissue tocopherol levels or vehicle tocopherol levels. Our interpretation of this study is that γ -tocopherol in the soy oil antagonized the function of the α -tocopherol that was administered. In another report, γ -tocopherol in tocopherol-stripped corn oil was administered daily by gavage to rats 2 weeks after one OVA sensitization and then the rats received two OVA challenges (302). In this report, there was a reduced number of eosinophils and lymphocytes in the bronchoalveolar lavage of the γ -tocopherol-treated mice after OVA challenge (302). However, the purity of the γ -tocopherol in the corn oil vehicle was not reported. Further, the leukocyte infiltration in the OVA response in these rats was predominantly neutrophils rather than the expected predominant eosinophil infiltration (302).

Tocopherols have also been used to scavenge ROS after ozone challenge to the lung. In a study examining γ -tocopherol modulation of ozone exposure after OVA challenge, control rats, which did not receive ozone but received γ -tocopherol for 4 days beginning after the last OVA challenge, had reduced lung eosinophils at day 4 after OVA challenge (303). However,

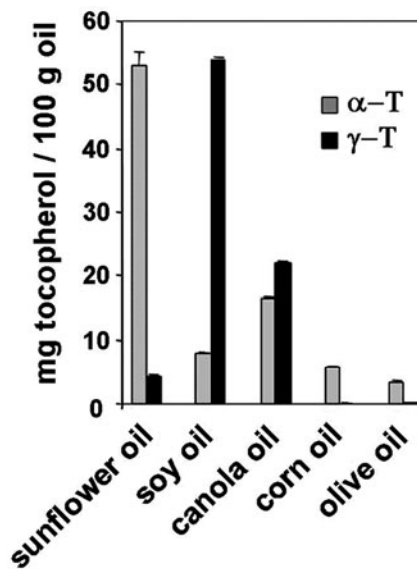


FIG. 12. α -tocopherol and γ -tocopherol in dietary oils. Adapted from ref. (30). Tocopherols were extracted from dietary oils and measured by high pressure liquid chromatography with an electrochemical detector.

since it takes a few days to raise tissue tocopherol levels, which, in this protocol, is after the peak of eosinophil infiltration, the effect on eosinophils at 4 days after the last OVA challenge was during the resolution phase of eosinophil inflammation. It has also been reported that mice deficient in liver α -tocopherol transfer protein exhibit severe deficiency in tissue α - and γ -tocopherol as well as reduced IgE and reduced IL-5 after OVA challenge to the lung (172). In these mice, it is not known whether severe tocopherol deficiency during mouse development alters leukocyte hematopoiesis or leukocyte responsiveness. The differences among the reports of tocopherol regulation of responses to OVA for VCAM-1-dependent eosinophil recruitment likely reflect differences in the forms of tocopherols, tocopherol concentrations, and time of administration of tocopherols in these studies.

Reports also conflict as to whether the antioxidant tocopherols modulate mediators of leukocyte recruitment during inflammation, including prostaglandins, cytokines, chemokines, and adhesion molecules (81, 86, 101, 128, 129, 135, 150, 164, 203, 213, 228, 251, 252, 296, 310, 314–316, 325). With regard to adhesion molecules, *in vitro*, α -tocopherol is reported to block IL-1 β -induced ICAM-1 expression on human aortic endothelial cells but not on human umbilical vein endothelial cells, and then, in another report, α -tocopherol does not inhibit TNF- α -stimulated ICAM-1 expression on human umbilical vein endothelial cells (314, 325). oxLDL or 25-hydroxycholesterol-induced VCAM-1 expression on human aortic endothelial cell is blocked by 200 μ M α -tocopherol or 10 μ M tocotrienols *in vitro* (208, 322). *In vivo*, we reported that purified natural d- α -tocopherol and d- γ -tocopherol at physiological levels do not alter OVA-induced VCAM-1 expression on lung venules (30). We suggest that variations in reports on outcomes of tocopherol treatments *in vitro* and *in vivo* result, at least in part, from differences in isoforms and purity of tocopherols, in concentrations of the tocopherols within different cells, and in experimental systems. This is important considering our report indicating that forms of tocopherols have cell-type-specific opposing regulatory functions on leukocyte recruitment since tocopherols directly affected endothelial cells but not leukocytes during leukocyte transmigration.

VIII. Clinical Implications for Vitamin E Regulation of Inflammation Involving VCAM-1

Reports of clinical studies on vitamin E primarily focus on the α -tocopherol isoform without adjustment for the dietary contribution of γ -tocopherol to the outcomes of these studies. For interpretation of the clinical studies, it is especially important to take into consideration the dietary contribution of tocopherols because γ -tocopherol is more abundant in western diets. The average plasma concentration of α -tocopherol is the same among many countries (304). However, the American diet is rich in γ -tocopherol found in soy oil, the major form of vegetable oil in the United States. In contrast, γ -tocopherol is low in other oils (sunflower and olive oil) commonly used in some of the European countries (Fig. 12) (30, 130, 304). Consistent with this, in the United States and the Netherlands, the average plasma γ -tocopherol level is 2–6 times higher than that reported for six European countries, Japan, and China (Table 4) (304). This fold increase in plasma γ -tocopherol is similar to fold increase in plasma γ -tocopherol in the rodent studies in which γ -tocopherol opposed the regulatory func-

TABLE 4. HUMAN PLASMA TOCOPHEROL

Human plasma	γ T (μ M)	α T (μ M)	Reference
United States (four reports)	2.5	22	130
	5.4	22	32
	5.2	27	254
	7	20	67
Netherlands	2.3	25	261
France	1.2	26	214
Italy	1.2	24	220
Austria	1.4	21	283
Ireland	1.8	26	214
Spain (two reports)	1.7	27	214
	1.7	27	248
Lithuania	1.6	22	155
China (three reports)	1.4	19	279
	2.4	19	240
		22	156
Japan (two reports)	1.7	23	226
	2.0	23	96

tions of α -tocopherol, even at 1/10 the concentration of α -tocopherol (30).

A consistent feature of inflammation in allergic asthma is the recruitment of eosinophils and mast cells. The recruitment of these cells is regulated by VCAM-1 and tocopherols (4, 5, 8, 30, 36, 57, 107, 108, 250). In clinical studies of asthma, it is reported that α -tocopherol supplementation of asthmatic patients is beneficial in Italy and Finland, but disappointingly α -tocopherol is not beneficial for asthmatic patients in studies in the United States or the Netherlands (78, 266, 277, 284, 308). These clinical outcomes are consistent with an interpretation that there is little benefit of α -tocopherol for inflammation in the presence of elevated plasma γ -tocopherol because γ -tocopherol is elevated 2–6-fold in people in the United States and the Netherlands (Table 4). Therefore, differences in outcome of the clinical reports on vitamin E modulation of asthma in European countries and the United States may, in part, reflect the opposing regulatory functions of α - and γ -tocopherol forms of vitamin E consumed in diets and supplements. Although there are many other differences regarding the environment and genetics of the people in these countries and it is acknowledged that other dietary factors, including unsaturated fatty acids may modulate asthma (11, 126, 148, 189, 194, 266), the clinical data are consistent with the animal studies demonstrating opposing functions of the tocopherol isoforms on leukocyte recruitment (30).

It has also been suggested that changes in environmental factors, including vitamin E consumption, may contribute to the increased incidence of asthma. The incidence of asthma in several countries, including the United States and the Netherlands, has dramatically increased in the last 40 years (95, 292, 300). It is thought that there must be environmental factors contributing to this increase since it is too rapid for genetic changes. The prevalence of asthma is higher in the United States than in Western Europe, Mediterranean countries, Japan, and China (6, 35, 158, 178). The World Health Organization has reported that the prevalence of asthma from 1950 to the present has increased in many countries, including countries with high rates of asthma, intermediate rates of asthma, or low rates of asthma (35). The increases in prevalence occur as countries assume Western

lifestyles (35). The dietary changes in the United States in the last 40 years with increased consumption of γ -tocopherol in vegetable oil may, in part, be a contributing factor to changes in asthma prevalence. In addition, in a Scottish cohort, it is reported that reduced maternal intake of vitamin E (likely referring to α -tocopherol) is associated with increased asthma and wheezing in children up to 5 years old (75). Then, in this same report, it was discussed that from 1967 to 2004, there was a significant increase in vegetable oil intake by Scottish (75), which we interpret as indicative of an increase in dietary γ -tocopherol since vegetable oil is rich in γ -tocopherol (Fig. 12). Therefore, since α -tocopherol levels are low in asthmatics (136, 139, 257, 264) and since α -tocopherol can reduce inflammation, an increase in α -tocopherol in the presence of low γ -tocopherol may be necessary to promote optimal health in asthmatics in combination with other regimens to treat inflammation.

The tocopherol isoform levels may also affect the inflammation in other diseases that involve VCAM-1 such as osteoarthritis and atherosclerosis (125, 171, 173, 207, 256). Although there are conflicting reports for tocopherol regulation in these diseases, we suggest that this may result from opposing functions of tocopherol isoforms present in the subjects in these studies. It has been reported that plasma γ -tocopherol is positively associated with osteoarthritis, whereas plasma α -tocopherol is negatively associated with osteoarthritis (134). In contrast, in another report on knee osteoarthritis, vitamin E supplementation (α -tocopherol) did not relieve symptoms but they did not measure α -tocopherol or γ -tocopherol levels (37). With regard to coronary heart disease and stroke, the benefits of tocopherols are also inconsistent among the studies (76, 265); further, measurements of levels of both α -tocopherol and γ -tocopherol are commonly not reported (76, 80, 190, 202, 203, 265). Studies of tocopherols and heart disease are complex since different dietary oils contain not only different forms of tocopherols but also different lipids that affect heart disease. It has been reported that plasma γ -tocopherol levels are not associated with heart disease or in other reports are associated with an increase in relative risk for myocardial infarction [reviewed in (76)]. In contrast, α -tocopherol intake is either not associated with heart disease or, in other reports, is associated with reduced death from heart disease (80, 190, 202, 265). Therefore, although the clinical reports on vitamin E association with heart disease are inconsistent, for those reports with an effect on heart disease, γ -tocopherol is associated with an increase, whereas α -tocopherol is associated with a decrease in parameters of heart disease. Similar to the antioxidant protective function for α -tocopherol on cardiovascular function, the safflower seed-derived antioxidants *N*-(*p*-coumaroyl)serotonin and *N*-feruloylserotonin reduce parameters of cardiovascular disease, including high-glucose-induced VCAM-1 expression and monocyte adhesion, arterial stiffness in hypercholesterolemic rabbits, atherosclerotic lesions in apolipoprotein-deficient mice, and cardiovascular risk factors (sVCAM-1 and oxLDL) in patients (137, 153, 154, 229). In summary, the opposing functions of α -tocopherol and γ -tocopherol in animal models (30) are consistent with the different outcomes for the clinical studies of tocopherols in heart disease. Future clinical studies of vitamin E regulation of inflammatory diseases should include a systematic design to examine opposing functions of the isoforms of vitamin E

on inflammation, leukocyte recruitment, and disease parameters.

