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Neutrophil–Endothelial Cell Interactions

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This chapter discusses endothelial cell research with application to neutrophil–endothelial interactions; it focuses on research that has utilized various cytometric methodologies in the investigations. Implicit in the intent are the reader's basic knowledge of endothelial cell physiology and pathology and interest in utilizing new or alternative cytometric techniques or a creative combination of techniques, particularly those enabling single cell measurement.

Endothelial research originated with light microscopists who recognized the existence of a thin layer of cells that line blood vessels. Yet it was not until the development of in vitro endothelial culturing techniques by Pomerat, Maruyama, and others that the complex role played by endothelial cells in physiology and pathology came to be appreciated (Jaffe et al., 1973; Pomerat and Slick, 1963; Maruyama, 1963). Light microscopy remained the mainstay of endothelial research until the development of electron microscopy greatly enhanced our visual understanding of the endothelial substructure. The molecular understanding of endothelial cells was expanded by the development of monoclonal antibodies recognizing specific epitopes. Fluorochromes conjugated to monoclonal antibodies provided simultaneous identification of one or more epitopes on endothelial cells. More recently other fluorochromes have been used

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to investigate facets of intracellular physiology such as reactive oxygen species (ROS), pH, glutathione content, and calcium flux. With physiology comes the realization that endothelial cells are actively changing in response to their environment and to stimuli. Accordingly, with flow cytometry and confocal microscopy we now have the technology to assess cellular physiology and pathology in terms of active, real-time processes, with three-dimensional capabilities afforded by laser scanning confocal microscopy and computer reconstruction. An additional cytometric tool, termed intravital microscopy, is the rediscovered technique of videomicroscopy in combination with in vivo techniques. Intravital microscopy provides the capability for real-time visualization of previously hypothesized mechanisms. Finally, the integration of fluorescence and confocal microscopy with intravital/videomicroscopy has opened new horizons for the study of cell–cell interactions.

MARGINATION TO EMIGRATION

The circulating neutrophil undergoes a complex series of interactions with endothelial cells before emerging as a tissue neutrophil. The initial neutrophil–endothelial interactions were classically referred to as margination. Recently this process has been more aptly described as "tethering" by several groups who have described the visual tethering of the neutrophil to endothelial cells for an instant, essentially providing a deceleration mechanism that initiates leukocyte rolling along the endothelium. Molecular tools and the characterization of several adhesion molecule antibodies along with intravital microscopy have created a truly visual appreciation of this process, shown schematically in Figure 1. As the initial molecular tethers are created, the slowing neutrophil begins to roll along the endothelial surface and continues to decelerate as more tethers are created between endothelial and leukocyte selectins and their respective ligands (Tables 1a and 1b). Tethers are created between the neutrophil L-selectin (also referred to as LECAM-1, LAM-1, or CD62L) and carbohydrate ligands on endothelial cells (Imai et al., 1990; von Andrian et al., 1991; Ley et al., 1991). Another "tethering" receptor is P-selectin (also referred to as GMP-140 and CD62P), which is expressed on endothelial cells and like L-selectin, initiates deceleration and rolling of neutrophils along the endothelial surface. P-Selectin is stored in Weibel–Palade bodies in endothelial cells (and in secretory granules in platelets) and is rapidly translocated within minutes of cell activation to the plasma membrane via exocytosis (McEver et al., 1989; Lorant et al., 1991). The ligand for P-selectin is also a carbohydrate ligand (fucosylated and sialylated), with evidence that it is the same sialyl-Lewis x (sLe^x, CD15s) ligand to which E-selectin binds, and is also associated with L-selectin on neutrophils (Polley et al., 1991; Picker et al., 1991; Foxall et al., 1992). Using P-selectin–deficient mice, Mayadas and co-workers (1993) demonstrated virtually total absence of leukocyte rolling in mesenteric venules, increased circulating neutrophil numbers, and decreased recruitment of neutrophils to the peritoneal cavity during inflammation. The third selectin molecule identified in neutrophil–endothelial interactions is E-selectin (also referred to as ELAM-1, LECAM-2, or CD62E,) which is also expressed on endothelial cells. The ligand for E-selectin is the carbohydrate ligand sLe^x located on neutrophils (Walz et al., 1990; Phillips et al.,

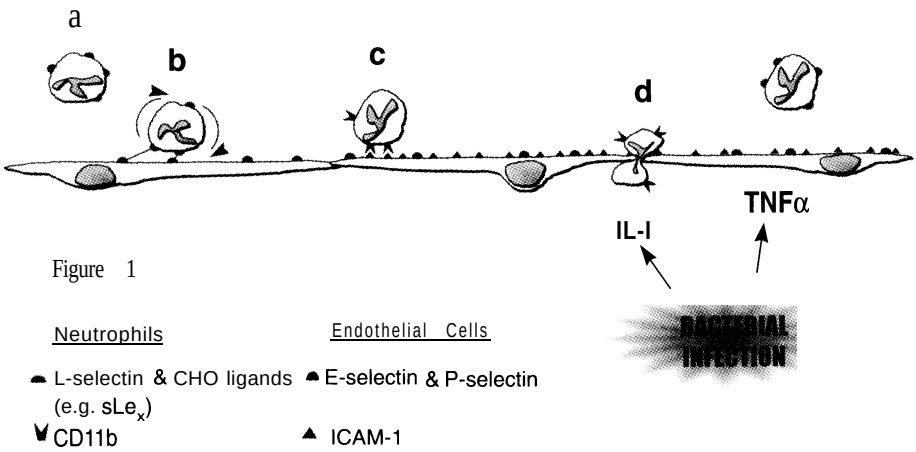

Fig. 1. Diagram showing the circulating neutrophil (a) and the initiation of rolling (b) as molecular tethers are formed between selectin and CHO ligands on neutrophils and endothelial cells. If an adequate number of tethers are formed, the neutrophil completely decelerates. With chemotactic stimulation of the neutrophil, L-selectin is rapidly shed whereas other receptors such as E-selectin, CD1 1 b, and ICAM- 1 are upregulated by cytokines and other inflammatory mediators (c). Firm neutrophil/endothelial cell adhesion is mediated by CD 11 b and ICAM- 1 and is followed by emigration of the neutrophil through the endothelium (d).

1990). Other reports have suggested that L-selectin, CD66, and β_2 integrins may also be ligands for E-selectin (Picker et al., 1991; Kuijpers et al., 1992; Kotovuori et al., 1993, Pate1 et al., 1995). The interaction of sLe^x with soluble E-selectin has been very elegantly demonstrated by Jacob et al. (1995) using fluorescence polarization. Their results confirmed weak binding between the carbohydrate and protein entities, consistent with other carbohydrate-protein interactions; however, they also acknowledged additional binding interactions, such as clustering, that may be factors. It is also important to recognize that this deceleration and rolling process is reversible. Without a critical mass of tethering attachments between the neutrophil and endothelial cell, the neutrophil can escape back into the circulating pool. Of utmost importance in developing this critical mass of attachments is the upregulation and expression of P- and E-selectin on endothelial cells and the development of a firm adhesion via β_2 integrins and ICAM- 1 molecules.

Once the neutrophil has come to a complete stop, chemotactic stimulation (e.g., GM-CSF, TNF-α, fMLP, LTB4, and IL-8) or ROS induces a rapid loss of L-selectin from the neutrophil surface (Smith et al., 1991; Griffin et al., 1990; Rao et al., 1994; Huber et al., 1991; Fraticelli et al., 1996) (Fig. 1). This loss is temporally associated with an upregulation of CD1 1 b, which develops a firm adhesion with its endothelial ligand ICAM-1 (CD54) (Diamond et al., 1990; Griffin et al., 1990; Simon et al., 1995; Fraticelli et al., 1996). However, CD1 lb is only a subunit of the heterodimeric integral membrane protein CD 11 b/CD 18, which consists of an alpha and a beta subunit. On neutrophils, the two heterodimers of most importance for adhesion to endothelial cells are CD 11 a/CD 18 (also referred to as LFA- 1 or $a_L\beta_2$) and CD 1 lb/CD 18 (also referred to as MAC-l or $a_M\beta_2$). These β_2 integrins, which have unique alpha and

TABLE 1A. Endothelial and Neutrophil Adhesion Molecules and their Respective Ligands

Endothelial Adhesion Molecules	Neutrophil Counter Ligand
P-Selectin (CD62P)	s-Le^x (CD 15s)
E-Selectin (CD62E)	s-Le^x, CD66, L-selectin, β2 integrins

identical beta subunits, both bind to ICAM-1 on endothelial cells. Additionally, CD 11 a/CD 18 binds to ICAM- 1 and ICAM-2 (CD 102) on endothelial cells (Staunton et al., 1989). CD1 1c/CD1 8 (p150,95 or a,&), the third β_2 integrin on neutrophils, is generally considered to make a smaller contribution to adhesion to endothelial cells (Carlos and Harlan, 1994). A definitive endothelial ligand has not been well defined; however, many ligands that may act as a bridge between endothelial cells and neutrophils have been identified, including iC3b and fibrinogen (Stacker and Springer, 1991; Loike et al., 1991; Bilsland et al., 1994). CD1 1b/CD1 8 has also been shown to bind to iC3b, fibrinogen, and factor X, which may also bridge to endothelial cells (Beller et al., 1982; Wright et al., 1988; Altieri et al.,1988). Once this firm adhesion is established between the neutrophil integrins and the endothelial counter ligands, migration begins through the endothelium and the transition from the circulating pool to the tissue is complete.

Cytokine Modulation of Adhesion

The adhesion described above is very complex, yet it is only a fraction of the entire process. The elements missing are the sensory stimuli (the chemoattractants and cytokines) to which the neutrophil and endothelial cells respond by altering binding avidity or expression or shedding of adhesion molecules.

