INTRODUCTION

Independently of their protective influence, leukocytes can have harmful effects in pathological situations such as diabetes (Benhamou et al., 1990), ischemia-reperfusion injury (Hernandez et al., 1987; Schmid-Schönbein and Engler, 1990; Thornton et al., 1989), inflammatory disorders (Fiebig et al., 1991; Grant, 1973; Jutila, 1992) and vascular permeability disorders (Kubes et al., 1991a; Schoenberg et al., 1991). Their action is mainly due to a rolling phenomenon along venular walls, adhesion to vascular endothelial cells and migration across the endothelium. A number of factors govern the interactions between leukocytes and endothelial cells, including the expression of molecules on the surface of activated leukocytes and/or endothelial cells, certain products of neutrophil activation, and shear forces within blood vessels that tend to sweep leukocytes along the endothelial cell surface (Harlan, 1985; Jutila, 1992; Ley, 1989; Tonnesen, 1989; Zimmerman et al., 1992).

In this Commentary, we describe recent progress in the understanding of neutrophil adhesion to endothelial cells, the influence of shear stresses and rolling along the venous wall, extravascular migration and microvascular permeability. The diverse structures and mechanisms of expression of the various proadhesive molecules will be only briefly touched on, as an excellent review of this subject has been published by Zimmerman et al. (1992). Nevertheless, a brief description is of interest for the following discussion of hydrodynamic stress and the factors that influence neutrophil/endothelial cell interactions. A schematic representation is given in Fig. 1 of the main components involved in leukocyte/endothelial cell interactions and described here.

ADHESION MOLECULES

The adhesion of leukocytes to endothelial cells requires regulated expression of molecules on one or both types of cell. Some molecules are expressed by the leukocyte, and leukocyte-specific adhesion proteins are expressed on the surface of the vascular endothelium. These adhesion molecules have diverse structures and mechanisms of expression; they act either by tethering the two cells together or as signals that induce activation-dependent processes (Zimmerman et al., 1992).

Proadhesive molecules expressed by neutrophils

Two types of adhesion proteins expressed by the circulating neutrophil appear to be important: leukocyte integrins and leukocyte L-selectin.

The $\beta_2$-integrins are LFA-1 (CD11a-CD18), Mac-1 (CD11b-CD18) and p150-95 (CD11c-CD18). They are present in the polymorphonuclear plasma membrane and regulate adhesion to endothelial cells by binding endothelial cell adhesion molecules. The L-selectin (previously called LAM-1, LECAM-1) is a membrane glycoprotein.

LFA-1, Mac-1 and p150-95 share a common $\beta$ subunit resulting from mutations in the $\beta$ (CD18) chain (leukocyte adhesion deficiency syndrome) can result in the absence of cell surface expression of all three members of the leukocyte integrins. Such a deficiency can reduce neutrophil accumulation at extravascular sites of infection, demonstrating a critical role for these glycoproteins in migration and adhesion (Larson and Springer, 1990; Springer, 1990).

Additional evidence that leukocyte integrins are important has come from monoclonal antibody (mAb) blocking studies. They have shown that these molecules are involved in leukocyte aggregation and adhesion to endothelial cells (Arnaout, 1990; Springer, 1990; Wright et al., 1983).

All three $\beta_2$-integrins are constitutively expressed on the surface of leukocytes and are normally in a functionally 'inactive' state (Arnaout, 1990; Humphries, 1990; Springer, 1990). Conversion of the inactive to the active form occurs rapidly in response to chemotactic factors, cytokines, antigen and mitogens. No new copies of the molecules are required at the cell surface, and it is thus thought that a conformational change in the molecule controls this conversion (Arnaout, 1990; Humphries, 1990), perhaps resulting from the phosphorylation of the $\beta$ subunit.

The $\beta_2$-integrins LFA-1 and Mac-1 bind to the intercellular adhesion molecule-1 (ICAM-1) on endothelium, whereas LFA-1, but not Mac-1, binds to intercellular adhe-
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Fig. 1. Schematic explanation of the involvement of proadhesive molecules expressed by leukocytes and endothelial cells, together with the role of xanthine oxidase, oxidants, PAF, LTB₄ and NO, in leukocyte/endothelial cell interactions.