IX. Concluding Remarks

During several inflammatory diseases, VCAM-1 expression is induced on peripheral tissue endothelium by several mediators, including cytokines or turbulent shear stress, that signal through ROS. Once VCAM-1 is expressed, it is a scaffold on which leukocytes migrate but VCAM-1 also activates signals in endothelial cells that are required for VCAM-1-dependent leukocyte migration (1, 2, 62, 63, 72). Thus, the endothelium plays an active role in the regulation of VCAM-1-dependent leukocyte migration. Crosslinking of VCAM-1 activates calcium fluxes and Rac-1, which then activates endothelial cell NOX2 (a form of NADPH oxidase). Nox2 catalyzes the production of superoxide from oxygen using the cofactor NADPH. The superoxide dismutates to H_2O_2 . Moreover, VCAM-1 induces the production of only $1\ \mu M$ H_2O_2 (65, 73, 184), which is a 100–1000-fold lower concentration of ROS than the concentration of ROS produced by leukocytes or that induce oxidative vascular damage. The VCAM-1-induced H_2O_2 diffuses rapidly activating endothelial cell-associated MMPs and a delayed activation of lymphocyte-associated MMPs. The endothelial-associated MMPs but not leukocyte-associated MMPs are required for VCAM-1-dependent transendothelial migration. These endothelial cell-associated MMPs degrade matrix and endothelial cell surface receptors in cell junctions (9, 260). In addition to the MMP extracellular targets of oxidation, H_2O_2 diffuses through membranes at $100\ \mu m/s$ (183) to oxidize and transiently activate endothelial cell PKC α (1). PKC α then phosphorylates and activates endothelial cell PTP1B (1, 72). As PTP1B is not oxidized, it indicates specificity for ROS targets during VCAM-1 signaling. The rapid activation of VCAM-1 signals is consistent with the local rapid process of leukocyte transendothelial migration. Further, these signals through ROS, MMPs, PKC α , and PTP1B are required for VCAM-1-dependent leukocyte transendothelial migration *in vitro* (1, 57, 72, 73). Both the MMPs and the PKC α /PTP1B pathways contribute to the VCAM-1-dependent leukocyte transendothelial migration, because inhibition of both pathways exhibits a greater inhibitory effect on migration than inhibition of either pathway alone (72). Since VCAM-1 is located on both the apical and lateral surface of endothelial cells, VCAM-1 may continue to provide localized endothelial cell signals as leukocytes migrate through the lateral junctions. The VCAM-1 signaling through NOX2 in endothelial cells also has a role in leukocyte recruitment *in vivo* since the nonhematopoietic knockout of NOX2 blocks VCAM-1-dependent eosinophil recruitment in allergic lung inflammation with accumulation of eosinophil binding to the luminal surface of venules (2, 30, 140). VCAM-1-dependent leukocyte recruitment is also regulated *in vivo* by the antioxidants bilirubin and vitamin E isoforms. Moreover, isoforms of vitamin E have novel opposing regulatory functions during VCAM-1-mediated leukocyte recruitment *in vivo* and *in vitro*. These opposing regulatory functions of isoforms of vitamin E are consistent with the seemingly disparate outcomes of vitamin E in clinical studies and animal studies of inflammation. The differential tocopherol isoform regulation of inflammation provides a basis toward designing drugs

and diets that more effectively modulate these pathways and improve health. Moreover, VCAM-1 signals provide targets for designing approaches to modulate VCAM-1-dependent processes during inflammatory disease.

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References

1. Abdala-Valencia H and Cook-Mills JM. VCAM-1 signals activate endothelial cell protein kinase C α via oxidation. *J Immunol* 177: 6379–6387, 2006.
2. Abdala-Valencia H, Earwood J, Bansal S, Jansen M, Babcock G, Garvy B, Wills-Karp M, and Cook-Mills JM. Non-hematopoietic NADPH oxidase regulation of lung eosinophilia and airway hyperresponsiveness in experimentally induced asthma. *Am J Physiol Lung Cell Mol Physiol* 292: L1111–L1125, 2007.
3. Abecassis I, Olofsson B, Schmid M, Zalzman G, and Karniguian A. RhoA induces MMP-9 expression at CD44 lamellipodial focal complexes and promotes HMEC-1 cell invasion. *Exp Cell Res* 291: 363–376, 2003.
4. Abonia JP, Austen KF, Rollins BJ, Joshi SK, Flavell RA, Kuziel WA, Koni PA, and Gurish MF. Constitutive homing of mast cell progenitors to the intestine depends on autologous expression of the chemokine receptor CXCR2. *Blood* 105: 4308–4313, 2005.
5. Abonia JP, Hallgren J, Jones T, Shi T, Xu Y, Koni P, Flavell RA, Boyce JA, Austen KF, and Gurish MF. Alpha-4 integrins and VCAM-1, but not MAdCAM-1, are essential for recruitment of mast cell progenitors to the inflamed lung. *Blood* 108: 1588–1594, 2006.
6. Ait-Khaled N, Enarson D, and Bousquet J. Chronic respiratory diseases in developing countries: the burden and strategies for prevention and management. *Bull World Health Organ* 79: 971–979, 2001.
7. Al-Mehdi AB, Zhao G, Dodia C, Tozawa K, Costa K, Muzykantov V, Ross C, Blecha F, Dinauer M, and Fisher AB. Endothelial NADPH oxidase as the source of oxidants in lungs exposed to ischemia or high K $^{+}$. *Circ Res* 83: 730–737, 1998.
8. Alcaide P, Jones TG, Lord GM, Glimcher LH, Hallgren J, Arinobu Y, Akashi K, Paterson AM, Gurish MA, and Luscinskas FW. Dendritic cell expression of the transcription factor T-bet regulates mast cell progenitor homing to mucosal tissue. *J Exp Med* 204: 431–439, 2007.
9. Alexander JS and Elrod JW. Extracellular matrix, junctional integrity and matrix metalloproteinase interactions in endothelial permeability regulation. *J Anat* 200: 561–574, 2002.
10. Allavena R, Noy S, Andrews M, and Pullen N. CNS elevation of vascular and not mucosal addressin cell adhesion molecules in patients with multiple sclerosis. *Am J Pathol* 176: 556–562, 2010.
11. Almqvist C, Garden F, Xuan W, Mirhshahi S, Leeder SR, Oddy W, Webb K, and Marks GB. Omega-3 and omega-6 fatty acid exposure from early life does not affect atopy and asthma at age 5 years. *J Allergy Clin Immunol* 119: 1438–1444, 2007.
12. Alon R and Dustin ML. Force as a facilitator of integrin conformational changes during leukocyte arrest on blood vessels and antigen-presenting cells. *Immunity* 26: 17–27, 2007.
13. Alon R and Feigelson S. From rolling to arrest on blood vessels: leukocyte tap dancing on endothelial integrin ligands and chemokines at sub-second contacts. *Sem Immunol* 14: 93–104, 2002.
14. Alon R, Grabovsky V, Feigelson S. Chemokine induction of integrin adhesiveness on rolling and arrested leukocytes local signaling events or global stepwise activation? *Microcirculation* 10: 297–311, 2003.
15. Alon R, Kassner PD, Carr MW, Finger EB, Hemler ME, and Springer TA. The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J Cell Biol* 128: 1243–1253, 1995.
16. Anderie I, Schulz I, and Schmid A. Direct interaction between ER membrane-bound PTP1B and its plasma membrane-anchored targets. *Cell Signal* 19: 582–592, 2007.
17. Arora S, Gunther A, Wennerblom B, Ueland T, Andreassen AK, Gude E, Endresen K, Geiran O, Wilhelmsen N, Andersen R, et al. Systemic markers of inflammation are associated with cardiac allograft vasculopathy and an increased intimal inflammatory component. *Am J Transplant* 10: 1428–1436, 2010.
18. Atkinson J, Epand RF, and Epand RM. Tocopherols and tocotrienols in membranes: a critical review. *Free Radic Biol Med* 44: 739–764, 2008.
19. Azzi A and Stocker A. Vitamin E: non-antioxidant roles. *Prog Lipid Res* 39: 231–255, 2000.
20. Ballinger SW, Patterson C, Yan CN, Doan R, Burow DL, Young CG, Yakes FM, Van Houten B, Ballinger CA, Freeman BA, et al. Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. *Circ Res* 86: 960–966, 2000.
21. Balogh P, Aydar Y, Tew JG, and Szakal AK. Appearance and phenotype of murine follicular dendritic cells expressing VCAM-1. *Anat Rec* 268: 160–168, 2002.
22. Banning A, Schnurr K, Bol GF, Kupper D, Muller-Schmehl K, Viita H, Yla-Herttuala S, and Brigelius-Flohe R. Inhibition of basal and interleukin-1-induced VCAM-1 expression by phospholipid hydroperoxide glutathione peroxidase and 15-lipoxygenase in rabbit aortic smooth muscle cells. *Free Radic Biol Med* 36: 135–144, 2004.
23. Baron JL, Madri JA, Ruddle NH, Hashim G, and Janeway CA Jr. Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. *J Exp Med* 177: 57–68, 1993.
24. Barreiro O, Yanez-Mo M, Sala-Valdes M, Gutierrez-Lopez MD, Ovalle S, Higginbottom A, Monk PN, Cabanas C, and Sanchez-Madrid F. Endothelial tetraspanin microdomains regulate leukocyte firm adhesion during extravasation. *Blood* 105: 2852–2861, 2005.
25. Barreiro O, Yanez-Mo M, Serrador JM, Montoya MC, Vicente-Manzanares M, Tejedor R, Furthmayr H, and Sanchez-Madrid F. Dynamic interaction of VCAM-1 and ICAM-1 with moesin and ezrin in a novel endothelial docking structure for adherent leukocytes. *J Cell Biol* 157: 1233–1245, 2002.
26. Barreiro O, Zamai M, Yanez-Mo M, Tejera E, Lopez-Romero P, Monk PN, Gratton E, Caiolfa VR, and Sanchez-Madrid F. Endothelial adhesion receptors are recruited to adherent leukocytes by inclusion in preformed tetraspanin nanoplateforms. *J Cell Biol* 183: 527–542, 2008.
27. Barringhaus KG, Phillips JW, Thatte JS, Sanders JM, Czarnik AC, Bennett DK, Ley KF, and Sarembock IJ. Alpha4-beta1 integrin (VLA-4) blockade attenuates both early and late leukocyte recruitment and neointimal growth