The binding avidity of neutrophil L-selectin can be upregulated by G-CSF, GM-CSF, and TNF-a (Spertini et al., 1991). As mentioned above, however, neutrophils shed L-selectin receptors following this upregulation. E-Selectin on endothelial cells is an inducible selectin whose expression can be upregulated by IL-l, TNF-α, and lipopolysaccharide (Bevilacqua et al., 1987; Gimbrone, Jr., et al., 1989). Following intradermal lipopolysaccharide injection, E-selectin expression is evident after 30 min, peaks at 8 h, and is sustained for up to 72 h; as expected, neutrophil tissue lo-

TABLE 1B. Endothelial and Neutrophil Adhesion Molecules and their Respective Ligands

Neutrophil Adhesion Molecules	Endothelial Counter Ligand
L-Selectin (CD62L)	s-Le^x (CD 15s)
CD11a/CD18	ICAM- 1 (CD54), ICAM-2 (CD1 02)
CD1 lb/CD18	ICAM-1 (CD54), [iC3b, fibrinogen, factor X]
CD11c/CD18	?, [iC3b, fibrinogen]

calization parallels the E-selectin expression (Silber et al., 1994). Kuijpers et al. (1994) investigated the effect of TNF-α stimulation on E-selectin expression in human umbilical vein endothelial cells (HUVEC) monolayers. Using confocal microscopy, they determined that monoclonal antibodies (mAbs) to E-selectin were internalized in a rapid, energy-dependent fashion, in contrast to ICAM- 1 and VCAM- 1, which remained surface-bound. After internalization, E-selectin was identified in intracellular tubular compartments and was co-localized with cathepsin B. A fluorescently labeled sLe^x-oligosaccharide was similarly internalized. The internalization process was not inhibited by protein kinase C, CAMP-dependent protein kinase A, tyrosine kinase, or cytochalasin B. However, colchicine and vinblastine inhibited formation of the intracellular tubular structures and increased surface expression, thus indicating a tubulin-driven process (Kuijpers et al., 1994). In *Rickettsia rickettsii*-infected endothelial cells, E-selectin expression has also been demonstrated to aid in recruitment of neutrophils (Spom et al., 1993). Tat protein, an HIV gene product, has been shown to increase E-selectin expression on central nervous system-derived endothelial cells (Hofman et al., 1994).

Subramaniam and co-workers (1993) compared P-selectin and E-selectin and confirmed that E-selectin was not stored but synthesized after induction by cytokines, and after a brief cell surface exposure was internalized and degraded by lysosomes. In contrast, P-selectin was stored, recycled to storage granules, and reused. The release of P-selectin from Weibel–Palade bodies is stimulated by thrombin, histamine, phorbol esters, and trypsin, but not by IL-l (Collins et al., 1993; Geng et al., 1990). Asako et al. (1994) infused histamine into rat mesentery and demonstrated increased leukocyte rolling and decreased velocity and increased albumin leakage. The effects were inhibited by anti-P-selectin mAb, soluble sLe^x oligosaccharide, and H_1 but not H_2 receptor antagonists. Recent evidence also indicates that inhibition of nitric oxide ($\dot{N}O\cdot$) promotes P-selectin expression and leukocyte rolling whereas $NO\cdot$ donors can reduce P-selectin expression on endothelial cells (Davenpeck, et al., 1994, Gauthier et al., 1994) (Fig. 2).

Some β_2 integrin/ICAM-1 adhesions are also modulated by various inflammatory cytokines. CD 11 a/CD 18 is not upregulated by inflammatory mediators. However, CD 11 b/CD 18 molecules are stored in secondary and tertiary granules of neutrophils and the integrins are mobilized and quantitatively upregulated by TNF-α, LTB,, and phorbol esters (Miller et al., 1987; Jones et al., 1988; Freyer et al., 1988; Huber et al., 1991). After stimulation of endothelial cells with TNF-α, IL- la, IL-l β, y-IFN, or LPS, a dose-dependent increase in ICAM-1 expression is detectable within 2 h and peaks 24 h after activation (Fabry et al., 1992; de Fougerolles et al., 1991). Although unstimulated HUVEC have a lo-fold greater expression of ICAM-2 than ICAM- 1, ICAM-2 expression is unaffected by inflammatory cytokines (de Fougerolles et al., 199 1). Fabry et al. (1992) demonstrated expression of ICAM- 1 and ICAM-2 in 30-40 and 15–20%, respectively, of nonactivated murine brain microvascular endothelial cells. Additionally, they showed that transforming growth factor beta (TGF-β) did not increase ICAM-1 expression in murine brain microvascular endothelial cells and that ICAM-2 was not upregulated by pro-inflammatory cytokines. In contrast, Suzuki et al. (1994) have recently reported that TGF-β 1 increased the expression of ICAM- 1

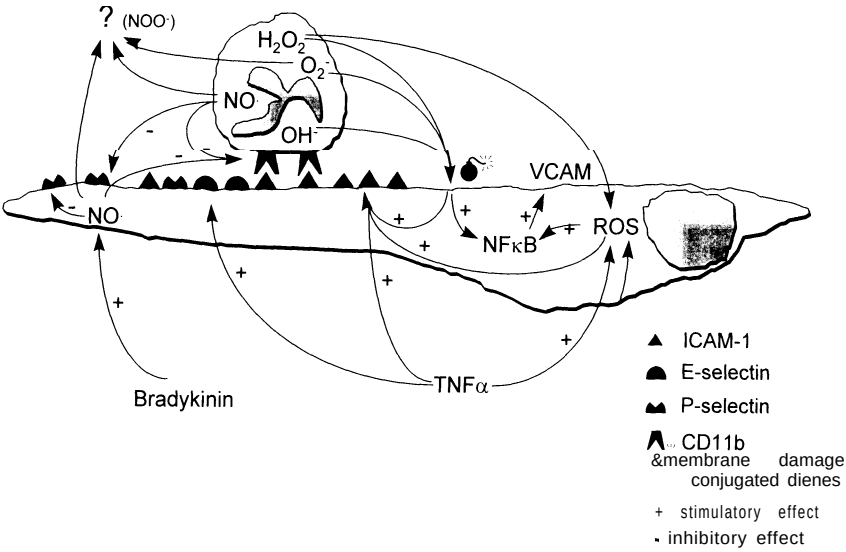

Fig. 2. Diagram of some of the known and unknown interactions between neutrophils and endothelial cells. Nitric oxide (NO·) and reactive oxygen species (ROS) are produced by both neutrophils and endothelial cells.

on HUVEC and induced the potential for neutrophil-mediated lung injury as indicated by increases in ^{125}I-labeled albumin and neutrophils in lung tissue and bronchoalveolar lavage fluid. Margiotta et al. (1992) reported increased ICAM- 1 expression in human saphenous vein endothelial cells exposed to Dacron, an observation with some obvious implications for vascular prostheses and other implants.

Another key cytokine in neutrophil and endothelial cell interactions is IL-8. Interleukin-8 has been reported to inhibit neutrophil adhesion to endothelial cells and yet induce neutrophil migration into tissues (Gimbrone et al., 1989; Huber et al., 1991; Kunkel et al., 1991). As part of these investigations, IL-8 has been shown to induce L-selectin shedding and β_2 integrin upregulation (Huber et al., 1991). Solomkin et al. (1994) investigated these findings in vivo and reported that 3 h after humans were infused with endotoxin, IL-8 concentrations were elevated but TNF-α concentrations were not. Neutrophils isolated from these subjects had decreased L-selectin expression, which would reduce their ability to adhere to activated endothelial cells. Thus an inflammatory lesion with IL-8 as the predominant cytokine may impair neutrophil delivery to that area. In addition to lipopolysaccharide and TNF-α, IL-l induces production of IL-8 by endothelial cells that can be inhibited by IL- lra (Kaplanski et al., 1994). Westlin et al. (1992) used confocal microscopy to demonstrate that IL-8 induced rapid F-actin polymerization in neutrophils and that the rate of depolymerization was inversely related to the IL-8 concentration; chemotactic factors C5a and fMLP showed similar effects. The authors concluded that these agonists may help regulate actin polymerization in neutrophils, which is crucial for neutrophil adhesion to endothelium.

Interleukin-2 has recently been proposed in many antineoplastic therapies; however, various toxicities including a "vascular leak" phenomenon have proved problematic. Recognizing that IL-2 has limited direct effects on neutrophils but may have mediating ones through TNF-a, Edwards et al. investigated this effect (1992). Using fluorescein isothiocyanate-labeled albumin and intravital microscopy, they verified macromolecular leakage and increased leukocyte-endothelial adhesion that was inhibited by a polyclonal antibody against TNF-α.

Many other interleukins have been investigated for their effect on endothelial cells, but interleukins that affect non-neutrophilic granulocytes (basophils and eosinophils) are of specific interest. Interleukin-4 and IL- 13 induce the expression of VCAM- 1 on endothelial cells but not an increase in ELAM- 1 or ICAM-1 expression (Schleimer et al., 1992; Bochner et al., 1995). The ligand for VCAM-1 on eosinophils and basophils is VLA-4, which is lacking on neutrophils; this may partly explain the enrichment of basophils and eosinophils versus neutrophils in allergic diseases (Schleimer et al., 1992; Bochner et al., 1991; Walsh et al., 1991; Bochner et al., 1995). Interleukin-3 has been shown to increase surface expression of CD 11 b on basophils and adhesion to HUVEC but has no effect on neutrophil adhesiveness (Bochner et al., 1990). Interleukin-5 induces upregulation of eosinophil CR3 but not neutrophil CR3 and thus IL-5 enhances eosinophil (not neutrophil) adhesion to endothelial cells via β_2 integrins (Walsh et al., 1990). In contrast, IL-l can induce increased adhesion of eosinophils, basophils, and neutrophils by inducing expression of VCAM-1, ICAM-1, and E-selectin (Bochner et al., 1991; Walsh et al., 1991).

In neutrophil-endothelial cell experiments, with current techniques it is very difficult if not impossible to control for all variables, but crucial to realize their effect. Silvestro et al. recently reported (1994) that heparin and its desulfated derivatives inhibit neutrophil adhesion to endothelial cells in a dose-dependent manner. The effects observed appeared to be related to inhibitory properties on the neutrophils and not the endothelial cells. Others have reported the direct or indirect inhibitory effect of heparin on the complement system, which may obviously also affect neutrophil adhesion or neutrophil activation (Cheung et al., 1994; Moen et al., 1995). Temperature has also been shown to have a significant effect on CD 11 b and CD 1 lc expression in neutrophils (Le Deist et al., 1994).