ICAM-1 is a single glycoprotein with five immunoglobulin-like domains. ICAM-1 induction is one of the mechanisms regulating inflammatory cell interactions; it occurs on a time scale of hours and requires mRNA and protein synthesis (Springer, 1990). ICAM-1 is little expressed on endothelial cells but is greatly increased at inflammatory sites and by stimulation with lipopolysaccharides (Pohlman et al., 1986, 1987) and cytokines such as interferon-γ (Dustin et al., 1986; Pohlman et al., 1987), interleukin-1 (IL-1) (Dustin et al., 1986; Groves et al., 1992) and tumour necrosis factor-α (TNF-α) (Lo et al., 1992; Rohleim et al., 1988). These cytokines activate endothelial cells, increasing their adhesiveness for neutrophils in vitro and leading to neutrophil migration across endothelial cell monolayers grown on solid surfaces or on microporous filters (Furie and McHugh, 1989; Morzycki et al., 1990; Morzycki and Issekutz, 1991; Moser et al., 1989; Smith et al., 1989). However, the source and regulation of such cytokine production at the site of injury has not been clearly identified. Concerning IL-1, one group (Hawrylowicz et al., 1989) has found that thrombin-activated platelets express IL-1, which in turn induces ICAM-1 expression (Hawrylowicz et al., 1991). This finding suggests that platelets may initiate and regulate some of the inflammatory functions of the vascular endothelium. In addition, it is conceivable that platelets may affect other cells with which they interact, such as leukocytes.

ICAM-2 is very similar to ICAM-1 but contains only two immunoglobulin-like domains, and thus has a lower molecular mass than ICAM-1. ICAM-2 is expressed at a higher surface density on resting endothelium but is not increased after cytokine activation (De Fougerolles et al., 1991; Staunton et al., 1989). The interactions of LFA-1 with ICAM-1 and ICAM-2 occur within an intermembrane distance roughly equal to or less than 27 and 36 nm, respectively (Springer, 1990). The different distances suggest that membrane contacts could occur in different membrane domains within the zone of adhesion between two cells or
that the flexibility of ICAM-1 may play an important role in solid binding of ICAM-2. The binding position may also be of importance: Mac-1 binds to the third immunoglobulin repeat of ICAM-1, whereas LFA-1 binds to the first (Diamond et al., 1991).

L-selectin, LAM-1, also appears to play an important role in leukocyte adhesion. L-selectin was originally defined as a molecule expressed by lymphocytes and serves as a peripheral lymph node homing receptor (Jutila et al., 1990a; Lewinsohn et al., 1987; Smith et al., 1991). L-selectin has an amino-terminal domain that is similar to mammalian type-C lectins, an epidermal growth factor (EGF)-like sequence, multiple repeats of a complement binding-like domain, a transmembrane region and a short cytoplasmic domain (McEver, 1991). L-selectin is rapidly shed by proteolytic cleavage near the transmembrane span when leukocytes are activated.

L-selectin is also a major mediator of leukocyte attachment to activated endothelium: L-selectin mediates the adhesion of both lymphocytes and neutrophils to TNF-activated endothelial cells. It has been suggested that L-selectin is involved in the initial attachment of these types of leukocytes to endothelium (Griffin et al., 1990; Jutila et al., 1989; Smith et al., 1991; Spertini et al., 1991) and in optimal neutrophil transendothelial migration (Spertini et al., 1991).

The important role of the interaction between L-selectin and its endothelial ligand has been illustrated by using a soluble form of a specific cell adhesion molecule, a soluble immunoglobulin chimera containing an L-selectin domain. This soluble form blocks neutrophil recruitment to inflammatory sites (Watson et al., 1991). In consequence, soluble forms of a single type of adhesion molecule could be effective for the inhibition of neutrophil-mediated inflammation and it could be of interest to test other soluble adhesion molecules such as integrins or other lectin cell adhesion molecules (for example, E- and P-selectin). These studies could lead to the development of new therapeutic drugs.

The molecules described above are the most common proteins expressed by leukocytes, especially neutrophils, and involved in adhesion processes. However, other proadhesive molecules are present in mononuclear cells. For example, molecules not related to the β2-integrin family of heterodimer leukocyte adhesion molecule and L-selectin participate in the binding of monocytes to cytokine-stimulated endothelial cells.

**Molecules expressed by endothelial cells**

Two molecules recently recognized as leukocyte adhesion molecules have been identified: endothelial leukocyte adhesion molecule-1 (ELAM-1) and CD62 (PADGEM or GMP-140). ELAM-1 and CD62 are now included in a family of adhesion molecules called selectins; the names of ELAM-1 and CD62 have been changed to E-selectin and P-selectin, respectively (Beverlauca et al., 1989). These two molecules are expressed through different mechanisms when the endothelial cell is activated. E- and P-selectin have molecular structures similar to L-selectin, and their functions appear to be regulated by their lectin activities.