- following carotid injury in apolipoprotein E (-/-) mice. *J Vasc Res* 41: 252–260, 2004.
28. Basuroy S, Seth A, Elias B, Naren AP, and Rao R. MAPK interacts with occludin and mediates EGF-induced prevention of tight junction disruption by hydrogen peroxide. *Biochem J* 393: 69–77, 2006.
 29. Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, and Kahn BB. Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nat Med* 12: 917–924, 2006.
 30. Berdnikovs S, Abdala-Valencia H, McCary C, Somand M, Cole R, Garcia A, Bryce P, and Cook-Mills J. Isoforms of Vitamin E have Opposing Immunoregulatory Functions during Inflammation by Regulating Leukocyte Recruitment. *J Immunol* 182: 4395–4405, 2009.
 31. Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, Weissman IL, Hamann A, and Butcher EC. Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell* 74: 185, 1993.
 32. Block G, Norkus E, Hudes M, Mandel S, and Helzlsouer K. Which plasma antioxidants are most related to fruit and vegetable consumption? *Am J Epidemiol* 154: 1113–1118, 2001.
 33. Bochner BS, Luscinskas FW, Gimbrone MA Jr., Newman W, Sterbinsky SA, Derse-Anthony CP, Klunk D, and Schleimer RP. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J Exp Med* 173: 1553–1557, 1991.
 34. Boivin D, Bilodeau D, and Beliveau R. Regulation of cytoskeletal functions by Rho small GTP-binding proteins in normal and cancer cells. *Can J Physiol Pharmacol* 74: 801–810, 1996.
 35. Bousquet J, Bousquet PJ, Godard P, and Daures JP. The public health implications of asthma. *Bull World Health Organ* 83: 548–554, 2005.
 36. Boyce JA, Mellor EA, Perkins B, Lim YC, and Luscinskas FW. Human mast cell progenitors use alpha4-integrin, VCAM-1, and PSGL-1 E-selectin for adhesive interactions with human vascular endothelium under flow conditions. *Blood* 99: 2890–2896, 2002.
 37. Brand C, Snaddon J, Bailey M, and Cicutini F. Vitamin E is ineffective for symptomatic relief of knee osteoarthritis: a six month double blind, randomised, placebo controlled study. *Ann Rheum Dis* 60: 946–949, 2001.
 38. Braun M, Pietsch P, Schror K, Baumann G, and Felix SB. Cellular adhesion molecules on vascular smooth muscle cells. *Cardiovasc Res* 41: 395–401, 1999.
 39. Brautigam DL. Great expectations: protein tyrosine phosphatases in cell regulation. *Biochim Biophys Acta* 1114: 63–77, 1992.
 40. Brautigam DL and Pinault FM. Serine phosphorylation of protein tyrosine phosphatase (PTP1B) in HeLa cells in response to analogues of cAMP or diacylglycerol plus okadaic acid. *Mol Cell Biochem* 127–128: 121–129, 1993.
 41. Briscoe DM, Yeung AC, Schoen FJ, Allred EN, Stavrakis G, Ganz P, Cotran RS, Pober JS, and Schoen EL. Predictive value of inducible endothelial cell adhesion molecule expression for acute rejection of human cardiac allografts. *Transplantation* 59: 204–211, 1995.
 42. Brower V. Like a snake in the grass. *EMBO Rep* 5: 555–558, 2004.
 43. Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. *Arch Biochem Biophys* 300: 535–543, 1993.
 44. Campbell DJ, Woodward M, Chalmers JP, Colman SA, Jenkins AJ, Kemp BE, Neal BC, Patel A, and MacMahon SW. Soluble vascular cell adhesion molecule 1 and N-terminal pro-B-type natriuretic peptide in predicting ischemic stroke in patients with cerebrovascular disease. *Arch Neurol* 63: 60–65, 2006.
 45. Campbell JJ, Qin S, Bacon KB, Mackay CR, and Butcher EC. Biology of chemokine and classical chemoattractant receptors: differential requirements for adhesion-triggering versus chemotactic responses in lymphoid cells. *J Cell Biol* 134: 255–266, 1996.
 46. Carluccio MA, Ancora MA, Massaro M, Carluccio M, Scoditti E, Distante A, Storelli C, and De Caterina R. Homocysteine induces VCAM-1 gene expression through NF-kappaB and NAD(P)H oxidase activation: protective role of Mediterranean diet polyphenolic antioxidants. *Am J Physiol Heart Circ Physiol* 293: H2344–H2354, 2007.
 47. Carman CV and Springer TA. A Transmigratory cup in leukocyte diapedesis both through individual vascular endothelial cells and between them. *J Cell Biol* 167: 377–388, 2004.
 48. Carr MW, Alon R, and Springer TA. The C-C chemokine MCP-1 differentially modulates the avidity of beta 1 and beta 2 integrins on T lymphocytes. *Immunity* 4: 179–187, 1996.
 49. Cassie S, Masterson MF, Polukoshko A, Viskovic MM, and Tibbles LA. Ischemia/reperfusion induces the recruitment of leukocytes from whole blood under flow conditions. *Free Radic Biol Med* 36: 1102–1111, 2004.
 50. Cernuda-Morollon E, Gharbi S, and Millan J. Discriminating between the paracellular and transcellular routes of diapedesis. *Methods Mol Biol* 616: 69–82, 2010.
 51. Chan BM, Elices MJ, Murphy E, and Hemler ME. Adhesion to vascular cell adhesion molecule 1 and fibronectin. Comparison of alpha 4 beta 1 (VLA-4) and alpha 4 beta 7 on the human B cell line JY. *J Biol Chem* 267: 8366–8370, 1992.
 52. Chan JR, Hyduk SJ, and Cybulsky MI. Detecting rapid and transient upregulation of leukocyte integrin affinity induced by chemokines and chemoattractants. *J Immunol Methods* 273: 43–52, 2003.
 53. Chen L, Lin SX, Amin S, Overbergh L, Maggiolino G, and Chan LS. VCAM-1 blockade delays disease onset, reduces disease severity and inflammatory cells in an atopic dermatitis model. *Immunol Cell Biol* 88: 334–342, 2010.
 54. Chen XL, Zhang Q, Zhao R, Ding X, Tummala PE, and Medford RM. Rac1 and superoxide are required for the expression of cell adhesion molecules induced by tumor necrosis factor-alpha in endothelial cells. *J Pharmacol Exp Ther* 305: 573–580, 2003.
 55. Cheung AT, Tomic MM, Chen PC, Miguelino E, Li CS, and Devaraj S. Correlation of microvascular abnormalities and endothelial dysfunction in type-1 diabetes mellitus (T1DM): a real-time intravital microscopy study. *Clin Hemorheol Microcirc* 42: 285–295, 2009.
 56. Chiba R, Nakagawa N, Kurasawa K, Tanaka Y, Saito Y, and Iwamoto I. Ligation of CD31 (PECAM-1) on endothelial cells increases adhesive function of alphavbeta3 integrin and enhances beta1 integrin-mediated adhesion of eosinophils to endothelial cells. *Blood* 94: 1319–1329, 1999.
 57. Chin JE, Hatfield CA, Winterrowd GE, Brashler JR, Vonderfecht SL, Fidler SF, Griffin RL, Kolbasa KP, Krzesicki RF, Sly LM, et al. Airway recruitment of leukocytes in mice is dependent on alpha4-integrins and vascular cell adhesion molecule-1. *Am J Physiol* 272: L219–L229, 1997.

58. Chiu HH, Crowe DT, Renz ME, Presta LG, Jones S, Weissman IL, and Fong S. Similar but nonidentical amino acid residues on vascular cell adhesion molecule-1 are involved in the interaction with $\alpha_4\beta_7$ under different activity states. *J Immunol* 155: 5257–5267, 1995.
59. Chiu JJ, Lee PL, Chen CN, Lee CI, Chang SF, Chen LJ, Lien SC, Ko YC, Usami S, and Chien S. Shear stress increases ICAM-1 and decreases VCAM-1 and E-selectin expressions induced by tumor necrosis factor- α in endothelial cells. *Arterioscler Thromb Vasc Biol* 24: 73–79, 2004.
60. Christen S, Woodall AA, Shigenaga MK, Southwell-Keely PT, Duncan MW, and Ames BN. gamma-tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: physiological implications. *Proc Natl Acad Sci U S A* 94: 3217–3222, 1997.
61. Clifford DB, DeLuca A, Simpson DM, Arendt G, Giovannoni G, and Nath A. Natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: lessons from 28 cases. *Lancet Neurol* 9: 438–446, 2010.
62. Cook-Mills JM. Hydrogen peroxide activation of endothelial cell-associated MMPs during VCAM-1-dependent leukocyte migration. *Cell Mol Biol* 52: 8–16, 2006.
63. Cook-Mills JM, and Deem TL. Active endothelial cell function during inflammation. *J Leuk Biol* 77: 487–495, 2005.
64. Cook-Mills JM, Gallagher JS, and Feldbush TL. Isolation and characterization of high endothelial cell lines derived from mouse lymph nodes. *In Vitro Cell Develop Biol* 32: 167–177, 1996.
65. Cook-Mills JM, Johnson JD, Deem TL, Ochi A, Wang L, and Zheng Y. Calcium mobilization and Rac1 activation are required for VCAM-1 (vascular cell adhesion molecule-1) stimulation of NADPH oxidase activity. *Biochem J* 378: 539–547, 2004.
66. Cook-Mills JM, Wirth JJ, and Fraker PJ. Possible roles for zinc in destruction of *Trypanosoma cruzi* by toxic oxygen metabolites produced by mononuclear phagocytes. In: *Antioxidant Nutrients and Immune Function*, edited by Bendich A, Phillips M, and Tengerdy R. New York, NY: Plenum Press, 1990, pp. 111–121.
67. Cooney RV, Custer LJ, Okinaka L, and Franke AA. Effects of dietary sesame seeds on plasma tocopherol levels. *Nutr Cancer* 39: 66–71, 2001.
68. Costanzo A, Moretti F, Burgio VL, Bravi C, Guido F, Leverero M, and Puri PL. Endothelial activation by angiotensin II through NF κ B and p38 pathways: involvement of NF κ B-inducible kinase (NIK), free oxygen radicals, and selective inhibition by aspirin. *J Cell Physiol* 195: 402–410, 2003.
69. Cybulsky MI, Fries JW, Williams AJ, Sultan P, Davis VM, Gimbrone MA Jr., and Collins T. Alternative splicing of human VCAM-1 in activated vascular endothelium. *Am J Pathol* 138: 815–820, 1991.
70. Cybulsky MI, Fries JW, Williams AJ, Sultan P, Eddy R, Byers M, Shows T, Gimbrone MA Jr., and Collins T. Gene structure, chromosomal location, and basis for alternative mRNA splicing of the human VCAM1 gene. *Proc Natl Acad Sci U S A* 88: 7859–7863, 1991.
71. Dadke S and Chernoff J. Protein-tyrosine phosphatase 1B as a potential drug target for obesity. *Curr Drug Targets Immune Endocr Metabol Disord* 3: 299–304, 2003.
72. Deem TL, Abdala-Valencia H, and Cook-Mills JM. VCAM-1 activation of PTP1B in endothelial cells. *J Immunol* 178: 3865–3873, 2007.
73. Deem TL and Cook-Mills JM. Vascular cell adhesion molecule-1 (VCAM-1) activation of endothelial cell matrix metalloproteinases: role of reactive oxygen species. *Blood* 104: 2385–2393, 2004.
74. DeLeo FR and Quinn MT. Assembly of the phagocyte NADPH oxidase: molecular interaction of oxidase proteins. *J Leuk Biol* 60: 677–691, 1996.
75. Devereux G. Early life events in asthma—diet. *Pediatr Pulmonol* 42: 663–673, 2007.
76. Dietrich M, Traber MG, Jacques PF, Cross CE, Hu Y, and Block G. Does gamma-tocopherol play a role in the primary prevention of heart disease and cancer? A review. *J Am Coll Nutr* 25: 292–299, 2006.
77. Dorseuil O, Quinn MT, and Bokoch GM. Dissociation of Rac translocation from p47phox/p67phox movements in human neutrophils by tyrosine kinase inhibitors. *Jf Leuk Biol* 58: 108–113, 1995.
78. Dow L, Tracey M, Villar A, Coggon D, Margetts BM, Campbell MJ, and Holgate ST. Does dietary intake of vitamins C and E influence lung function in older people? *Am J Respir Crit Care Med* 154: 1401–1404, 1996.
79. Dube N and Tremblay ML. Involvement of the small protein tyrosine phosphatases TC-PTP and PTP1B in signal transduction and diseases: from diabetes, obesity to cell cycle, and cancer. *Biochim Biophys Acta* 1754: 108–117, 2005.
80. Dutta A and Dutta SK. Vitamin E and its role in the prevention of atherosclerosis and carcinogenesis: a review. *J Am Coll Nutr* 22: 258–268, 2003.
81. Egger T, Schuligoi R, Wintersperger A, Amann R, Malle E, and Sattler W. Vitamin E (alpha-tocopherol) attenuates cyclo-oxygenase 2 transcription and synthesis in immortalized murine BV-2 microglia. *Biochem J* 370: 459–467, 2003.
82. El Shikh ME, El Sayed RM, Wu Y, Szakal AK, and Tew JG. TLR4 on follicular dendritic cells: an activation pathway that promotes accessory activity. *J Immunol* 179: 4444–4450, 2007.
83. Elangbam CS, Qualls CW Jr., and Dahlgren RR. Cell adhesion molecules—update. *Vet Pathol* 34: 61–73, 1997.
84. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 283: 1544–1548, 1999.
85. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, and Lobb RR. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* 60: 577–584, 1990.
86. Erl W, Weber C, Wardemann C, and Weber PC. alpha-Tocopheryl succinate inhibits monocytic cell adhesion to endothelial cells by suppressing NF-kappa B mobilization. *Am J Physiol* 273: H634–H640, 1997.
87. Esparza J, Vilardell C, Calvo J, Juan M, Vives J, Urbano-Marquez A, Yague J, and Cid MC. Fibronectin upregulates gelatinase B (MMP-9) and induces coordinated expression of gelatinase A (MMP-2) and its activator MT1-MMP (MMP-14) by human T lymphocyte cell lines. A process repressed through RAS/MAP kinase signaling pathways. *Blood* 94: 2754–2766, 1999.
88. Etienne-Manneville S, Manneville JB, Adamson P, Wilbourn B, Greenwood J, and Couraud PO. ICAM-1-coupled cytoskeletal rearrangements and transendothelial lymphocyte migration involve intracellular calcium signaling in brain endothelial cell lines. *J Immunol* 165: 3375–3383, 2000.