Cytoskeletal Factors Regulating Adhesion, Emigration, and Transport

The intention behind the term "integrin" receptor was to describe an integration of the extracellular matrix with the intracellular cytoskeleton via these receptors (Tamkun et al., 1986). However, relatively little research has focused on these interactions, especially compared to the information known about the β_2 integrin subunit, ICAM, and the cytoskeleton. Several reports have indicated different responses by neutrophils in solution compared to adherent neutrophils. Nathan et al. (1989) demonstrated that adherent neutrophils released H_2O_2 in response to TNF-α, whereas neutrophils in suspension failed to respond accordingly. It was further shown that the production of ROS upon TNF-α stimulation was mediated through β_2 integrin receptors via tyrosine phosphorylation of paxillin (Fuortes et al., 1994). Richter et al. (1990)

investigated TNF-a-induced degranulation in adherent neutrophils and demonstrated that degranulation depended on CD 11 b/CD 1 &triggered oscillatory cycles of unbound cytosolic calcium and that this could be inhibited by anti-CD 18 mAb. β_1 and β_3 cytoplasmic domains also contain a tyrosine kinase phosphorylation site, suggesting a functional or regulatory interaction of these integrins (Tamkun et al., 1986). Asako et al. (1992) assessed the role of the cytoskeleton on adhesion and emigration by utilizing intravital microscopy of rat mesentery and phalloidin, a microfilament stabilizer. Their findings demonstrated reduced emigration but normal adherence with low-dose phalloidin and reduced leukocyte adherence and emigration with high-dose phalloidin. Another group evaluated macromolecular transport in bovine endothelial monolayers with a fluid-phase endocytic tracer, fluorescein isothiocyanate-labeled dextran-70 (Liu et al., 1993). Using pharmacological modulation, they determined that translocation occurred by chains of vesicles that required microtubules and that microfilaments hinder the vesicle transport. Clearly this research area is ripe with opportunities to utilize the capabilities of intracellular and extracellular fluorescent markers, especially with the visual discrimination possible with confocal microscopy and physiological impact of time-resolved studies.

THE EFFECTS OF NUCLEAR FACTOR-κB, REACTIVE OXYGEN SPECIES, AND NITRIC OXIDE ON ENDOTHELIAL CELL PHYSIOLOGY AND PATHOLOGY

Nuclear Factor-κB

Many publications have linked nuclear factor-κB (NF-κB) activation to reactive oxygen species (Staal et al., 1990; Schreck et al., 1991). Weber et al. (1994) investigated the role of antioxidants in inhibiting VCAM-1 and ICAM-1 expression in TNF-α-stimulated endothelial cells. The antioxidant pyrrolidine dithiocarbamate (PDTC) inhibited NF-κB and blocked VCAM- 1 but not ICAM-1 expression in TNF-α-stimulated HUVEC, suggesting that only VCAM-1 was controlled by NF-κB. Moynagh et al. (1994) reported similar findings on human glial cells, yet a binding site for NF-κB has been identified in the S-regulatory region of the human gene for ICAM- 1 (Voraberger et al., 1991). Aspirin has been reported to inhibit NF-κB by preventing the degradation of the NF-κB inhibitor I kappa B (Kopp and Ghosh, 1994). Accordingly, aspirin has been shown to induce the inhibition of VCAM-1 and E-selectin but not ICAM-1 by TNF-a-stimulated HUVEC (Weber et al., 1995). Salicylates have since been shown to reduce transendothelial migration of neutrophils (Pierce et al., 1996).

ICAM-1 and L-Selectin

The ability of H_2O_2 to increase adhesion to HUVEC through ICAM-1 has been investigated. A dose-dependent response was observed with a two- to threefold increase in ICAM-1 expression noted between 0.5 and 1 h after H_2O_2 challenge (Lo et al.,

1993). Suzuki et al. (199 1) showed an increase in leukocyte adhesion induced by neutrophil oxidants H_2O_2 and monochloramine (NH_2Cl) that was inhibited by an anti-CD18 mAb. Sellak et al. (1994) evaluated adherence of unstimulated neutrophils on HUVEC exposed to nonlytic concentrations of ROS. Their data suggested increased adhesion involving ICAM- 1 but independent of ICAM- 1 upregulation and included a non-ICAM- 1 mechanism that recognized L-selectin on neutrophils. However, it has been demonstrated that ROS induce a rapid loss of L-selectin from the neutrophil surface (Fraticelli et al., 1996).

When investigating ICAM expression on endothelial cells and the effects of neutrophils, it is important to remember that many cells other than endothelial cells express ICAM- 1. For example, Ikeda et al. (1994) investigated the expression of ICAM-1 in keratinocytes and showed that ROS increased epidermal ICAM- 1 expression and that some antioxidants prevented the expression. Furthermore, it has been demonstrated using intracellular cardiac myocyte ROS with 2′,7′-dichlorofluorescein and myocyte ICAM- 1 expression that binding of CD1 1b/CD1 8 and ICAM- 1 was necessary for activation of the neutrophil oxidative burst and thus ROS-mediated injury (Entman et al., 1992).

In neutrophils, a link between L-selectin binding and ROS generation was shown when Waddell et al. (1994) determined that cross-linking L-selectin with DREG and secondary antibody failed to generate ROS but enhanced the response of fMLP and TNF-α to produce ROS. Additionally an intracellular rise in Ca^{2+} was measured upon L-selectin cross-linking. Similar signaling roles for other adhesion molecules in neutrophils and endothelial cells are likely.

Reactive Oxygen Species Generation

Acombination of two fluorochromes, 2′,7′-dichlorofluorescin diacetate (DCFH-DA) and hydroethidine (HE), has been used to measure rat pulmonary artery endothelial ROS generation after stimulation with H_2O_2 (Carter et al., 1994). It was determined that superoxide anion (O_2^-) was generated partially by the xanthine oxidase pathway after stimulation with H_2O_2. Additional studies with confocal microscopy have suggested that O_2^- generation occurs primarily in mitochondria. Using DCFH-DA and laser scanning confocal microscopy, Egawa et al. (1994) demonstrated an increased intracellular oxidation state in immortalized human endothelial cells (ECV304) after stimulation with TNF-α. This effect was blocked by free-radical scavengers *N*-acetylcysteine (a precursor of glutathione) and pyrrolidine dithiocarbamate. These findings appear to accord with results suggesting that TNF-α induced free-radical production that was necessary for NF-κB induction (Menon et al., 1993). Suematsu et al. (1993) used intravital fluorescence microscopy and DCFH-DA-loaded rat mesentery to evaluate oxidative changes in microvessels during neutrophil activation with platelet activating factor (PAF). By alternating light sources, they observed both neutrophil adhesion and significant increases in DCF fluorescence in endothelial cells as early as 10 min after PAF super-fusion. An iron chelator and hydroxyl radical scavenger both significantly attenuated the PAF-induced oxidative changes, suggesting a possible role for hydroxyl radical in endothelial oxidative stress. Al-Mehdi et al. (1994)

also used DCFH-DA to determine if oxidizing species were generated in lung ischemia-reperfusion (I/R) experiments in which rat lungs were isolated, oxygen ventilated, and perfused with artificial media. Dichlorofluorescin diacetate was added to the perfusate and tissue fluorescence following the ischemia/reperfusion injury was measured with a confocal laser scanning microscope. The authors showed that oxidizing species were generated after the I/R injury with predominant localization in endothelial, type II, Clara, and ciliated cells and macrophages.

The ROS generated by neutrophils, macrophages, and other cells obviously have potential for direct damage to cell membranes. Block (199 1) investigated the direct effects of H_2O_2 on membrane fluidity and function of porcine pulmonary artery endothelial cells. A 30-min exposure to 50 μM H_2O_2 increased conjugated dienes and significantly altered membrane fluidity 0.5 and 6 h post-exposure, but conditions returned to normal within 24 h (Block, 1991) (Fig. 2). Lipid membrane peroxidation and functional alterations were inhibited by pretreatment with a-tocopherol or dimethylthiourea.

Bradykinin is a vasoactive peptide and inflammatory mediator that also stimulates nitric oxide ($NO\cdot$) generation (Fig. 2). Bradykinin has also been shown to stimulate O_2^- generation via a mechanism that is partially inhibited by cyclooxygenase inhibitors (Holland et al., 1990). Shimizu et al. (1994) used DCFH-DA to assess ROS generation by endothelial cells after stimulation by bradykinin. The oxidation was inhibited by hydroxyl radical (OH-) scavengers and cyclooxygenase inhibitors but unaffected by inhibitors of $NO\cdot$. Furthermore, using Fluo-3 to measure intracellular calcium, they identified an intracellular calcium peak following bradykinin stimulation. These findings suggested that the ROS generated after bradykinin stimulation was OH- and not associated with $NO\cdot$ generation but instead may be related to a cyclooxygenase pathway.

In addition to neutrophils and other phagocytic cells, cancer cells (specifically Walker 256 cells) have been shown to generate ROS and thus may directly damage endothelial cells (Soares et al., 1994). Masuda et al. (1990) investigated the mechanism of endothelial injury in inherited-cataract rats. Using bromodeoxyuridine pulse labeling they showed a reduced cell cycle traverse rate for inherited-cataract rats and that neutrophil-generated ROS may be responsible for endothelial cell injury.