89. Fernvik E, Hallden G, Lundahl J, Raud J, Alkner U, van H-HM, and Gronneberg R. Allergen-induced accumulation of eosinophils and lymphocytes in skin chambers is associated with increased levels of interleukin-4 and sVCAM-1. *Allergy* 54: 455–463, 1999.
90. Fialkow L, Chan CK, and Downey GP. Inhibition of CD45 during neutrophil activation. *J Immunol* 158: 5409–5417, 1997.
91. Fialkow L, Chan CK, Rotin D, Grinstein S, and Downey GP. Activation of the mitogen-activated protein kinase signaling pathway in neutrophils. Role of oxidants. *J Biol Chem* 269: 31234–31242, 1994.
92. Fiore E, Fusco C, Romero P, and Stamenkovic I. Matrix metalloproteinase 9 (MMP-9/gelatinase B) proteolytically cleaves ICAM-1 and participates in tumor cell resistance to natural killer cell-mediated cytotoxicity. *Oncogene* 21: 5213–5223, 2002.
93. Fischer S, Wiesnet M, Renz D, and Schaper W. H₂O₂ induces paracellular permeability of porcine brain-derived microvascular endothelial cells by activation of the p44/42 MAP kinase pathway. *Eur J Cell Biol* 84: 687–697, 2005.
94. Forman HJ and Torres M. Reactive oxygen species and cell signaling: respiratory burst in macrophage signaling. *Am J Respir Crit Care Med* 166: S4–S8, 2002.
95. Friebele E. The attack of asthma. *Environ Health Perspect* 104: 22–25, 1996.
96. Fukui K, Ostapenko VV, Abe K, Nishide T, Miyano M, Mune M, Yukawa S, and Nishide I. Changes in plasma alpha and gamma tocopherol levels before and after long-term local hyperthermia in cancer patients. *Free Radic Res* 40: 893–899, 2006.
97. Garton KJ, Gough PJ, Philalay J, Wille PT, Blobel CP, Whitehead RH, Dempsey PJ, and Raines EW. Stimulated shedding of vascular cell adhesion molecule 1 (VCAM-1) is mediated by tumor necrosis factor-alpha-converting enzyme (ADAM 17). *J Biol Chem* 278: 37459–37464, 2003.
98. Gearing AJ, Hemingway I, Pigott R, Hughes J, Rees AJ, and Cashman SJ. Soluble forms of vascular adhesion molecules, E-selectin, ICAM-1, and VCAM-1: pathological significance. *Annal N Y Acad Sci* 667: 324–331, 1992.
99. Gerszten RE, Lusinskas FW, Ding HT, Dichek DA, Stoolman LM, Gimbrone MA Jr., and Rosenzweig A. Adhesion of memory lymphocytes to vascular cell adhesion molecule-1-transduced human vascular endothelial cells under simulated physiological flow conditions *in vitro*. *Circ Res* 79: 1205–1215, 1996.
100. Gibbs RA, Weinstock GM, Metzker ML, Muzny DM, Sodergren EJ, Scherer S, Scott G, Steffen D, Worley KC, Burch PE, et al. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature* 428: 493–521, 2004.
101. Grammas P, Hamdheydari L, Benaksas EJ, Mou S, Pye QN, Wechter WJ, Floyd RA, Stewart C, and Hensley K. Anti-inflammatory effects of tocopherol metabolites. *Biochem Biophys Res Commun* 319: 1047–1052, 2004.
102. Grayson MH, Van der Vieren M, Sterbinsky SA, Michael Gallatin W, Hoffman PA, Staunton DE, and Bochner BS. alpha5beta2 integrin is expressed on human eosinophils and functions as an alternative ligand for vascular cell adhesion molecule 1 (VCAM-1). *J Exp Med* 188: 2187–2191, 1998.
103. Guaiquil V, Swendeman S, Yoshida T, Chavala S, Campochiaro PA, and Blobel CP. ADAM9 is involved in pathological retinal neovascularization. *Mol Cell Biol* 29: 2694–2703, 2009.
104. Gum RJ, Gaede LL, Koterski SL, Heindel M, Clampit JE, Zinker BA, Trevillyan JM, Ulrich RG, Jirousek MR, and Rondinone CM. Reduction of protein tyrosine phosphatase 1B increases insulin-dependent signaling in ob/ob mice. *Diabetes* 52: 21–28, 2003.
105. Gurtner GC, Davis V, Li H, McCoy MJ, Sharpe A, and Cybulsky MI. Targeted disruption of the murine VCAM1 gene: essential role of VCAM-1 in chorioallantoic fusion and placentation. *Genes Dev* 9: 1–14, 1995.
106. Hag AM, Kristoffersen US, Pedersen SF, Gutte H, Lebech AM, and Kjaer A. Regional gene expression of LOX-1, VCAM-1, and ICAM-1 in aorta of HIV-1 transgenic rats. *PLoS ONE* 4: e8170, 2009.
107. Hakugawa J, Bae SJ, Tanaka Y, and Katayama I. The inhibitory effect of anti-adhesion molecule antibodies on eosinophil infiltration in cutaneous late phase response in Balb/c mice sensitized with ovalbumin (OVA). *J Dermatol* 24: 73–79, 1997.
108. Hallgren J, Jones TG, Abonia JP, Xing W, Humbles A, Austen KF, and Gurish MF. Pulmonary CXCR2 regulates VCAM-1 and antigen-induced recruitment of mast cell progenitors. *Proc Natl Acad Sci U S A* 104: 20478–20483, 2007.
109. Halpner AD, Handelman GJ, Harris JM, Belmont CA, and Blumberg JB. Protection by vitamin C of loss of vitamin E in cultured rat hepatocytes. *Arch Biochem Biophys* 359: 305–309, 1998.
110. Hastings NE, Feaver RE, Lee MY, Wamhoff BR, and Blackman BR. Human IL-8 regulates smooth muscle cell VCAM-1 expression in response to endothelial cells exposed to atheroprone flow. *Arterioscler Thromb Vasc Biol* 29: 725–731, 2009.
111. Hecht D and Zick Y. Selective inhibition of protein tyrosine phosphatase activities by H₂O₂ and vanadate *in vitro*. *Biochem Biophys Res Commun* 188: 773–779, 1992.
112. Heinonen KM, Dube N, Bourdeau A, Lapp WS, and Tremblay ML. Protein tyrosine phosphatase 1B negatively regulates macrophage development through CSF-1 signaling. *Proc Natl Acad Sci U S A* 103: 2776–2781, 2006.
113. Hemler ME. Tetraspanin functions and associated microdomains. *Nat Rev Mol Cell Biol* 6: 801–811, 2005.
114. Henderson LM and Chappell JB. Dihydrorhodamine 123: a fluorescent probe for superoxide generation? *Eur J Biochem* 217: 973–980, 1993.
115. Hendriks HR, Korn C, Duijvestijn AM, and Kraal G. Changes in lymphocyte binding and expression of HIS 22 of high endothelial venules in rat lymph nodes after occlusion of afferent lymph flow. *Adv Exp Med Biol* 237: 485–490, 1988.
116. Hermann C, Zeiher AM, and Dimmeler S. Shear stress inhibits H₂O₂-induced apoptosis of human endothelial cells by modulation of the glutathione redox cycle and nitric oxide synthase. *Arterioscler Thromb Vasc Biol* 17: 3588–3592, 1997.
117. Herren B, Levkau B, Raines EW, and Ross R. Cleavage of beta-catenin and plakoglobin and shedding of VE-cadherin during endothelial apoptosis: evidence for a role for caspases and metalloproteinases. *Mol Biol Cell* 9: 1589–1601, 1998.
118. Hession C, Moy P, Tizard R, Chisholm P, Williams C, Wysk M, Burkly L, Miyake K, Kincade P, and Lobb R. Cloning of murine and rat vascular cell adhesion molecule-1. *Biochem Biophys Res Commun* 183: 163–169, 1992.
119. Hession C, Tizard R, Vassallo C, Schiffer SB, Goff D, Moy P, Chi-Rosso G, Luhowskyj S, Lobb R, and Osborn L.

- Cloning of an alternate form of vascular cell adhesion molecule-1 (VCAM1). *J Biol Chem* 266: 6682–6685, 1991.
120. Hogg N, Browning J, Howard T, Winterford C, Fitzpatrick D, and Gobe G. Apoptosis in vascular endothelial cells caused by serum deprivation, oxidative stress and transforming growth factor-beta. *Endothelium* 7: 35–49, 1999.
121. Hortelano S, Lopez-Fontal R, Traves PG, Villa N, Grashoff C, Bosca L, and Luque A. ILK mediates LPS-induced vascular adhesion receptor expression and subsequent leucocyte trans-endothelial migration. *Cardiovasc Res* 86: 283–292, 2010.
122. Huang J and May JM. Ascorbic acid spares alpha-tocopherol and prevents lipid peroxidation in cultured H4IIE liver cells. *Mol Cell Biochem* 247: 171–176, 2003.
123. Huo Y, Hafezi-Moghadam A, and Ley K. Role of vascular cell adhesion molecule-1 and fibronectin connecting segment-1 in monocyte rolling and adhesion on early atherosclerotic lesions. *Circ Res* 87: 153–159, 2000.
124. Iademarco MF, McQuillan JJ, Rosen GD, and Dean DC. Characterization of the promoter for vascular cell adhesion molecule-1 (VCAM-1). *J Biol Chem* 267: 16323–16329, 1992.
125. Iiyama K, Hajra L, Iiyama M, Li H, DiChiara M, Medoff BD, and Cybulsky MI. Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. *Circ Res* 85: 199–207, 1999.
126. Jaudszus A, Krokowski M, Mockel P, Darcan Y, Avagyan A, Matricardi P, Jahreis G, and Hamelmann E. Cis-9,trans-11-conjugated linoleic acid inhibits allergic sensitization and airway inflammation via a PPARgamma-related mechanism in mice. *J Nutr* 138: 1336–1342, 2008.
127. Jha HC, Divya A, Prasad J, and Mittal A. Plasma circulatory markers in male and female patients with coronary artery disease. *Heart Lung* 39: 296–303, 2010.
128. Jialal I, Devaraj S, and Kaul N. The effect of alpha-tocopherol on monocyte proatherogenic activity. *J Nutr* 131: 389S–394S, 2001.
129. Jiang Q and Ames BN. Gamma-tocopherol, but not alpha-tocopherol, decreases proinflammatory eicosanoids and inflammation damage in rats. *FASEB J* 17: 816–822, 2003.
130. Jiang Q, Christen S, Shigenaga MK, and Ames BN. gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. *Am J Clin Nutr* 74: 714–722, 2001.
131. Johnston B and Butcher EC. Chemokines in rapid leukocyte adhesion triggering and migration. *Semin Immunol* 14: 83–92, 2002.
132. Jones SA, O'Donnell VB, Wood JD, Broughton JP, Hughes EJ, and Jones OT. Expression of phagocyte NADPH oxidase components in human endothelial cells. *Am J Physiol* 271: H1626–H1634, 1996.
133. Jones SC, Banks RE, Haidar A, Gearing AJ, Hemingway IK, Ibbotson SH, Dixon MF, and Axon AT. Adhesion molecules in inflammatory bowel disease. *Gut* 36: 724–730, 1995.
134. Jordan JM, De Roos AJ, Renner JB, Luta G, Cohen A, Craft N, Helmick CG, Hochberg MC, and Arab L. A case-control study of serum tocopherol levels and the alpha- to gamma-tocopherol ratio in radiographic knee osteoarthritis: the Johnston County Osteoarthritis Project. *Am J Epidemiol* 159: 968–977, 2004.
135. Jung WJ and Sung MK. Effects of major dietary antioxidants on inflammatory markers of RAW 264.7 macrophages. *Biofactors* 21: 113–117, 2004.
136. Kalayci O, Besler T, Kilinc K, Sekerel BE, and Saraclar Y. Serum levels of antioxidant vitamins (alpha tocopherol, beta carotene, and ascorbic acid) in children with bronchial asthma. *Turk J Peds* 42: 17–21, 2000.
137. Katsuda S, Suzuki K, Koyama N, Takahashi M, Miyake M, Hazama A, and Takazawa K. Safflower seed polyphenols (N-(p-coumaroyl)serotonin and N-feruloylserotonin) ameliorate atherosclerosis and distensibility of the aortic wall in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits. *Hypertens Res* 32: 944–949, 2009.
138. Kavanagh DP, Durant LE, Crosby HA, Lalor PF, Frampton J, Adams DH, and Kalia N. Haematopoietic stem cell recruitment to injured murine liver sinusoids depends on (alpha)4(beta)1 integrin/VCAM-1 interactions. *Gut* 59: 79–87, 2010.
139. Kelly FJ, Mudway I, Blomberg A, Frew A, and Sandstrom T. Altered lung antioxidant status in patients with mild asthma. *Lancet* 354: 482–483, 1999.
140. Keshavan P, Deem TL, Schwemberger SJ, Babcock GF, Cook-Mills JM, and Zucker SD. Unconjugated bilirubin inhibits VCAM-1-mediated transendothelial leukocyte migration. *J Immunol* 174: 3709–3718, 2005.
141. Kevil CG, Oshima T, and Alexander JS. The role of p38 MAP kinase in hydrogen peroxide mediated endothelial solute permeability. *Endothelium* 8: 107–116, 2001.
142. Khan BV, Harrison DG, Olbrych MT, Alexander RW, and Medford RM. Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc Natl Acad Sci U S A* 93: 9114–9119, 1996.
143. Kilger G, Needham LA, Nielsen PJ, Clements J, Vestweber D, and Holzmann B. Differential regulation of $\alpha 4$ integrin-dependent binding to domains 1 and 4 of vascular cell adhesion molecule-1. *J Biol Chem* 270: 5979–5984, 1995.
144. Kim SR, Bae YH, Bae SK, Choi KS, Yoon KH, Koo TH, Jang HO, Yun I, Kim KW, Kwon YG, et al. Visfatin enhances ICAM-1 and VCAM-1 expression through ROS-dependent NF-kappaB activation in endothelial cells. *Biochim Biophys Acta* 1783: 886–895, 2008.
145. Kimm H, Yun JE, Jo J, and Jee SH. Low serum bilirubin level as an independent predictor of stroke incidence: a prospective study in Korean men and women. *Stroke* 40: 3422–3427, 2009.
146. Kinashi T, St. Pierre Y, and Springer TA. Expression of glycosphosphatidylinositol-anchored and -non-anchored isoforms of vascular cell adhesion molecule 1 in murine stromal and endothelial cells. *J Leuk Biol* 57: 168–173, 1995.
147. Kitani A, Nakashima N, Izumihara T, Inagaki M, Baoui X, Yu S, Matsuda T, and Matsuyama T. Soluble VCAM-1 induces chemotaxis of Jurkat and synovial fluid T cells bearing high affinity very late antigen-4. *J Immunol* 161: 4931–4938, 1998.
148. Knapp S, Florquin S, Golenbock DT, and van der Poll T. Pulmonary lipopolysaccharide (LPS)-binding protein inhibits the LPS-induced lung inflammation *in vivo*. *J Immunol* 176: 3189–3195, 2006.
149. Ko GT, Chan JC, Woo J, Lau E, Yeung VT, Chow CC, Li JK, So WY, and Cockram CS. Serum bilirubin and cardiovascular risk factors in a Chinese population. *J Cardiovasc Risk* 3: 459–463, 1996.
150. Konger RL. A new wrinkle on topical vitamin E and photo-inflammation: Mechanistic studies of a hydrophilic gamma-tocopherol derivative compared with alpha-tocopherol. *J Invest Dermatol* 126: 1447–1449, 2006.
151. Koopman G, Parmentier HK, Schuurman HJ, Newman W, Meijer CJ, and Pals ST. Adhesion of human B cells to follicular

- dendritic cells involves both the lymphocyte function-associated antigen 1/intercellular adhesion molecule 1 and very late antigen 4/vascular cell adhesion molecule 1 pathways. *J Exp Med* 173: 1297–1304, 1991.
152. Korenaga R, Ando J, Kosaki K, Isshiki M, Takada Y, and Kamiya A. Negative transcriptional regulation of the VCAM-1 gene by fluid shear stress in murine endothelial cells. *Am J Physiol* 273: C1506–C1515, 1997.
 153. Koyama N, Kuribayashi K, Seki T, Kobayashi K, Furuhashi Y, Suzuki K, Arisaka H, Nakano T, Amino Y, and Ishii K. Serotonin derivatives, major safflower (*Carthamus tinctorius* L.) seed antioxidants, inhibit low-density lipoprotein (LDL) oxidation and atherosclerosis in apolipoprotein E-deficient mice. *J Agric Food Chem* 54: 4970–4976, 2006.
 154. Koyama N, Suzuki K, Furukawa Y, Arisaka H, Seki T, Kuribayashi K, Ishii K, Sukegawa E, and Takahashi M. Effects of safflower seed extract supplementation on oxidation and cardiovascular risk markers in healthy human volunteers. *Br J Nutr* 101: 568–575, 2009.
 155. Kristenson M, Kucinskiene Z, Schafer-Elinder L, Leanderson P, and Tagesson C. Lower serum levels of beta-carotene in Lithuanian men are accompanied by higher urinary excretion of the oxidative DNA adduct, 8-hydroxydeoxyguanosine. The LiVicordia study. *Nutrition* 19: 11–15, 2003.
 156. Kumagai Y, Pi JB, Lee S, Sun GF, Yamanushi T, Sagai M, and Shimojo N. Serum antioxidant vitamins and risk of lung and stomach cancers in Shenyang, China. *Cancer Lett* 129: 145–149, 1998.
 157. Kumar AG, Dai XY, Kozak CA, Mims MP, Gotto AM, and Ballantyne CM. Murine VCAM-1. Molecular cloning, mapping, and analysis of a truncated form. *J Immunol* 153: 4088–4098, 1994.
 158. Kusunoki T, Morimoto T, Nishikomori R, Yasumi T, Heike T, Fujii T, and Nakahata T. Changing prevalence and severity of childhood allergic diseases in Kyoto, Japan, from 1996 to 2006. *Allergol Int* 58: 543–548, 2009.
 159. Lalor PF, Shields P, Grant A, and Adams DH. Recruitment of lymphocytes to the human liver. *Immunol Cell Biol* 80: 52–64, 2002.
 160. Laudanna C and Alon R. Right on the spot. Chemokine triggering of integrin-mediated arrest of rolling leukocytes. *Thromb Haemost* 95: 5–11, 2006.
 161. Laudanna C, Kim JY, Constantin G, and Butcher E. Rapid leukocyte integrin activation by chemokines. *Immunol Rev* 186: 37–46, 2002.
 162. Leavesley DI, Oliver JM, Swart BW, Berndt MC, Haylock DN, and Simmons PJ. Signals from platelet/endothelial cell adhesion molecule enhance the adhesive activity of the very late antigen-4 integrin of human CD34+ hemopoietic progenitor cells. *J Immunol* 153: 4673–4683, 1994.
 163. Lechleitner S, Gille J, Johnson DR, and Petzelbauer P. Interferon enhances tumor necrosis factor-induced vascular cell adhesion molecule 1 (CD106) expression in human endothelial cells by an interferon-related factor 1-dependent pathway. *J Exp Med* 187: 2023–2030, 1998.
 164. Lee E, Choi MK, Lee YJ, Ku JL, Kim KH, Choi JS, and Lim SJ. Alpha-tocopheryl succinate, in contrast to alpha-tocopherol and alpha-tocopheryl acetate, inhibits prostaglandin E2 production in human lung epithelial cells. *Carcinogenesis* 27: 2308–2315, 2006.
 165. Lee HS, Namkoong K, Kim DH, Kim KJ, Cheong YH, Kim SS, Lee WB, and Kim KY. Hydrogen peroxide-induced alterations of tight junction proteins in bovine brain microvascular endothelial cells. *Microvasc Res* 68: 231–238, 2004.
 166. Lee S, Chung J, Ha IS, Yi K, Lee JE, Kang HG, Choi I, Oh KH, Kim JY, Surh CD, et al. Hydrogen peroxide increases human leukocyte adhesion to porcine aortic endothelial cells via NFkappaB-dependent up-regulation of VCAM-1. *Int Immunol* 19: 1349–1359, 2007.
 167. Lee YW, Kuhn H, Hennig B, Neish AS, Toborek M. IL-4-induced oxidative stress upregulates VCAM-1 gene expression in human endothelial cells. *J Mol Cell Cardiol* 33: 83–94, 2001.
 168. Leusen JH, de Klein A, Hilarius PM, Ahlin A, Palmblad J, Smith CI, Diekmann D, Hall A, Verhoeven AJ, and Roos D. Disturbed interaction of p21-rac with mutated p67-phox causes chronic granulomatous disease. *J Exp Med* 184: 1243–1249, 1996.
 169. Leusen JH, Verhoeven AJ, and Roos D. Interactions between the components of the human NADPH oxidase: intrigues in the phox family. *J Lab Clin Med* 128: 461–476, 1996.
 170. Ley K. Molecular mechanisms of leukocyte recruitment in the inflammatory process. *Cardiovasc Res* 32: 733–742, 1996.
 171. Libby P, DiCarli M, and Weissleder R. The vascular biology of atherosclerosis and imaging targets. *J Nucl Med* 51 Suppl 1: 33S–37S, 2010.
 172. Lim Y, Vasu VT, Valacchi G, Leonard S, Aung HH, Schock BC, Kenyon NJ, Li CS, Traber MG, and Cross CE. Severe vitamin E deficiency modulates airway allergic inflammatory responses in the murine asthma model. *Free Radic Res* 42: 387–396, 2008.
 173. Linhartova K, Sterbakova G, Racek J, Cerbak R, Porazikova K, and Rokyta R. Linking soluble vascular adhesion molecule-1 level to calcific aortic stenosis in patients with coronary artery disease. *Exp Clin Cardiol* 14: e80–e83, 2009.
 174. Lobb R, Chi-Rosso G, Leone D, Rosa M, Newman B, Luthowsky J, Osborn L, Schiffer S, Benjamin C, Douglas I, et al. Expression and functional characterization of a soluble form of vascular cell adhesion molecule 1. *Biochem Biophys Res Commun* 178: 1498–1504, 1991.
 175. Lorenzon P, Vecile E, Nardon E, Ferrero E, Harlan JM, Tedesco F, and Dobrina A. Endothelial cell E- and P-selectin and vascular cell adhesion molecule-1 function as signaling receptors. *J Cell Biol* 142: 1381–1391, 1998.
 176. Ludwig RJ, Hardt K, Hatting M, Bistran R, Diehl S, Radeke HH, Podda M, Schon MP, Kaufmann R, Henschler R, et al. Junctional adhesion molecule (JAM)-B supports lymphocyte rolling and adhesion through interaction with alpha4beta1 integrin. *Immunology* 128: 196–205, 2009.
 177. Luo SF, Fang RY, Hsieh HL, Chi PL, Lin CC, Hsiao LD, Wu CC, Wang JS, and Yang CM. Involvement of MAPKs and NF-kappaB in tumor necrosis factor alpha-induced vascular cell adhesion molecule 1 expression in human rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum* 62: 105–116, 2009.
 178. Ma Y, Zhao J, Han ZR, Chen Y, Leung TF, and Wong GW. Very low prevalence of asthma and allergies in schoolchildren from rural Beijing, China. *Pediatr Pulmonol* 44: 793–799, 2009.
 179. Mackay F, Loetscher H, Stueber D, Gehr G, and Lesslauer W. Tumor necrosis factor alpha (TNF-alpha)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. *J Exp Med* 177: 1277–1286, 1993.
 180. Mamdouh Z, Mikhailov A, and Muller WA. Transcellular migration of leukocytes is mediated by the endothelial lateral border recycling compartment. *J Exp Med* 206: 2795–2808, 2009.