Nitric Oxide

Nitric oxide ($NO\cdot$) has been shown to exhibit a multitude of physiological and pathological effects. The interactions between phagocytic cells (neutrophils and macrophages) and endothelial cells become very complex when one attempts to understand the effects of ROS, including $NO\cdot$, not least because neutrophils, macrophages, and endothelial cells produce both $NO\cdot$ and superoxide anion (O_2^-), and these ROS can react with each other to form peroxynitrite ($ONOO^-$). An in-depth discussion of peroxynitrite is beyond the scope of this chapter (see Chapter 9), yet potentially very important considering the oxidizing capability of this molecule and the potential for cellular damage; for a general review we refer the reader to Szabó (1996). The complexity of these interactions is also difficult to segregate when one realizes the

relative nonspecificity of many fluorochromes to measure individual oxidizing agents. The authors have shown that rat pulmonary artery endothelial cells and human neutrophils loaded with DCFH-DA (2′,7′-dichlorofluorescin diacetate, a fluorochrome commonly used in phagocytic cell experiments to measure H_2O_2) demonstrated a significant increase in DCF fluorescence after the addition of 100 n*M* NO· or caged nitric oxide (potassium nitrosylpentachlororuthenate) (Robinson et al., 1994). The dose-dependent response observed suggested that NO· was directly oxidizing DCFH to DCF; however, another possible explanation was that peroxynitrite or hydroxyl radical produced by the combination of nitric oxide and O_2^- oxidized DCFH to DCF. In other studies 3-morpholinosydnonimine N-ethylcarbamide (SIN-1) was used to generate NO· and O_2^- simultaneously, with the conclusion that hydroxyl radical was formed (Hogg et al., 1992).

Intravital microscopy has been used to assess the effect of two NO· inhibitors (N^{ω}-monomethyl-L-arginine (L-NMMA) and N^{ω}-nitro-L-arginine methyl ester (L-NAME)) on leukocyte adhesion in feline mesenteric vessels (Kubes et al., 1991). The study demonstrated a 15-fold increase in leukocyte adhesion induced by the NO· inhibition that was blocked by an anti-CD1 8 mAb or L-arginine. Gaboury et al. (1993) evaluated the effect of NO· on leukocyte adhesion in a superoxide-generating system of hypoxanthine and xanthine oxidase (HX-X0) infusion, PAF infusion, or LTB, infusion into mesenteric vessels. They demonstrated that both superoxide dismutase and SIN-1 inhibited leukocyte adhesion in the HX-X0 infusion and PAF infusion but not the LTB, infusion and concluded that the anti-adhesive properties of NO· were likely related to the ability of NO· to inactivate O_2^-. Niu et al. (1994) recently investigated neutrophil adhesion in HUVEC by blocking NO· synthesis with L-NAME. At 1 h, these researchers observed no effect, but at 4 h after L-NAME administration, a dose-dependent increase in adhesion that was prevented with L-arginine and NO· donors. The adhesion was inhibited by mAb against CD 18 and ICAM-1, and by PAF receptor antagonist. In addition, intracellular oxygen radical scavengers inhibited the adhesion but extracellular oxygen radical scavengers had no effect. An increase in intracellular endothelial ROS generation in L-NAME-treated cells was confirmed with an increase in DCFH oxidation to DCF in NO·-blocked cells. Recently Biffl et al. (1996) demonstrated that NO· reduces endothelial expression of constitutive ICAM-1, but does not affect lipopolysaccharide-induced upregulation of ICAM-1 on endothelial cells. As mentioned above, inhibition of NO· has been shown to promote P-selectin expression and leukocyte rolling whereas nitric oxide (NO·) donors reduce P-selectin expression on endothelial cells (Davenpeck et al., 1994, Gauthier et al., 1994).

ENDOTHELIAL RESEARCH AND CONFOCAL MICROSCOPY

Technical Aspects of Live Endothelial Confocal Microscopy Research

Although confocal microscopy has been available for several years, performing experiments on live endothelial cells under physiological conditions creates special

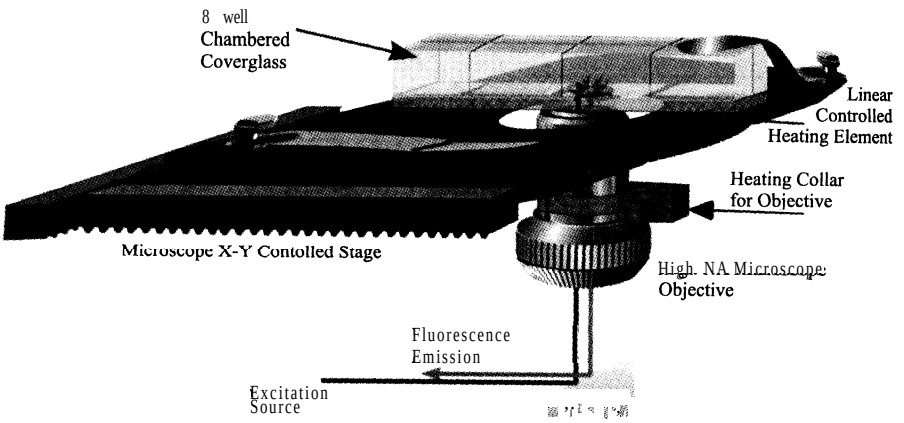

Fig. 3. Inverted microscope setup for endothelial cell studies. Reproduced with permission from Purdue Cytometry CDROM Volume 3, 1997.

problems. For proper optical assessment, these experiments require the use of an inverted scope with a heated stage that must not only be maintained within 0.1°C but also prevent electrical on-off cycle oscillations that result in specimen-focusing aberrations and subsequent poor image quality. Additionally, the cells must be grown in culture chambers made with optically pure coverglass (Lab-Tek® culture chambers are one commercial option) to allow close positioning of high-NA (numerical aperture) fluorescence microscope objectives. If an oil or water interface is used between the objective and coverglass, a heated objective is also recommended to prevent local heat loss and subsequent altered physiological conditions for the cells of most interest (those directly over the objective). Heated chambers that enclose the entire stage and objectives are an alternative but can be cumbersome and physically interfering to the operator. Figure 3 shows a successful setup using a BioRad MRC 1024 confocal microscope, a Nikon 300 inverted microscope, and endothelial cells cultured in a Lab-Tek® eight-well chambered coverglass.

Confocal Studies Assessing Endothelial Damage and Vascular Leakage

The development and evolution of confocal microscopy has provided many new techniques for scientists seeking understanding of endothelial cell physiology and pathology. Many fluorochromes are available to assess intracellular changes in a single cell and extracellular effects. For example, macromolecular leakage of intact endothelium has been studied utilizing a mesenteric window in rats injected with fluorescein isothiocyanate (FITC)-labeled albumin (Thurston et al., 1995). After being harvested and fixed, tissues were stained with rhodamine phalloidin to label filamentous actin and assess endothelial cytoskeleton. Thurston showed that focal leaks occurred in small areas of cell-cell contact and involved disruption of the endothelial peripheral actin rim. "Mid-leaks" involved one endothelial cell with extended disruption of

the peripheral actin rim, and "extended leaks" involved two or more adjacent endothelial cells (Thurston et al., 1995). Phalloidin labeled with FITC has been used to examine cytoskeletal organization of F-actin microfilaments during cell migration and translocation of cornea1 endothelial repair (Ichijima et al., 1993). After cornea1 injury, tissues were harvested, fixed, and labeled with FITC-phalloidin. Results indicated two separate, injury-dependent mechanisms of cornea1 repair: cell spreading and cell migration. Nagashima et al. (1994) used confocal microscopy to study the role of leukotriene C_4 on brain edema. They isolated cerebral capillary endothelial cells from rats and cultured the cells on an optically clear collagen membrane mounted in a plastic frame. Their study found an increase in permeability and no intracellular calcium flux with leukotriene C,.

Andries and Brutsaert (1994) used confocal microscopy to investigate the selective permeability of ventricular endocardial endothelium versus myocardial capillary endothelium. Intercellular clefts were shorter and deeper in endocardial endothelium than in capillary endothelium, suggesting that endocardial endothelium may be less permeable. However, Lucifer Yellow-labeled dextran 10,000 penetrated endocardial endothelium faster than capillary endothelium, but dextran 40,000 did not penetrate either endothelium. The authors concluded the structural differences observed may function to limit diffusion in the endocardial endothelial owing to the high hydrostatic pressure in the heart (Andries and Brutsaert, 1994).

Three-color analysis is also possible with a single-laser confocal microscope. Uchihara et al. (1995) used phycoerythrin-labeled HLA-DR, FITC-labeled von Willebrand factor (vWF), and cyanin 5-labeled amyloid beta protein to assess histopathological changes in brain tissue of Alzheimer's disease. Using an argon laser, these investigators sequentially imaged the three antigens and performed a computer reconstruction of the individual images for simultaneous evaluation of three distinct structures: microglia, endothelium, and A beta deposits.

Endothelial Responses to Shear Stress

Endothelial adhesion to substratum in real time has been assessed using tandem scanning confocal microscopy (Davies et al., 1993). By subtraction analysis of consecutive images the researchers recorded continuous remodeling of focal adhesion sites of endothelial cells during periods of less than 1 min yet with less than 10% variation in cell adhesion for the entire cell. Cytoskeletal disruption with cytochalasin or exposure of the endothelial cells to proteolytic enzymes resulted in a rapid reduction in cell adhesion. Later experiments by this same group assessed quantitative adhesion of endothelial cells exposed to shear stress (Davies et al., 1994). They demonstrated focal adhesion-site remodeling starting within 7-9 h in the direction of laminar flow (10 $dyne/cm^2$). Additionally they observed redistribution of intracellular stress fibers and coalescence of small focal adhesions into larger focal adhesions, resulting in fewer total adhesion sites per cell.

Walpola et al. (1993) evaluated the effect of low and high shear stress in vivo by partially ligating the left carotid artery in rabbits. There was an adaptive reduction in left carotid (low shear) artery diameter, desquamation of endothelial cells, and fewer

and shorter stress fibers than in normal cells. Although the right carotid artery (high shear) diameter was unchanged, their findings indicated an increase in stress fibers and a reduction in peripheral actin staining. Another group demonstrated an inducible cell membrane complex that developed strong adhesion to elastin fibers (Perdomo et al., 1994).

Surface Receptor Studies and Confocal Microscopy

Syn-capping of human T-lymphocyte adhesion molecules during interaction with endothelial cells has been identified through confocal microscopy (Rosenman et al., 1993). Of particular interest was the finding that CD2 co-capped with CD44 and CD 11 a/CD 18, suggesting functional cooperation of these molecules. Furthermore, CD2, CD44, and L-selectin redistributed to pseudopodia when exposed to HUVEC, but CD 1 la/CD18 remained globally distributed on the cell surface. Similar studies with neutrophils and endothelial cells exposed to ROS and activated with cytokines could provide key information to help understand physiological and pathological neutrophil-endothelial interactions.

Both confocal microscopy and immunogold electron microscopy were used to localize tumor necrosis factor (TNF) receptors on HUVEC in time-lapse experiments to demonstrate physiological changes (Bradley et al., 1995). In this study the authors identified a 75-kDa receptor on the cell surface, endocytosis, and transport to lysosomes through the coated pits and coated vesicles. Additionally they showed that TNF may accelerate clustering and internalization of the 75-kDa receptor. The 55-kDa receptor, expressed at much lower concentrations, was mostly unaffected by TNF.

The alpha 6 beta 4 integrin complex is expressed by epithelial cells and localized in hemidesmosomes (Stepp et al., 1990). Alpha 6 beta 4 was recently reported to be expressed in a subset of endothelial cells in mice (Kennel et al., 1992). However, Cremona et al. (1994) demonstrated by confocal microscopy that alpha 6 beta 4 was not expressed by endothelial cells, because they did not express von Willebrand factor. The higher resolution of confocal microscopy compared to conventional immunofluorescence permitted determination of the mAb to alpha 6 beta 4-labeled smooth muscle cells of small vessels.

ENDOTHELIAL RESEARCH IN SPECIFIC CLINICAL DISEASES

Trauma, Sepsis, and Adult Respiratory Distress Syndrome

Most clinicians and researchers agree that neutrophil-endothelial interaction is also a key contributor to the pathophysiology of adult respiratory distress syndrome (ARDS), which can be associated with many disease syndromes, including sepsis and trauma. Vogel et al. (1993) investigated the role of trauma and plasma products in the induction of neutrophil-independent endothelial cell damage. They determined that both fibrin degradation products (FDP-D and FDP-E) significantly decreased neutrophil adhesion to human endothelial cells in vitro and that endothelial cell membrane integrity was disturbed by serum from trauma patients. Another study evaluat-

ed microvascular function and rheological changes in hyperdynamic sepsis and showed that increased leukocyte adherence to microvascular endothelial cells contributed to rheological changes and compromised capillary cross-sectional area (Astiz et al., 1995). Fabian et al. (1994) investigated the effect of blocking adhesion through the CD 18 adhesion complex after traumatic shock and endotoxemia. An anti-CD18 mAb decreased lung injury and improved hemodynamic parameters in endotoxic pigs. Using intravital microscopy, Harris et al. (1994) assessed leukocyte–endothelial adhesion in feline mesenteric venules exposed to lipopolysaccharide. An anti-CD 1 l/CD 18 mAb and superoxide dismutase (SOD) significantly- attenuated leukocyte adhesion to endothelial cells, whereas an anti-ICAM-1 mAb and platelet activating factor receptor antagonist (PAFra) had no effect. Additionally, anti-CD1 l/CD 18, anti-ICAM- 1, SOD, and PAFra did not affect leukocyte emigration or leukocyte rolling. Kurose and co-workers induced intestinal mucosal erosions and increased tissue neutrophils and protein leakage with intravenous lactoferrin infusion; these alterations were reduced by anti-P-selectin mAb but not by anti-E-selectin (Kurose et al., 1994b).

Several disease models for pulmonary injury exist. One utilizes the intrapulmonary deposition of IgG immune complexes to elicit damage and has been used to investigate the effect of blocking several adhesion molecules by intravenous or intratracheal administration of mAb (Mulligan et al., 1995). The data suggested therapeutic compartmentalization. Intravenous mAbs against CD 1 la, L-selectin, and ICAM- 1 effectively reduced pulmonary neutrophils and lung injury. In contrast, with intratracheal administration (alveolar compartment), only CD 11 b and ICAM- 1 were protective and only CD 1 lb significantly reduced TNF-α concentrations in bronchoalveolar lavage fluid. In a different model of cobra venom factor-induced acute lung injury, Mulligan and co-workers reported in 1993 that sLe^x significantly reduced lung injury and tissue neutrophil accumulation.

Reactive oxygen species are believed to be a major contributor to the pathogenesis of pulmonary oxygen toxicity. In addition, ICAM- 1 expression is increased on inflamed alveolar epithelium, whereas an anti-ICAM- 1 mAb in pulmonary oxygen toxicity can reduce pulmonary neutrophil infiltration and pulmonary damage (Wegner et al., 1992).

Ischemia/Reperfusion Injuries

Another clinical syndrome that appears to involve endothelial cells directly is ischemia/reperfusion injuries. The primary pathophysiological process is still the subject of much debate; however, it is generally agreed that neutrophil-endothelial interactions and ROS generation contribute to the pathology. Although some scientists still debate the existence of the ischemia/reperfusion injury, it is important to recognize that experimental findings depend somewhat on the choice of animal species and organ.

Granger and co-workers have used intravital microscopy to observe neutrophil adhesion to endothelial cells following ischemia/reperfusion (I/R) injuries and assess the molecular components of adhesion, the role of ROS, and potential therapies. They

demonstrated decreased I/R-induced capillary permeability, leukocyte adhesion, and extravasation with adenosine and similar responses with dimethyl sulfoxide (Grisham et al., 1989; Sekizuka et al., 1989). Kurose et al. (1994a) used intravital fluorescence microscopy to evaluate the ability of several mAbs to block the reperfusion-induced leukocyte–endothelial interactions in rat mesentery. They verified that the magnitude of leukocyte adhesion was related to the duration of ischemia, as was albumin leakage. Monoclonal antibodies against CD 11 b and CD 18 consistently reduced leukocyte adhesion, whereas anti-ICAM-1 was effective only after 10 and 30 min of ischemia and anti-L-selectin only after 10 min of ischemia. P- and E-selectin did not reduce adhesion. Post-ischemic venules contained platelet-leukocyte aggregates that were blocked by mAbs against P-selectin, CD 11 b, CD 18, and ICAM- 1. A feline model of small intestine transplantation has been used to assess I/R injuries and the therapeutic potential of mAbs against several adhesion molecules (Slocum and Granger, 1993). Catalase, superoxide dismutase, mAbs against ICAM- 1, and P-selectin had no protective effect, although anti-CD 18 mAb prevented much of the microvascular dysfunction. In another study, HUVEC exposed to anoxia followed by reoxygenation (A/R) did not increase ICAM-1 or ELAM-1 expression; however, neutrophil adherence was blocked by mAbs against CD1 la, CD1 lb, CD18, and ICAM- 1 but not ELAM-1 (Yoshida et al., 1992). Furthermore, A/R HUVEC-conditioned medium increased CD 11 b and CD 18 expression on neutrophils whereas catalase and PAF antagonist diminished the increased adhesion. These results suggested that hydrogen peroxide and PAF mediated increased neutrophil adherence via CD1 la/CD 18 and CD 11 b/CD 18 with ICAM- 1 on endothelial cells. Farhood et al. (1995) used a rat model of hepatic I/R to assess ICAM-1 expression and the therapeutic efficacy of an anti-ICAM-1 mAb. ICAM-1 mRNA was evident in the ischemic liver lobes during ischemia and in the ischemic and nonischemic lobes during reperfusion. Therapeutically, anti-ICAM- 1 mAb reduced hepatocyte injury as indicated by a 5 1% reduction in plasma alanine aminotransferase concentrations and reduced hepatic necrosis; however, no significant reduction in hepatic neutrophil infiltration occurred during reperfusion.

The endothelial changes following myocardial I/R injuries have been studied in several animal models, and recently extensive research has been published on the feline model. Ma et al. (1992) investigated the cardioprotective effect of anti-ICAM-1 in a feline myocardial I/R injury. Their results indicated reduced myocardial necrosis, reduced tissue neutrophil infiltration, and increased endothelial-dependent relaxation. Another feline myocardial I/R study demonstrated cardioprotective effects of P-selectin (Weyrich et al., 1993). These results suggested that cardioprotection was directly related to reduced neutrophil adhesion to endothelial cells and demonstrated significant upregulation of P-selectin in endothelial cells that lined coronary vessels. Weyrich et al. (1995) also demonstrated sequential expression of P-selectin, ICAM-1, and E-selectin, respectively, after feline myocardial I/R injury and suggested that P-selectin and ICAM- 1 expression coordinated with neutrophil trafficking. Cardioprotection has also been demonstrated with anti-L-selectin mAb, sLe^x oligosaccharide, and L-arginine (Ma et al., 1993; Buerke et al., 1994; Weyrich et al., 1992). Using the Langendorff model of an isolated perfused rat heart, Kamikubo (1993) demonstrated that in the early phase of myocardial ischemia, myocardial dysfunction

and cytokine gene expression can occur without the infiltration of inflammatory cells. Kamikubo also measured an increased in the expression of IL-6, IL 8, y-IFN, TNF-α, and IL- la, yet found no detectable increase in the expression of IL- 1 β, MCP- 1, and IL- lra measured by semiquantitative reverse transcription/polymerase chain reaction (RT-PCR).

CONCLUSION

Endothelial and neutrophil research continues to evolve with the cytometric tools and molecular techniques available today. Integrating endothelial and neutrophil (and other cell) research at the cytoskeletal and molecular level with techniques discussed above will allow us more fully to understand these cellular interactions and signalling processes. Creative combinations of these techniques and future developments and refinements will initiate new cytometric evaluations of cellular interactions. These capabilities will certainly yield novel understandings of endothelial pathophysiology and endothelial-neutrophil interactions.

REFERENCES

Al-Mehdi A, Shuman H, Fisher AB (1994): Fluorescence microtopography of oxidative stress in lung ischemia-reperfusion. Lab Invest 70:579–587.

Altieri DC, Morrissey JH, Edgington TS (1988): Adhesive receptor Mac- 1 coordinates the activation of factor X on stimulated cells of monocytic and myeloid differentiation: an alternative initiation of the coagulation protease cascade. Proc Natl Acad Sci USA 85:7462–7466.