181. Marshall D and Haskard DO. Clinical overview of leukocyte adhesion and migration: where are we now? *Semin Immunol* 14: 133–140, 2002.
182. Marui N, Offermann MK, Swerlick R, Kunsch C, Rosen CA, Ahmad M, Alexander RW, and Medford RM. Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 92: 1866–1874, 1993.
183. Mathai JC and Sitaramam V. Stretch sensitivity of transmembrane mobility of hydrogen peroxide through voids in the bilayer. Role of cardiolipin. *J Biol Chem* 269: 17784–17793, 1994.
184. Matheny HE, Deem TL, and Cook-Mills JM. Lymphocyte migration through monolayers of endothelial cell lines involves VCAM-1 signaling via endothelial cell NADPH oxidase. *J Immunol* 164: 6550–6559, 2000.
185. Matsuno O, Miyazaki E, Nureki S, Ueno T, Ando M, Ito K, Kumamoto T, and Higuchi Y. Elevated soluble ADAM8 in bronchoalveolar lavage fluid in patients with eosinophilic pneumonia. *Int Arch Allergy Immunol* 142: 285–290, 2007.
186. Matsuno O, Miyazaki E, Nureki S, Ueno T, Kumamoto T, and Higuchi Y. Role of ADAM8 in experimental asthma. *Immunol Lett* 102: 67–73, 2006.
187. May MJ, Entwistle G, Humphries MJ, and Ager A. VCAM-1 is a CS1 peptide-inhibitable adhesion molecule expressed by lymph node high endothelium. *J Cell Sci* 106: 109–119, 1993.
188. McEver RP. Selectins: lectins that initiate cell adhesion under flow. *Curr Opin Cell Biology* 14: 581–586, 2002.
189. McKeever TM, Lewis SA, Cassano PA, Ocke M, Burney P, Britton J, and Smit HA. The relation between dietary intake of individual fatty acids, FEV1 and respiratory disease in Dutch adults. *Thorax* 63: 208–214, 2008.
190. Meydani M. Vitamin E modulation of cardiovascular disease. *Ann N Y Acad Sci* 1031: 271–279, 2004.
191. Meydani SN, Shapiro AC, Meydani M, Macauley JB, and Blumberg JB. Effect of age and dietary fat (fish, corn and coconut oils) on tocopherol status of C57BL/6Nia mice. *Lipids* 22: 345–350, 1987.
192. Meyer JW, Holland JA, Ziegler LM, Chang MM, Beebe G, and Schmitt ME. Identification of a functional leukocyte-type NADPH oxidase in human endothelial cells: a potential atherogenic source of reactive oxygen species. *Endothelium* 7: 11–22, 1999.
193. Meyer TN, Schwesinger C, Ye J, Denker BM, and Nigam SK. Reassembly of the tight junction after oxidative stress depends on tyrosine kinase activity. *J Biol Chem* 276: 22048–22055, 2001.
194. Mickleborough TD, Ionescu AA, and Rundell KW. Omega-3 Fatty acids and airway hyperresponsiveness in asthma. *J Altern Complement Med* 10: 1067–1075, 2004.
195. Middleton J, Patterson AM, Gardner L, Schmutz C, and Ashton BA. Leukocyte extravasation: chemokine transport and presentation by the endothelium. *Blood* 100: 3853–3860, 2002.
196. Min JK, Kim YM, Kim SW, Kwon MC, Kong YY, Hwang IK, Won MH, Rho J, and Kwon YG. TNF-related activation-induced cytokine enhances leukocyte adhesiveness: induction of ICAM-1 and VCAM-1 via TNF receptor-associated factor and protein kinase C-dependent NF-kappaB activation in endothelial cells. *J Immunol* 175: 531–540, 2005.
197. Miyake K, Medina K, Ishihara K, Kimoto M, Auerbach R, and Kincade PW. A VCAM-like adhesion molecule on murine bone marrow stromal cells mediates binding of lymphocyte precursors in culture. *J Cell Biol* 114: 557–565, 1991.
198. Montes-Sanchez D, Ventura JL, Mitre I, Frias S, Michan L, Espejel-Nunez A, Vadillo-Ortega F, and Zentella A. Glycosylated VCAM-1 isoforms revealed in 2D western blots of HUVECs treated with tumoral soluble factors of breast cancer cells. *BMC Chem Biol* 9: 7, 2009.
199. Moran EJ, Sarshar S, Cargill JF, Shahbaz MM, Lio A, Mjalli AMM, and Armstrong RW. Radio frequency tag encoded combinatorial library method for the discovery of tripeptide-substituted cinnamic acid inhibitors of the protein tyrosine phosphatase PTP1B. *J Am Chem Soc* 117: 10787–10788, 1995.
200. Moy P, Lobb R, Tizard R, Olson D, and Hession C. Cloning of an inflammation-specific phosphatidyl inositol-linked form of murine vascular cell adhesion molecule-1. *J Biol Chem* 268: 8835–8841, 1993.
201. Muller WA. Mechanisms of transendothelial migration of leukocytes. *Circ Res* 105: 223–230, 2009.
202. Munteanu A and Zingg JM. Cellular, molecular and clinical aspects of vitamin E on atherosclerosis prevention. *Mol Aspects Med* 28: 538–590, 2007.
203. Munteanu A, Zingg JM, and Azzi A. Anti-atherosclerotic effects of vitamin E—myth or reality? *J Cell Mol Med* 8: 59–76, 2004.
204. Murphy G, Willenbrock F, Crabbe T, O’Shea M, Ward R, Atkinson S, O’Connell J, and Docherty A. Regulation of matrix metalloproteinase activity. *Ann N Y Acad Sci* 732: 31–41, 1994.
205. Murray D, Morrin M, and McDonnell S. Increased invasion and expression of MMP-9 in human colorectal cell lines by a CD44-dependent mechanism. *Anticancer Res* 24: 489–494, 2004.
206. Nadali G, Chiliosi M, and Semenzato G. Circulating soluble adhesion molecules: more observations on the increased levels in disease. *Immunol Today* 15: 140–141, 1994.
207. Nahrendorf M, Keliher E, Panizzi P, Zhang H, Hembrador S, Figueiredo JL, Aikawa E, Kelly K, Libby P, and Weissleder R. 18F-4V for PET-CT imaging of VCAM-1 expression in atherosclerosis. *JACC Cardiovasc Imaging* 2: 1213–1222, 2009.
208. Naito Y, Shimozawa M, Kuroda M, Nakabe N, Manabe H, Katada K, Kokura S, Ichikawa H, Yoshida N, Noguchi N, et al. Tocotrienols reduce 25-hydroxycholesterol-induced monocyte-endothelial cell interaction by inhibiting the surface expression of adhesion molecules. *Atherosclerosis* 180: 19–25, 2005.
209. Needham LA, Van DS, Pigott R, Edwards RM, Shepherd M, Hemingway I, Jack L, and Clements JM. Activation dependent and independent VLA-4 binding sites on vascular cell adhesion molecule-1. *Cell Adh Commun* 2: 87–99, 1994.
210. Neish AS, Williams AJ, Palmer HJ, Whitley MZ, and Collins T. Functional analysis of the human vascular cell adhesion molecule 1 promoter. *J Exp Med* 176: 1583–1593, 1992.
211. O’Connor PW. Use of natalizumab in multiple sclerosis patients. *Can J Neurol Sci* 37: 98–104, 2010.
212. O’Keeffe LM, Muir G, Piterina AV, McGloughlin T. Vascular cell adhesion molecule-1 expression in endothelial cells exposed to physiological coronary wall shear stresses. *J Biomech Eng* 131: 081003, 2009.
213. Okamoto N, Murata T, Tamai H, Tanaka H, and Nagai H. Effects of alpha tocopherol and probucol supplements on

- allergen-induced airway inflammation and hyperresponsiveness in a mouse model of allergic asthma. *Int Arch Allergy Immunol* 141: 172–180, 2006.
214. Olmedilla B, Granado F, Southon S, Wright AJ, Blanco I, Gil-Martinez E, Berg H, Corridan B, Roussel AM, Chopra M, *et al.* Serum concentrations of carotenoids and vitamins A, E, and C in control subjects from five European countries. *Br J Nutr* 85: 227–238, 2001.
 215. Osborn L, Hession C, Tizard R, Vassallo C, Luhowskyj S, Chi-Rosso G, and Lobb R. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell* 59: 1203–1211, 1989.
 216. Osborn L, Vassallo C, and Benjamin CD. Activated endothelium binds lymphocytes through a novel binding site in the alternately spliced domain of vascular cell adhesion molecule-1. *J Exp Med* 176: 99–107, 1992.
 217. Ostrow JD, Mukerjee P, and Tiribelli C. Structure and binding of unconjugated bilirubin: relevance for physiological and pathophysiological function. *J Lipid Res* 35: 1715–1737, 1994.
 218. Ou R, Zhang M, Huang L, Flavell RA, Koni PA, and Moskophidis D. Regulation of immune response and inflammatory reactions against viral infection by VCAM-1. *J Virol* 82: 2952–2965, 2008.
 219. Oymar K and Bjerknes R. Differential patterns of circulating adhesion molecules in children with bronchial asthma and acute bronchiolitis. *Ped Allergy Immunol* 9: 73–79, 1998.
 220. Palli D, Masala G, Vineis P, Garte S, Saieva C, Krogh V, Panico S, Tumino R, Munnia A, Riboli E, *et al.* Biomarkers of dietary intake of micronutrients modulate DNA adduct levels in healthy adults. *Carcinogenesis* 24: 739–746, 2003.
 221. Pallotta I, Lovett M, Rice W, Kaplan DL, and Balduini A. Bone marrow osteoblastic niche: a new model to study physiological regulation of megakaryopoiesis. *PLoS ONE* 4: e8359, 2009.
 222. Peduto L. ADAM9 as a potential target molecule in cancer. *Curr Pharm Des* 15: 2282–2287, 2009.
 223. Pepinsky B, Hession C, Chen L-L, Moy P, Burkly L, Jakubowski A, Chow EP, Benjamin C, Chi-Rosso G, Luhowskyj S, *et al.* Structure/function studies on vascular cell adhesion molecule-1. *J Biol Chem* 267: 17820–17826, 1992.
 224. Pepinsky B, Hession C, Chen LL, Moy P, Burkly L, Jakubowski A, Chow EP, Benjamin C, Chi-Rosso G, Luhowskyj S, *et al.* Structure/function studies on vascular cell adhesion molecule-1. *J Biol Chem* 267: 17820–17826, 1992.
 225. Pero RS, Borchers MT, Spicher K, Ochkur SI, Sikora L, Rao SP, Abdala-Valencia H, O'Neill KR, Shen H, McGarry MP, *et al.* Galphai2-mediated signaling events in the endothelium are involved in controlling leukocyte extravasation. *Proc Natl Acad Sci U S A* 104: 4371–4376, 2007.
 226. Persson C, Sasazuki S, Inoue M, Kurahashi N, Iwasaki M, Miura T, Ye W, and Tsugane S. Plasma levels of carotenoids, retinol and tocopherol and the risk of gastric cancer in Japan: a nested case-control study. *Carcinogenesis* 29: 1042–1048, 2008.
 227. Peter K, Weirich U, Nordt TK, Ruef J, and Bode C. Soluble vascular cell adhesion molecule-1 (VCAM-1) as potential marker of atherosclerosis. *Thromb Haemost* 82 Suppl 1: 38–43, 1999.
 228. Pietersma A, deJong N, deWit LE, Kraak-Slee RG, Koster JF, and Sluiter W. Evidence against the involvement of multiple radical generating sites in the expression of the vascular cell adhesion molecule-1. *Free Rad Res* 28: 137–150, 1998.
 229. Piga R, Naito Y, Kokura S, Handa O, and Yoshikawa T. Inhibitory effect of serotonin derivatives on high glucose-induced adhesion and migration of monocytes on human aortic endothelial cells. *Br J Nutr* 102: 264–272, 2009.
 230. Piga R, Naito Y, Kokura S, Handa O, and Yoshikawa T. Short-term high glucose exposure induces monocyte-endothelial cells adhesion and transmigration by increasing VCAM-1 and MCP-1 expression in human aortic endothelial cells. *Atherosclerosis* 193: 328–334, 2007.
 231. Pober JS, Kluger MS, and Schechner JS. Human endothelial cell presentation of antigen and the homing of memory/effector T cells to skin. *Ann N Y Acad Sci* 941: 12–25, 2001.
 232. Polte T, Newman W, and Gopal TV. Full length vascular cell adhesion molecule 1 (VCAM-1). *Nucleic Acids Res* 18: 5901, 1990.
 233. Polte T, Newman W, Raghunathan G, and Gopal TV. Structural and functional studies of full-length vascular cell adhesion molecule-1: internal duplication and homology to several adhesion proteins. *DNA Cell Biol* 10: 349–357, 1991.
 234. Pot DA, Woodford TA, Remboutsika E, Haun RS, and Dixon JE. Cloning, bacterial expression, purification, and characterization of the cytoplasmic domain of rat LAR, a receptor-like protein tyrosine phosphatase. *J Biol Chem* 266: 19688–19696, 1991.
 235. Qasem AR, Bucolo C, Baiula M, Sparta A, Govoni P, Bedini A, Fasci D, and Spampinato S. Contribution of alpha4beta1 integrin to the antiallergic effect of levocabastine. *Biochem Pharmacol* 76: 751–762, 2008.
 236. Qu Y, Shi X, Zhang H, Sun W, Han S, Yu C, and Li J. VCAM-1 siRNA reduces neointimal formation after surgical mechanical injury of the rat carotid artery. *J Vasc Surg* 50: 1452–1458, 2009.
 237. Qureshi MH, Cook-Mills J, Doherty DE, and Garvy BA. TNF-alpha-dependent ICAM-1- and VCAM-1-mediated inflammatory responses are delayed in neonatal mice infected with *[I]Pneumocystis carinii*. *J Immunol* 171: 4700–4707, 2003.
 238. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, and Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases *in vitro*. Implications for atherosclerotic plaque stability. *J Clin Invest* 98: 2572–2579, 1996.
 239. Rao SP, Wang Z, Zuberi RI, Sikora L, Bahaie NS, Zuraw BL, Liu FT, and Sriramarao P. Galectin-3 functions as an adhesion molecule to support eosinophil rolling and adhesion under conditions of flow. *J Immunol* 179: 7800–7807, 2007.
 240. Ratnasinghe D, Tangrea JA, Forman MR, Hartman T, Gunter EW, Qiao YL, Yao SX, Barrett MJ, Giffen CA, Erozan Y, *et al.* Serum tocopherols, selenium and lung cancer risk among tin miners in China. *Cancer Causes Control* 11: 129–135, 2000.
 241. Rattan V, Sultana C, Shen Y, and Kalra VK. Oxidant stress-induced transendothelial migration of monocytes is linked to phosphorylation of PECAM-1. *Am J Physiol* 273: E453–E461, 1997.
 242. Reinhardt PH and Kubes P. Differential leukocyte recruitment from whole blood via endothelial adhesion molecules under shear conditions. *Blood* 92: 4691–4699, 1998.
 243. Ren G, Zhao X, Zhang L, Zhang J, L'Huillier A, Ling W, Roberts AI, Le AD, Shi S, Shao C, *et al.* Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in mesenchymal stem cells are critical for immunosuppression. *J Immunol* 184: 2321–2328, 2010.

244. Renz ME, Chiu HH, Jones S, Fox J, Kim KJ, Presta LG, and Fong S. Structural requirements for adhesion of soluble recombinant murine vascular cell adhesion molecule-1 to alpha 4 beta 1. *J Cell Biol* 125: 1395–1406, 1994.
245. Romanic AM and Madri JA. The induction of 72-kD gelatinase in T cells upon adhesion to endothelial cells is VCAM-1 dependent. *J Cell Biol* 125: 1165–1178, 1994.
246. Rondinone CM, Trevillyan JM, Clampit J, Gum RJ, Berg C, Kroeger P, Frost L, Zinker BA, Reilly R, Ulrich R, et al. Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis. *Diabetes* 51: 2405–2411, 2002.
247. Ruegg C, Postigo AA, Sikorski EE, Butcher EC, Pytela R, and Erle DJ. Role of integrin alpha 4 beta 7/alpha 4 beta P in lymphocyte adherence to fibronectin and VCAM-1 and in homotypic cell clustering. *J Cell Biol* 117: 179–189, 1992.
248. Ruiz Rejon F, Martin-Pena G, Granado F, Ruiz-Galiana J, Blanco I, and Olmedilla B. Plasma status of retinol, alpha- and gamma-tocopherols, and main carotenoids to first myocardial infarction: case control and follow-up study. *Nutrition* 18: 26–31, 2002.
249. Ryter SW and Tyrrell RM. The heme synthesis and degradation pathways: role of oxidant sensitivity. *Free Rad Biol Med* 28: 289–309, 2000.
250. Sagara H, Matsuda H, Wada N, Yagita H, Fukuda T, Okumura K, Makino S, and Ra C. A monoclonal antibody against very late activation antigen-4 inhibits eosinophil accumulation and late asthmatic response in a guinea pig model of asthma. *Int Arch Allergy Immunol* 112: 287–294, 1997.
251. Sakamoto W, Fujie K, Handa H, Nishihira J, and Mino M. Vitamin E inhibits PGE2 and O2- production in rat peritoneal macrophages. *Biochim Biophys Acta* 1074: 251–255, 1991.
252. Sakamoto W, Fujie K, Nishihira J, Handa H, Ueda N, and Yamamoto S. Effect of vitamin E on expression of cyclooxygenase-2 in lipopolysaccharide-stimulated rat macrophages. *Biochim Biophys Acta* 1304: 139–144, 1996.
253. Salmeen A, Andersen JN, Myers MP, Tonks NK, and Barford D. Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. *Mol Cell* 6: 1401–1412, 2000.
254. Sato R, Helzlsouer KJ, Alberg AJ, Hoffman SC, Norkus EP, and Comstock GW. Prospective study of carotenoids, tocopherols, and retinoid concentrations and the risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 11: 451–457, 2002.
255. Scherbarth S and Orr FW. Intravital videomicroscopic evidence for regulation of metastasis by the hepatic microvasculature: effects of interleukin-1alpha on metastasis and the location of B16F1 melanoma cell arrest. *Cancer Res* 57: 4105–4110, 1997.
256. Schett G, Kiechl S, Bonora E, Zwerina J, Mayr A, Axmann R, Weger S, Oberhollenzer F, Lorenzini R, and Willeit J. Vascular cell adhesion molecule 1 as a predictor of severe osteoarthritis of the hip and knee joints. *Arthritis Rheum* 60: 2381–2389, 2009.
257. Schunemann HJ, Grant BJ, Freudenheim JL, Muti P, Browne RW, Drake JA, Klocke RA, and Trevisan M. The relation of serum levels of antioxidant vitamins C and E, retinol and carotenoids with pulmonary function in the general population. *Am J Respir Crit Care Med* 163: 1246–1255, 2001.
258. Schwinn MK, Gonzalez JM, Jr., Gabelt BT, Sheibani N, Kaufman PL, and Peters DM. Heparin II domain of fibronectin mediates contractility through an alpha4beta1 co-signaling pathway. *Exp Cell Res* 316: 1500–1512, 2010.
259. Sedlak TW and Snyder SH. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. *Pediatrics* 113: 1776–1782, 2004.
260. Seiki M. The cell surface: the stage for matrix metalloproteinase regulation of migration. *Curr Opin Cell Biol* 14: 624–632, 2002.
261. Sen CK, Khanna S, and Roy S. Tocotrienols in health and disease: the other half of the natural vitamin E family. *Mol Aspects Med* 28: 692–728, 2007.
262. Shamri R, Grabovsky V, Feigelson SW, Dwir O, Van Kooyk Y, and Alon R. Chemokine stimulation of lymphocyte alpha 4 integrin avidity but not of leukocyte function-associated antigen-1 avidity to endothelial ligands under shear flow requires cholesterol membrane rafts. *J Biol Chem* 277: 40027–40035, 2002.
263. Shi K, Egawa K, Maegawa H, Nakamura T, Ugi S, Nishio Y, and Kashiwagi A. Protein-tyrosine phosphatase 1B associates with insulin receptor and negatively regulates insulin signaling without receptor internalization. *J Biochem (Tokyo)* 136: 89–96, 2004.
264. Shvedova AA, Kisin ER, Kagan VE, and Karol MH. Increased lipid peroxidation and decreased antioxidants in lungs of guinea pigs following an allergic pulmonary response. *Toxicol Appl Pharmacol* 132: 72–81, 1995.
265. Siekmeier R, Steffen C, and Marz W. Role of oxidants and antioxidants in atherosclerosis: results of *in vitro* and *in vivo* investigations. *J Cardiovasc Pharmacol Ther* 12: 265–282, 2007.
266. Smit HA, Grievink L, and Tabak C. Dietary influences on chronic obstructive lung disease and asthma: a review of the epidemiological evidence. *Proc Nutr Soc* 58: 309–319, 1999.
267. Sommer D, Coleman S, Swanson SA, and Stemmer PM. Differential susceptibilities of serine/threonine phosphatases to oxidative and nitrosative stress. *Arch Biochem Biophys* 404: 271–278, 2002.
268. Soriano A, Salas A, Salas A, Sans M, Gironella M, Elena M, Anderson DC, Pique JM, and Panes J. VCAM-1, but not ICAM-1 or MAdCAM-1, immunoblockade ameliorates DSS-induced colitis in mice. *Lab Invest* 80: 1541–1551, 2000.
269. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76: 301–314, 1994.
270. Springer TA. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. *Ann Rev Physiol* 57: 827–872, 1995.
271. Stanley AC, Dalton JE, Rossotti SH, MacDonald KP, Zhou Y, Rivera F, Schroder WA, Maroof A, Hill GR, Kaye PM, et al. VCAM-1 and VLA-4 modulate dendritic cell IL-12p40 production in experimental visceral leishmaniasis. *PLoS Pathog* 4: e1000158, 2008.
272. Stocker R, Glazer AN, and Ames BN. Antioxidant activity of albumin-bound bilirubin. *Proc Natl Acad Sci U S A* 84: 5918–5922, 1987.
273. Stocker R, McDonagh AF, Glazer AN, and Ames BN. Antioxidant activities of bile pigments: biliverdin and bilirubin. *Method Enzymol* 186: 301–309, 1990.
274. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, and Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 235: 1043–1046, 1987.
275. Suchankova J, Voprsalova M, Kottova M, Semecky V, and Visnovsky P. Effects of oral alpha-tocopherol on lung