Andries LJ, Brutsaert DL (1994): Endocardial endothelium in the rat: junctional organization and permeability. Cell Tissue Res 277:391–400.

Asako H, Kurose I, Wolf R, et al. (1994): Role of H 1 receptors and P-selectin in histamine-induced leukocyte rolling and adhesion in postcapillary venules. J Clin Invest 93: 1508-l 5 15.

Asako H, Wolf RE, Granger DN, Korthuis RJ (1992): Phalloidin prevents leukocyte emigration induced by proinflammatory stimuli in rat mesentery. Am J Physiol 263:H 1637-H 1642.

Astiz ME, DeGent GE, Lin RY, Rackow EC (1995): Microvascular function and rheologic changes in hyperdynamic sepsis. Crit Care Med 23:265–27 1.

Beller DI, Springer TA, Schreiber RD (1982): Anti-Mac-l selectively inhibits the mouse and human type three complement receptor. J Exp Med 156: 1 000– 1009.

Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone MA, Jr. (1987): Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Natl Acad Sci USA 84:9238–9242.

Biffl WL, Moore EE, Moore FA, Barnett C (1996): Nitric oxide reduces endothelial expression of intercellular adhesion molecule (ICAM)- 1. J Surg Res 63:328–332.

Bilsland CA, Diamond MS, Springer TA (1994): The leukocyte integrin p 150,95 (CD 1 1c/CD 18) as a receptor for iC3b. Activation by a heterologous beta subunit and localization of a ligand recognition site to the I domain. J Immunol 152:4582–4589.

Block ER (199 1): Hydrogen peroxide alters the physical state and function of the plasma membrane of pulmonary artery endothelial cells. J Cell Physiol 146:362–369.

Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP (1995): IL- 13 selectively induces vascular cell adhesion molecule-l expression in human endothelial cells. J Immunol 154:799–803.

Bochner BS, Luscinskas FW, Gimbrone MA, Jr., Newman W, Sterbinsky SA (1991): 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-l 557.

Bochner BS, McKelvey AA, Sterbinsky SA, et al. (1990): IL-3 augments adhesiveness for endothelium and CD1 1 b expression in human basophils but not neutrophils. J Immunol 145: 1832-l 837.

Bradley JR, Thiru S, Pober JS (1995): Disparate localization of 55-kd and 75-kd tumor necrosis factor receptors in human endothelial cells. Am J Pathol 146:27–32.

Buerke M, Weyrich AS, Zheng Z, Gaeta FC, Forrest MJ, Lefer AM (1994): Sialyl Lewisx-containing oligosaccharide attenuates myocardial reperfusion injury in cats. J Clin Invest 93: 1140-l 148.

Carlos TM, Harlan JM (1994): Leukocyte-endothelial adhesion molecules. Blood 84:2068–2 101.

Carter WO, Narayanan PK, Robinson JP (1994): Intracellular hydrogen peroxide and superoxide anion detection in endothelial cells. J Leukoc Biol 55:253–258.

Cheung AK, Faezi-Jenkin B, Leypoldt JK (1994): Effect of thrombosis on complement activation and neutrophil degranulation during in vitro hemodialysis. J Am Soc Nephrol 5: 11 O-l 15.

Collins PW, Macey MG, Cahill MR, Newland AC (1993): von Willebrand factor release and P-selectin expression is stimulated by thrombin and trypsin but not IL- 1 in cultured human endothelial cells. Thromb Haemost 70:346–350.

Cremona 0, Savoia P, Marchisio PC, Gabbiani G, Chaponnier C (1994): The alpha 6 and beta 4 integrin subunits are expressed by smooth muscle cells of human small vessels: a new localization in mesenchymal cells. J Histochem Cytochem 42: 122 l-l 228.

Davenpeck KL, Gauthier TW, Lefer AM (1994): Inhibition of endothelial-derived nitric oxide promotes P-selectin expression and actions in the rat microcirculation. Gastroenterology 107: 1050– 1058.

Davies PF, Robotewskyj A, Griem ML (1993): Endothelial cell adhesion in real time. Measurements in vitro by tandem scanning confocal image analysis. J Clin Invest 91:2640–2652.

Davies PF, Robotewskyj A, Griem ML (1994): Quantitative studies of endothelial cell adhesion. Directional remodeling of focal adhesion sites in response to flow forces. J Clin Invest 93:2031–2038.

de Fougerolles AR, Stacker SA, Schwarting R, Springer TA (199 1): Characterization of ICAM-2 and evidence for a third counter-receptor for LFA- 1. J Exp Med 174:253–267.

Diamond MS, Staunton DE, de Fougerolles AR, et al. (1990): ICAM- 1 (CD54): a counter-receptor for Mac- 1 (CD 11 b/CD 18). J Cell Biol 111:3 129-3 139.

Edwards MJ, Abney DL, Heniford BT, Miller FN (1992): Passive immunization against tumor necrosis factor inhibits interleukin-2-induced microvascular alterations and reduces toxicity. Surgery 112:480–486.

Egawa K, Yoshiwara M, Nose K (1994): Effect of radical scavengers on TNF alpha-mediated activation of the uPA in cultured cells. Experientia 50:958–962.

Entman ML, Youker K, Shoji T, et al. (1992): Neutrophil induced oxidative injury of cardiac myocytes. A compartmented system requiring CD 11 b/CD 18-ICAM- 1 adherence. J Clin Invest 90: 1335-l 345.

Fabian TC, Croce MA, Stewart RM, Dockter ME, Proctor KG (1994): Neutrophil CD1 8 expression and blockade after traumatic shock and endotoxin challenge. Ann Surg 220:552–561.

Fabry Z, Waldschmidt MM, Hendrickson D, et al. (1992): Adhesion molecules on murine brain microvascular endothelial cells: expression and regulation of ICAM-1 and Lgp 55. J Neuroimmunol 36: l-l 1.

Farhood A, McGuire GM, Manning AM, Miyasaka M, Smith CW, Jaeschke H (1995): Intercellular adhesion molecule 1 (ICAM- 1) expression and its role in neutrophil-induced ischemia-reperfusion injury in rat liver. J Leukocyte Biol 57:368–374.

Foxall C, Watson SR, Dowbenko D, et al. (1992): The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis(x) oligosaccharide. J Cell Biol 117:895–902.

Fraticelli A, Serrano CV, Bochner BS, Capogrossi MC, Zweier JL (1996): Hydrogen peroxide and superoxide modulate leukocyte adhesion molecule expression and leukocyte endothelial adhesion. Biochim Biophys Acta 13 10:25 l-259.

Freyer DR, Morganroth ML, Rogers CE, Arnaout MA, Todd RF 3d (1988): Modulation of surface CD 1 l/CD 18 glycoproteins (Mo 1, LFA- 1, p 150, 95) by human mononuclear phagocytes. Clin Immunol Immunopathol 46:272–283.

Fuortes M, Jin WW, Nathan C (1994): Beta 2 integrin-dependent tyrosine phosphorylation of paxillin in human neutrophils treated with tumor necrosis factor. J Cell Biol 127: 1477-1483.

Gaboury J, Woodman RC, Granger DN, Reinhardt P, Kubes P (1993): Nitric oxide prevents leukocyte adherence: role of superoxide. Am J Physiol 265:H862–H867.

Gauthier TW, Davenpeck KL, Lefer AW (1994): Nitric oxide attenuates leukocyte-endothelial interaction via P-selectin in splanchnic ischemia-reperfusion. Am J Physiol 267:G562–G568.

Geng JG, Bevilacqua MP, Moore KL, McIntyre TM, Prescott SM, Kim (1990): Rapid neutrophil adhesion to activated endothelium mediated by GMP- 140. Nature 343:757–760.

Gimbrone MA, Jr., Obin MS, Brock AF, et al. (1989): Endothelial interleukin-8: a novel inhibitor of leukocyte-endothelial interactions. Science 246: 160 1- 1603.

Griffin JD, Spertini 0, Ernst TJ, et al. (1990): Granulocyte-macrophage colony-stimulating factor and other cytokines regulate surface expression of the leukocyte adhesion molecule- 1 on human neutrophils, monocytes, and their precursors. J Immunol 145:576–584.

Grisham MB, Hernandez LA, Granger DN (1989): Adenosine inhibits ischemia-reperfusion-induced leukocyte adherence and extravasation. Am J Physiol 257:H 1334-H 1339.

Harris NR, Russell JM, Granger DN (1994): Mediators of endotoxin-induced leukocyte adhesion in mesenteric postcapillary venules. Circ Shock 43: 155-l 60.

Hofman FM, Dohadwala MM, Wright AD, Hinton DR, Walker SM (1994): Exogenous tat protein activates central nervous system-derived endothelial cells. J Neuroimmunol 54: 19-28.

Hogg N, Darley Usmar VM, Wilson MT, Moncada S (1992): Production of hydroxyl radicals from the simultaneous generation of superoxide and nitric oxide. Biochem J 28 1:4 19-424.

Holland JA, Pritchard KA, Pappolla MA, Wolin MS, Rogers NJ, Sternerman MB (1990): Bradykinin induces superoxide anion release from human endothelial cells. J Cell Physiol 143:2 l-25.

Huber AR, Kunkel SL, Todd RF 3d, Weiss SJ (199 1): Regulation of transendothelial neutrophil migration by endogenous interleukin-8. Science 254:99–1 02 [published errata appear in Science 199 1 254(5032):63 1 and 199 1 254(5037): 1435].

Ichijima H, Petrol1 WM, Barry PA, et al. (1993): Actin filament organization during endothelial wound healing in the rabbit cornea: comparison between transcorneal freeze and mechanical scrape injuries. Invest Ophthalmol Vis Sci 34:2803–28 12.

Ikeda M, Schroeder KK, Mosher LB, Woods CW, Akeson AL (1994): Suppressive effect of antioxidants on intercellular adhesion molecule- 1 (ICAM- 1) expression in human epidermal keratinocytes. J Invest Dermatol 103:79 l-796.

Imai Y, True DD, Singer MS, Rosen SD (1990): Direct demonstration of the lectin activity of gp90MEL, a lymphocyte homing receptor. J Cell Biol 111: 1225-l 232.