- response in rat model of allergic asthma. *Respirology* 11: 414–421, 2006.
276. Sung HJ, Yee A, Eskin SG, and McIntire LV. Cyclic strain and motion control produce opposite oxidative responses in two human endothelial cell types. *Am J Physiol Cell Physiol* 293: C87–C94, 2007.
 277. Tabak C, Smit HA, Rasanen L, Fidanza F, Menotti A, Nissinen A, Feskens EJ, Heederik D, and Kromhout D. Dietary factors and pulmonary function: a cross sectional study in middle aged men from three European countries. *Thorax* 54: 1021–1026, 1999.
 278. Tadokoro T, Ikekita M, Toda T, Ito H, Sato T, Nakatani R, Hamaguchi Y, and Furukawa K. Involvement of galectin-3 with vascular cell adhesion molecule-1 in growth regulation of mouse BALB/3T3 cells. *J Biol Chem* 284: 35556–35563, 2009.
 279. Taylor PR, Qiao YL, Abnet CC, Dawsey SM, Yang CS, Gunter EW, Wang W, Blot WJ, Dong ZW, and Mark SD. Prospective study of serum vitamin E levels and esophageal and gastric cancers. *J Natl Cancer Inst* 95: 1414–1416, 2003.
 280. Terry RW, Kwee L, Levine JF, and Labow MA. Cytokine induction of an alternatively spliced murine vascular cell adhesion molecule (VCAM) mRNA encoding a glycosylphosphatidylinositol-anchored VCAM protein. *Proc Natl Acad Sci U S A* 90: 5919–5923, 1993.
 281. Thannickal VJ and Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol* 279: L1005–L1028, 2000.
 282. Tokuhira M, Hosaka S, Volin MV, Haines GK, 3rd, Katschke KJ Jr., Kim S, and Koch AE. Soluble vascular cell adhesion molecule 1 mediation of monocyte chemotaxis in rheumatoid arthritis. *Arthritis Rheum* 43: 1122–1133, 2000.
 283. Tomasch R, Wagner KH, and Elmadfa I. Antioxidative power of plant oils in humans: the influence of alpha- and gamma-tocopherol. *Ann Nutr Metab* 45: 110–115, 2001.
 284. Troisi RJ, Willett WC, Weiss ST, Trichopoulos D, Rosner B, and Speizer FE. A prospective study of diet and adult-onset asthma. *Am J Respir Crit Care Med* 151: 1401–1408, 1995.
 285. Tudor K-SRS, Deem TL, and Cook-Mills JM. Novel alpha 4-integrin ligands on an endothelial cell line. *Biochem Cell Biol* 78: 99–113, 2000.
 286. Tudor KSRS, Hess KL, and Cook-Mills JM. Cytokines modulate endothelial cell intracellular signal transduction required for VCAM-1-dependent lymphocyte transendothelial migration. *Cytokine* 15: 196–211, 2001.
 287. Tummala PE, Chen XL, Sundell CL, Laursen JB, Hammes CP, Alexander RW, Harrison DG, and Medford RM. Angiotensin II induces vascular cell adhesion molecule-1 expression in rat vasculature: a potential link between the renin-angiotensin system and atherosclerosis. *Circulation* 100: 1223–1229, 1999.
 288. Ueki S, Kihara J, Kato H, Ito W, Takeda M, Kobayashi Y, Kayaba H, and Chihara J. Soluble vascular cell adhesion molecule-1 induces human eosinophil migration. *Allergy* 64: 718–724, 2009.
 289. Usatyuk PV and Natarajan V. Role of mitogen-activated protein kinases in 4-hydroxy-2-nonenal-induced actin remodeling and barrier function in endothelial cells. *J Biol Chem* 279: 11789–11797, 2004.
 290. Van Buul JD, Fernandez-Borja M, Anthony EC, and Hordijk PL. Expression and localization of NOX2 and NOX4 in primary human endothelial cells. *Antioxid Redox Signal* 7: 308–317, 2005.
 291. van Buul JD, Voermans C, van den Berg V, Anthony EC, Mul FPJ, van Wetering S, van der Schoot EC, and Hordijk PL. Migration of human hematopoietic progenitor cells across bone marrow endothelium is regulated by vascular endothelial cadherin. *J Immunol* 168: 588–596, 2002.
 292. van Schayck CP and Smit HA. The prevalence of asthma in children: a reversing trend. *Eur Respir J* 26: 647–650, 2005.
 293. Van Wart HE and Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci U S A* 87: 5578–5582, 1990.
 294. van Wetering S, van den Berk N, van Buul JD, Mul FPJ, Lommerse I, Mous R, ten Klooster J-P, Zwaginga J-J, and Hordijk PL. VCAM-1-mediated Rac signaling controls endothelial cell-cell contacts and leukocyte transmigration. *Am J Physiol Cell Physiol* 285: C343–C352, 2003.
 295. Vandermeeren M, Janssens S, Borgers M, and Geysen J. Dimethylfumarate is an inhibitor of cytokine-induced E-selectin, VCAM-1, and ICAM-1 expression in human endothelial cells. *Biochem Biophys Res Commun* 234: 19–23, 1997.
 296. Vega-Lopez S, Kaul N, Devaraj S, Cai RY, German B, and Jialal I. Supplementation with omega3 polyunsaturated fatty acids and all-rac alpha-tocopherol alone and in combination failed to exert an anti-inflammatory effect in human volunteers. *Metabolism* 53: 236–240, 2004.
 297. Verdejo H, Roldan J, Garcia L, Del Campo A, Becerra E, Chiong M, Mellado R, Garcia A, Zalaquett R, Braun S, et al. Systemic vascular cell adhesion molecule-1 predicts the occurrence of post-operative atrial fibrillation. *Int J Cardiol*, 2010, PMID: 20447702 [Epub ahead of print].
 298. Vestweber D. Lymphocyte trafficking through blood and lymphatic vessels: more than just selectins, chemokines and integrins. *Eur J Immunol* 33: 1361–1364, 2003.
 299. Vidal-Vanaclocha F, Fantuzzi G, Mendoza L, Fuentes AM, Anasagasti MJ, Martin J, Carrascal T, Walsh P, Reznikov LL, Kim SH, et al. IL-18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. *Proc Natl Acad Sci U S A* 97: 734–739, 2000.
 300. Vollmer WM, Osborne ML, and Buist AS. 20-year trends in the prevalence of asthma and chronic airflow obstruction in an HMO. *Am J Respir Crit Care Med* 157: 1079–1084, 1998.
 301. Vonderheide RH, Tedder TF, Springer TA, and Staunton DE. Residues within a conserved amino acid motif of domains 1 and 4 of VCAM-1 are required for binding to VLA-4. *J Cell Biol* 125: 215–222, 1994.
 302. Wagner JG, Jiang Q, Harkema JR, Ames BN, Illek B, Roubey RA, and Peden DB. Gamma-tocopherol prevents airway eosinophilia and mucous cell hyperplasia in experimentally induced allergic rhinitis and asthma. *Clin Exp Allergy* 38: 501–511, 2008.
 303. Wagner JG, Jiang Q, Harkema JR, Illek B, Patel DD, Ames BN, and Peden DB. Ozone enhancement of lower airway allergic inflammation is prevented by gamma-tocopherol. *Free Radic Biol Med* 43: 1176–1188, 2007.
 304. Wagner KH, Kamal-Eldin A, and Elmadfa I. Gamma-tocopherol—an underestimated vitamin? *Ann Nutr Metab* 48: 169–188, 2004.
 305. Wang Q and Doerschuk CM. The p38 mitogen-activated protein kinase mediates cytoskeletal remodeling in pulmonary microvascular endothelial cells upon intracellular adhesion molecule-1 ligation. *J Immunol* 166: 6877–6884, 2001.

306. Wang Q, Pfeiffer GR 2nd, and Gaarde WA. Activation of SRC tyrosine kinases in response to ICAM-1 ligation in pulmonary microvascular endothelial cells. *J Biol Chem* 278: 47731–47743, 2003.
307. Weber C, Alon R, Moser B, and Springer TA. Sequential regulation of alpha 4 beta 1 and alpha 5 beta 1 integrin avidity by CC chemokines in monocytes: implications for transendothelial chemotaxis. *J Cell Biol* 134: 1063–1073, 1996.
308. Weiss ST. Diet as a risk factor for asthma. *Ciba Found Symp* 206: 244–257, 1997.
309. Weller PF, Rand TH, Goelz SE, Chi-Rosso G, and Lobb RR. Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule 1. *Proc Natl Acad Sci U S A* 88: 7430–7433, 1991.
310. Willam C, Schindler R, Frei U, and Eckardt KU. Increases in oxygen tension stimulate expression of ICAM-1 and VCAM-1 on human endothelial cells. *Am J Physiol* 276: H2044–H2052, 1999.
311. Wolf G. gamma-Tocopherol: an efficient protector of lipids against nitric oxide-initiated peroxidative damage. *Nutr Rev* 55: 376–378, 1997.
312. Wolf G. How an increased intake of alpha-tocopherol can suppress the bioavailability of gamma-tocopherol. *Nutr Rev* 64: 295–299, 2006.
313. Wu CY, Chi PL, Hsieh HL, Luo SF, and Yang CM. TLR4-dependent induction of vascular adhesion molecule-1 in rheumatoid arthritis synovial fibroblasts: roles of cytosolic phospholipase A(2)alpha/cyclooxygenase-2. *J Cell Physiol* 223: 480–491, 2010.
314. Wu D, Koga T, Martin KR, and Meydani M. Effect of vitamin E on human aortic endothelial cell production of chemokines and adhesion to monocytes. *Atherosclerosis* 147: 297–307, 1999.
315. Wu D, Liu L, Meydani M, and Meydani SN. Vitamin E increases production of vasodilator prostanoids in human aortic endothelial cells through opposing effects on cyclooxygenase-2 and phospholipase A2. *J Nutr* 135: 1847–1853, 2005.
316. Wu D, Meydani M, Beharka AA, Serafini M, Martin KR, and Meydani SN. *In vitro* supplementation with different tocopherol homologues can affect the function of immune cells in old mice. *Free Rad Biol Med* 28: 643–651, 2000.
317. Xu B, Wagner N, Pham LN, Magno V, Shan Z, Butcher EC, and Michie SA. Lymphocyte homing to bronchus-associated lymphoid tissue (BALT) is mediated by L-selectin/PNAd, alpha4beta1 integrin/VCAM-1, and LFA-1 adhesion pathways. *J Exp Med* 197: 1255–1267, 2003.
318. Yakubenko VP, Lobb RR, Plow EF, and Ugarova TP. Differential induction of gelatinase B (MMP-9) and gelatinase A (MMP-2) in T lymphocytes upon alpha(4)beta(1)-mediated adhesion to VCAM-1 and the CS-1 peptide of fibronectin. *Exp Cell Res* 260: 73–84, 2000.
319. Yamada Y, Arao T, Matsumoto K, Gupta V, Tan W, Fedynshyn J, Nakajima TE, Shimada Y, Hamaguchi T, Kato K, *et al.* Plasma concentrations of VCAM-1 and PAI-1: a predictive biomarker for post-operative recurrence in colorectal cancer. *Cancer Sci* 101: 1886–1890, 2010.
320. Yamaguchi M, Suwa H, Miyasaka M, and Kumada K. Selective inhibition of vascular cell adhesion molecule-1 expression by verapamil in human vascular endothelial cells. *Transplantation* 63: 759–764, 1997.
321. Yang Y, Cardarelli PM, Lehnert K, Rowland S, and Krisanssen GW. LPAM-1 (integrin alpha 4 beta 7)-ligand binding: overlapping binding sites recognizing VCAM-1, MAdCAM-1 and CS-1 are blocked by fibrinogen, a fibronectin-like polymer and RGD-like cyclic peptides. *Eur J Immunol* 28: 995–1004, 1998.
322. Yoshida N, Manabe H, Terasawa Y, Nishimura H, Enjo F, Nishino H, and Yoshikawa T. Inhibitory effects of vitamin E on endothelial-dependent adhesive interactions with leukocytes induced by oxidized low density lipoprotein. *Biofactors* 13: 279–288, 2000.
323. Yoshida Y, Saito Y, Jones LS, and Shigeri Y. Chemical reactivities and physical effects in comparison between tocopherols and tocotrienols: physiological significance and prospects as antioxidants. *J Biosci Bioeng* 104: 439–445, 2007.
324. Yu W-H, Woessner JF Jr., McNeish JD, and Stamenkovic I. CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. *Genes Develop* 16: 307–323, 2002.
325. Zapolska-Downar D, Zapolski-Downar A, Markiewski M, Ciechanowicz A, Kaczmarczyk M, and Naruszewicz M. Selective inhibition by alpha-tocopherol of vascular cell adhesion molecule-1 expression in human vascular endothelial cells. *Biochem Biophys Res Commun* 274: 609–615, 2000.
326. Zhang Z-Y. Protein tyrosine phosphatases: structure and function, substrate specificity, and inhibitor development. *Annu Rev Pharmacol Toxicol* 42: 209–234, 2002.
327. Zingg JM and Azzi A. Non-antioxidant activities of vitamin E. *Curr Med Chem* 11: 1113–1133, 2004.
328. Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, Xie N, Wilcox D, Jacobson P, Frost L, *et al.* PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. *Proc Natl Acad Sci U S A* 99: 11357–11362, 2002.
329. Zucker S, Drews M, Conner C, Foda HD, DeClerck YA, Langley KE, Bahou WF, Docherty AJ, and Cao J. Tissue inhibitor of metalloproteinase-2 (TIMP-2) binds to the catalytic domain of the cell surface receptor, membrane type 1-matrix metalloproteinase 1 (MT1-MMP). *J Biol Chem* 273: 1216–1222, 1998.

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Abbreviations Used

ADAM = a disintegrin and metalloprotease
AHR = airway hyperresponsiveness
ER = endoplasmic reticulum
FRET = fluorescence resonance energy transfer
G α i = G protein α i
GPI = glycosylphosphatidylinositol
H₂O₂ = hydrogen peroxide
HUVECs = human umbilical vein endothelial cells
ICAM-1 = intercellular adhesion molecule-1
Ig = immunoglobulin
IL = interleukin
MCP-1 = monocyte chemoattractant protein-1

mHEV cells = lymph node-derived high endothelial
venule-like cells
MMP = matrix metalloproteinase
OVA = chicken egg ovalbumin
oxLDL = oxidized low density lipoprotein
PECAM-1 = platelet-endothelial cell adhesion
molecule-1
PKC α = protein kinase C α
PTP1B = protein tyrosine phosphatase 1B
ROS = reactive oxygen species
sVCAM-1 = soluble vascular cell adhesion molecule-1
TIMP = tissue inhibitor of metalloproteinase
TLR = toll-like receptor
TNF α = tumor necrosis factor-alpha
VCAM-1 = vascular cell adhesion molecule-1