Jacob GS, Kirmaier C, Abbas SZ, Howard SC, Steininger CN, Welply (1995): Binding of sialyl Lewis x to E-selectin as measured by fluorescence polarization. Biochemistry 34: 12 10– 12 17.

Jaffe EA, Nachman RL, Becker CG, Minick CR (1973): Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J Clin Invest 52:2745–2756.

Jones DH, Anderson DC, Burr BL, et al. (1988): Quantitation of intracellular Mac- 1 (CD 11 b/CD 18) pools in human neutrophils. J Leukoc Biol 44:535–544.

Kamikubo Y (1993): [Cardiac dysfunction and endogenous cytokines in global ischemia and reperfusion injury]. Hokkaido Igaku Zasshi 68:8 13-826.

Kaplanski G, Farnarier C, Kaplanski S, et al. (1994): Interleukin- 1 induces interleukin-8 secretion from endothelial cells by a juxtacrine mechanism. Blood 84:4242–4248.

Kennel SJ, Godfrey V, Ch'ang LY, Lankford TK, Foote LJ, Makkinje A (1992): The beta 4 subunit of the integrin family is displayed on a restricted subset of endothelium in mice. J Cell Sci 101: 145-l 50.

Kopp E, Ghosh S (1994): Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 265:956–959.

Kotovuori P, Tontti E, Pigott R, et al. (1993): The vascular E-selectin binds to the leukocyte integrins CD1 1/CD18. Glycobiology 3:131–136.

Kubes P, Suzuki M, Granger DN (1991): Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 88:465 l-4655.

Kuijpers TW, Hoogerwerf M, van der Laan LJW, et al. (1992): CD66 nonspecific cross-reacting antigens are involved in neutrophil adherence to cytokine-activated endothelial cells. J Cell Biol 118:457–466.

Kuijpers TW, Raleigh M, Kavanagh T, et al. (1994): Cytokine-activated endothelial cells internalize E-selectin into a lysosomal compartment of vesiculotubular shape. A tubulin-driven process. J Immunol 152:5060–5069.

Kunkel SL, Standiford T, Kasahara K, Strieter RM (199 1): Interleukin-8 (IL-8): the major neutrophil chemotactic factor in the lung. Exp Lung Res 17: 17-23.

Kurose I, Anderson DC, Miyasaka M, et al. (1994a): Molecular determinants of reperfusion-induced leukocyte adhesion and vascular protein leakage. Circ Res 74:336–343.

Kurose I, Yamada T, Wolf R, Granger DN (1994b): P-selectin-dependent leukocyte recruitment and intestinal mucosal injury induced by lactoferrin. J Leukoc Biol 55:771–777.

Le Deist F, Menasche P, Kucharski K, Bel A, Piwnica A, Bloch G (1994): Hypothermia during cardiopulmonary bypass delays but does not prevent neutrophil-endothelial cell adhesion. Circulation 90: l-48.

Ley K, Gaehtgens P, Fennie C, Singer MS, Lasky LA, Rosen SD (199 1): Lectin-like cell adhesion molecule 1 mediates leukocyte rolling in mesenteric venules in vivo. Blood 77:2553–2555.

Liu SM, Magnusson KE, Sundqvist T (1993): Microtubules are involved in transport of macromolecules by vesicles in cultured bovine aortic endothelial cells. J Cell Physiol 156:3 1 l-3 16.

Lo SK, Janakidevi K, Lai L, Malik AB (1993): Hydrogen peroxide-induced increase in endothelial adhesiveness is dependent on ICAM- 1 activation. Am J Physiol 264:L406–4 12.

Loike JD, Sodeik B, Cao L, et al. (199 1): CD1 1 c/CD 18 on neutrophils recognizes a domain at the N terminus of the A alpha chain of fibrinogen. Proc Natl Acad Sci USA 88: 1044-1048.

Lorant DE, Pate1 KD, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA (199 1): Coexpression of GMP- 140 and PAF by endothelium stimulated by histamine or thrombin: a juxtacrine system for adhesion and activation of neutrophils. J Cell Biol 115:223–234.

Ma XL, Lefer DJ, Lefer AM, Rothlein R (1992): Coronary endothelial and cardiac protective effects of a monoclonal antibody to intercellular adhesion molecule- 1 in myocardial ischemia and reperfusion. Circulation 86:937–946.

Ma XL, Weyrich AS, Lefer DJ, et al. (1993): Monoclonal antibody to L-selectin attenuates neutrophil accumulation and protects ischemic reperfused cat myocardium. Circulation 88:649–658.

Margiotta MS, Robertson FS, Greco RS (1992): The adherence of endothelial cells to Dacron induces the expression of the intercellular adhesion molecule (ICAM- 1). Ann Surg 2 16:600–604.

Maruyama Y (1963): The human endothelial cell tissue culture. Z Zellforsch Mikrosk Anat 60:69–79.

Masuda M, Ihara N, Komiyama Y, Murakami T, Murata K (1990): Flow cytometric study of injuries in cultured endothelial cells by neutrophils of the inherited cataract rats. Atherosclerosis 84:245–254.

Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD (1993): Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. Cell 74:541–554.

McEver RP, Beckstead JH, Moore KL, Marshall Carlson L, Bainton DF (1989): GMP- 140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J Clin Invest 84:92–99.

Menon SD, Qin S, Guy GR, Tan YH (1993): Differential induction of nuclear NF-kappa B by protein phosphatase inhibitors in primary and transformed human cells. Requirement for both oxidation and phosphorylation in nuclear translocation. J Biol Chem 268:26805–268 12.

Miller LJ, Bainton DF, Borregaard N, Springer TA (1987): Stimulated mobilization of monocyte Mac- 1 and p150,95 adhesion proteins from an intracellular vesicular compartment to the cell surface. J Clin Invest 80:535–544.

Moen 0, Fosse E, Brockmeier V, et al. (1995): Disparity in blood activation by two different heparin-coated cardiopulmonary bypass systems. Ann Thorac Surg 60: 13 17– 1323.

Moynagh PN, Williams DC, O'Neill LA (1994): Activation of NF-kappa B and induction of vascular cell adhesion molecule- 1 and intracellular adhesion molecule- 1 expression in human glial cells by IL- 1. Modulation by antioxidants. J Immunol 153:268 l-2690.

Mulligan MS, Paulson JC, De Frees S, Zheng ZL, Lowe JB, Ward PA (1993): Protective effects of oligosaccharides in P-selectin-dependent lung injury. Nature 364: 149-l 5 1.

Mulligan MS, Vaporciyan AA, Warner RL, et al. (1995): Compartmentalized roles for leukocytic adhesion molecules in lung inflammatory injury. J Immunol 154: 1350-l 363.

Nagashima T, Shigin W, Mizoguchi A, Arakawa M, Yamaguchi M, Tamaki N (1994): The effect of leukotriene C4 on the permeability of brain capillary endothelial cell monolayer. Acta Neurochir Suppl Wien 60:55–57.

Nathan C, Srimal S, Farber C, et al. (1989): Cytokine-induced respiratory burst of human neutrophils: dependence on extracellular matrix proteins and CD 1 l/CD 18 integrins. J Cell Biol 109: 134 l-l 349.

Niu XF, Smith CW, Kubes P (1994): Intracellular oxidative stress induced by nitric oxide synthesis inhibition increases endothelial cell adhesion to neutrophils. Circ Res 74: 1133– 1140.

Patel KD, Moore KL, Nollert MU, McEver RP (1995): Neutrophils use both shared and distinct mechanisms to adhere to selectins under static and flow conditions. J Clin Invest 96: 1887-I 896.

Perdomo JJ, Gounon P, Schaeverbeke M, et al. (1994): Interaction between cells and elastin fibers: an ultrastructural and immunocytochemical study. J Cell Physiol 158:45 l-458.

Phillips ML, Nudelman E, Gaeta FC, et al. (1990): ELAM- 1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Lex. Science 250: 1130-l 132.

Picker LJ, Warnock RA, Burns AR, Doerschuk CM, Berg EL, Butcher EC (199 1): The neutrophil selectin LECAM- 1 presents carbohydrate ligands to the vascular selectins ELAM- 1 and GMP- 140. Cell 66:92 l-933 [published erratum appears in Cell 199 1 67(6):1267].

Pierce JW, Read MA, Ding H, Luscinskas FW, Collins T (1996): Salicylates inhibit I kappa B-alpha phosphorylation, endothelial-leukocyte adhesion molecule expression, and neutrophil transmigration. J Immunol 156:396 l-3969.

Polley MJ, Phillips ML, Wayner E, et al. (199 1): CD62 and endothelial cell-leukocyte adhesion molecule 1 (ELAM- 1) recognize the same carbohydrate ligand, sialyl-Lewis x. Proc Natl Acad Sci USA 88:6224–6228.

Pomerat CM, Slick WC (1963): Isolation and growth of endothelial cells in tissue culture. Nature 198:859–861.

Rao KM, Currie MS, Cohen HJ, Peters WP (1994): Alterations in L-selectin expression and elastase activity in neutrophils from patients receiving granulocyte colony-stimulating factor alone or in conjunction with high-dose chemotherapy with autologous bone marrow transplantation. Lymphokine Cytokine Res 13:383–390.

Richter J, Ng Sikorski J, Olsson I, Andersson T (1990): Tumor necrosis factor-induced degranulation in adherent human neutrophils is dependent on CD I 1 b/CD 18-integrin-triggered oscillations of cytosolic free Ca^{2+}. Proc Natl Acad Sci USA 87:9472–9476.

Robinson JP, Cordy W, Narayanan PK, Carter WO (1994): Fate of intracellular DCFH in human neutrophils after release of caged nitric oxide using UV confocal microscopy. Cytometry 7:76.

Rosenman SJ, Ganji AA, Tedder TF, Gallatin WM (1993): Syn-capping of human T lymphocyte adhesion/activation molecules and their redistribution during interaction with endothelial cells. J Leukoc Biol 53: l-10.

Schleimer RP, Sterbinsky SA, Kaiser J, et al. (1992): IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM- 1. J Immunol 148: 1086-1092.

Schreck R, Rieber P, Baeuerle PA (1991): Reactive oxygen intermediates as apparently widely used mes-

sengers in the activation of the NF-kappa B transcription factor and HIV- 1. EMBO J 10:2247-2258.

Sekizuka E, Benoit JN, Grisham MB, Granger DN (1989): Dimethylsulfoxide prevents chemoattractant-induced leukocyte adherence. Am J Physiol 256:H594–H597.

Sellak H, Franzini E, Hakim J, Pasquier C (1994): Reactive oxygen species rapidly increase endothelial ICAM- 1 ability to bind neutrophils without detectable upregulation. Blood 83:2669–2677.

Shimizu S, Ishii M, Yamamoto T, Kawanishi T, Momose K, Kuroiwa Y (1994): Bradykinin induces generation of reactive oxygen species in bovine aortic endothelial cells. Res Commun Chem Pathol Pharmacol 84:301–3 14.

Silber A, Newman W, Reimann KA, Hendricks E, Walsh D, Ringler DJ (1994): Kinetic expression of endothelial adhesion molecules and relationship to leukocyte recruitment in two cutaneous models of inflammation. Lab Invest 70: 163– 175.

Silvestro L, Viano I, Macario M, et al. (1994): Effects of heparin and its desulfated derivatives on leukocyte-endothelial adhesion. Semin Thromb Hemost 20:254–258.

Simon SI, Burns AR, Taylor AD, et al. (1995): L-selectin (CD62L) cross-linking signals neutrophil adhesive functions via Mac- 1 (CD 11 b/CD 18) beta 2-integrin. J Immunol 155: 1502-l 5 14.

Slocum MM, Granger DN (1993): Early mucosal and microvascular changes in feline intestinal transplants. Gastroenterology 105: 176 l-l 768.

Smith CW, Kishimoto TK, Abbassi 0, Hughes B, Rothlein R, McIntire (199 1): Chemotactic factors regulate lectin adhesion molecule 1 (LECAM- 1)-dependent neutrophil adhesion to cytokine-stimulated endothelial cells in vitro. J Clin Invest 87:609–6 18 (published erratum appears in J Clin Invest 1991 87(5):1873].

Soares FA, Shaughnessy SG, MacLarkey WR, Orr FW (1994): Quantification and morphologic demonstration of reactive oxygen species produced by Walker 256 tumor cells in vitro and during metastasis in vivo. Lab Invest 7 1:480–489.

Solomkin JS, Bass RC, Bjornson HS, Tindal CJ, Babcock GF (1994): Alterations of neutrophil responses to tumor necrosis factor alpha and interleukin-8 following human endotoxemia. Infect Immun 62:943–947.

Spertini 0, Kansas GS, Munro JM, Griffin JD, Tedder TF (199 1): Regulation of leukocyte migration by activation of the leukocyte adhesion molecule- 1 (LAM- 1) selectin. Nature 349:69 l-694.

Sporn LA, Lawrence SO, Silverman DJ, Marder VJ (1993): E-selectin-dependent neutrophil adhesion to Rickettsia rickettsii-infected endothelial cells. Blood 8 1:2406–24 12.

Staal FJ, Roederer M, Herzenberg LA (1990): Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. Proc Natl Acad Sci USA 87:9943–9947.

Stacker SA, Springer TA: Leukocyte integrin P1 50,95 (CD1 1c/CD18) functions as an adhesion molecule binding to a counter-receptor on stimulated endothelium. J Immunol 146:648–655.

Stacker, SA, Springer TA (199 1): Leukocyte integrin P1 50,95 (CD 11 c/CD 18) functions as an adhesion molecule binding to a counter-receptor on stimulated endothelium. J Immunol 146:648–655.

Staunton DE, Dustin ML, Springer TA (1988): Functional cloning of ICAM-2, a cell adhesion ligand for LFA- 1 homologous to ICAM- 1. Nature 339:6 l-64.

Stepp MA, Spurr Michaud S, Tisdale A, Elwell J, Gipson IK (1990): Alpha 6 beta 4 integrin heterodimer is a component of hemidesmosomes. Proc Natl Acad Sci USA 87:8970–8974.

Subramaniam M, Koedam JA, Wagner DD (1993): Divergent fates of P- and E-selectins after their expression on the plasma membrane. Mol Biol Cell 4:79 l-801.

Suematsu M, Schmid Schonbein GW, Chavez Chavez RH, et al. (1993): In vivo visualization of oxidative changes in microvessels during neutrophil activation. Am J Physiol 264:H88 1 –H89 1.

Suzuki M, Asako H, Kubes P, Jennings S, Grisham MB, Granger DN (1991): Neutrophil-derived oxidants promote leukocyte adherence in postcapillary venules. Microvasc Res 42: 125-l 38.

Suzuki Y, Tanigaki T, Heimer D, et al. (1994): TGF-beta 1 causes increased endothelial ICAM- 1 expression and lung injury. J Appl Physiol 77: 128 l-l 287.

Szabo C (1996): The pathophysiological role of peroxynitrite in shock, inflammation, and ischemia-reperfusion injury. Shock 6:79–88.

Tamkun JW, DeSimone DW, Fonda D, et al. (I 986): Structure of integrin, a glycoprotein involved in the transmembrane linkage between fibronectin and actin. Cell 46:27 l-282.

Thurston G, Baldwin AL, Wilson LM (1995): Changes in endothelial actin cytoskeleton at leakage sites in the rat mesenteric microvasculature. Am J Physiol 268:H3 16–H329.

Uchihara T, Kondo H, Akiyama H, Ikeda K (1995): Single-laser three-color immunolabeling of a histological section by laser scanning microscopy: application to senile plaque-related structures in postmortem human brain tissue. J Histochem Cytochem 43: 103-106.

Vogel P, v.d.Beek J, Marohl K, Fischer EG, Kirkpatrick CJ (1993): In vitro studies on PMN-independent endothelial cell damage in trauma: decrease of PMN-endothelial cell adherence by fibrinogen degradation products and disturbance of endothelial cell membrane integrity by trauma serum. Eur Surg Res 25:83–90.

von Andrian UH, Chambers JD, McEvoy LM, Bargatze RF, Arfors KE, Butcher EC (199 1): Two-step model of leukocyte-endothelial cell interaction in inflammation: distinct roles for LECAM- 1 and the leukocyte beta 2 integrins in vivo. Proc Natl Acad Sci USA 88:7538–7542.

Voraberger G, Schafer R, Stratowa C (1991): Cloning of the human gene for intercellular adhesion molecule 1 and analysis of its 5′-regulatory region. Induction by cytokines and phorbol ester. J Immunol 147:2777–2786.

Waddell TK, Fialkow L, Chan CK, Kishimoto TK, Downey GP (1994): Potentiation of the oxidative burst of human neutrophils. A signaling role for L-selectin. J Biol Chem 269: 18485-l 849 1.

Walpola PL, Gotlieb AI, Langille BL (1993): Monocyte adhesion and changes in endothelial cell number, morphology, and F-actin distribution elicited by low shear stress in vivo. Am J Pathol 142: 1392-1400.

Walsh GM, Hartnell A, Wardlaw AJ, Kurihara K, Sanderson CJ, Kay AB (1990): IL-5 enhances the in vitro adhesion of human eosinophils, but not neutrophils, in a leucocyte integrin (CD1 l/l 8)-dependent manner. Immunology 7 1:258–265.

Walsh GM, Mermod JJ, Hartnell A, Kay AB, Wardlaw AJ (199 1): Human eosinophil, but not neutrophil, adherence to IL- 1 -stimulated human umbilical vascular endothelial cells is alpha 4 beta 1 (very late antigen-4) dependent. J Immunol 146:3419–3423.

Walz G, Aruffo A, Kolanus W, Bevilacqua M, Seed B (1990): Recognition by ELAM- 1 of the sialyl-Lex determinant on myeloid and tumor cells. Science 250: 1132-l 135.

Weber C, Erl W, Pietsch A, Strobe1 M, Ziegler Heitbrock HW, Weber (1994): Antioxidants inhibit monocyte adhesion by suppressing nuclear factor-kappa B mobilization and induction of vascular cell adhesion molecule- 1 in endothelial cells stimulated to generate radicals. Arterioscler Thromb 14:1665–1673.

Weber C, Erl W, Pietsch A, Weber PC (1995): Aspirin inhibits nuclear factor-kappa B mobilization and monocyte adhesion in stimulated human endothelial cells. Circulation 9 1: 19 14-l 9 17.

Wegner CD, Wolyniec WW, LaPlante AM, et al. (1992): Intercellular adhesion molecule- 1 contributes to pulmonary oxygen toxicity in mice: role of leukocytes revised. Lung 170:267–279.

Westlin WF, Kiely JM, Gimbrone MA Jr (1992): Interleukin-8 induces changes in human neutrophil actin conformation and distribution: relationship to inhibition of adhesion to cytokine-activated endothelium. J Leukoc Biol 52:43 {endash} 5 1.

Weyrich AS, Buerke M, Albertine KH, Lefer AM (1995): Time course of coronary vascular endothelial adhesion molecule expression during reperfusion of the ischemic feline myocardium. J Leukoc Biol 57:45–55.

Weyrich AS, Ma XL, Lefer AM (1992): The role of L-arginine in ameliorating reperfusion injury after myocardial ischemia in the cat. Circulation 86:279–288.

Weyrich AS, Ma XL, Lefer DJ, Albertine KH, Lefer AM (1993): In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury [see comments]. J Clin Invest 9 1:2620–2629.

Wright SD, Weitz JI, Huang AJ, Levin SM, Silverstein SC, Loike JD (1988): Complement receptor type three (CD 11 b/CD 18) of human polymorphonuclear leukocytes recognizes fibrinogen. Proc Natl Acad Sci USA 85:7734–7738.

Yoshida N, Granger DN, Anderson DC, Rothlein R, Lane C, Kvietys PR (1992): Anoxia/reoxygenation-induced neutrophil adherence to cultured endothelial cells. Am J Physiol 262:H 189 1-H 1898.