P-selectin is a glycoprotein stored preformed in Weibel-Palade bodies of endothelial cells and translocated to the plasma membranes of these cells within seconds after activation by mediators of inflammation and hemostasis like thrombin and histamine (Hattori et al., 1989; Smith and Anderson, 1991), allowing these cells to bind neutrophils and monocytes at the site of tissue injury (Geng et al., 1990; Larsen et al., 1989). P-selectin can tether neutrophils to endothelial cells without requiring neutrophil activation or the β2-integrin system (Geng et al., 1990; Lorant et al., 1991).

E-selectin is also a glycoprotein but is not contained in the cytoplasm; it is predominantly found in cutaneous sites of inflammation (Picker et al., 1991), and is synthesized by endothelial cells in response to inflammatory agents (lipopolysaccharides or cytokines, such as tumor necrosis factor-α (TNF-α) and IL-1). Maximal expression is reached after 4 to 6 hours of activation. E-selectin promotes adhesion of neutrophils, monocytes and some lymphocytes (Beverlauca et al., 1989; Picker et al., 1991; Shimizu et al., 1991).

The counter-receptors for P- and E-selectins have not yet been characterized at the molecular level. The ligand for one of these proteins, E-selectin (LECAM-2 or ELAM-1), has been reported by several groups to contain a polylactosamine structure bearing a terminal sialic acid residue and at least one fucose residue (Phillips et al., 1991; Walz et al., 1991). The sialyl Lewis x tetrasaccharides are the smallest oligosaccharides recognized by the lectin (Tyrrell et al., 1991).

P-selectin (CD62 or GMP-140) also recognizes specific oligosaccharides ligands, lactosaminoglycans, including the sequence sialyl-Lewis x; however, the presence of sialyl Lewis x tetrasaccharides alone does not confer the ability to bind P-selectin (Zhou et al., 1991). A candidate P-selectin receptor that is a constitutively expressed glycoprotein different from other known sialylated membrane proteins on neutrophils has been described (Moore et al., 1991; Zhou et al., 1991).

**The simultaneous contribution of CD18 and E-selectin to neutrophil adherence**

Recently, using a three-dimensional model system for the simultaneous determination of leukocyte adherence and migration, Hakkert et al. (1991) have studied the contribution of CD11/CD18 and E-selectin in neutrophil adherence to and migration through cytokine (recombinant interleukin-1β (rIL-1β))-activated endothelial cells. The combined use of mAbs against E-selectin and CD18 significantly reduces neutrophil adherence. This finding indicates that additional independent mechanisms are involved. These authors finally demonstrated that neutrophil adherence to cytokine-activated endothelial cells is mediated by CD18-independent and by CD18-dependent mechanisms, and that migration across monolayers of cytokine-activated endothelial cells is predominantly CD18-dependent.

**Role of E- and L-selectins**

Spertini et al. (1991) suggested that L-selectin and E-selectin (described below) may interact in a cooperative fashion to promote optimum neutrophil transmigration across activated endothelium. This suggestion implies that L-selectin and E-selectin participate in separate pathways...
in the adhesion of neutrophils to cytokine-stimulated endothelium. In contrast, the findings of Kishimoto et al. (1991) provide evidence that L-selectin participates in the same adhesion pathways as E-selectin. These results have led to controversial conclusions, but the experimental conditions were not the same: Spertini et al. (1991) performed their experiments in vitro using rotating (non-static) conditions, while Kishimoto et al. (1991) operated in vitro under static conditions. Shear forces exerted on leukocytes should also be taken into account. For example, the following discussion contains evidence that L-selectin has an important role in leukocyte adhesion to endothelial cells under flow conditions. It is also conceivable that expression of adhesion molecules could be modulated by flow conditions.

**EFFECTS OF HYDRODYNAMIC AND ESSENTIAL FACTORS**

**Effect of shear stress on neutrophil adhesion to endothelial cells**

In general, shear force induced by blood flow appears to be an important factor in determining the outcome of leukocyte-adhesive interactions with the endothelium (House and Lipowsky, 1987). First, shear force appears to play an important role in the distribution of the different cells within the vessels. In small postcapillary venules with diameters just above leukocyte diameter, red cells tend to push leukocytes toward the venous wall (Schmid-Schönbein et al., 1980). In larger venules aggregates of red cells, which occupy the axial flow portion in the vessel, displace leukocytes to a marginal region. This marginal position of leukocytes in flowing blood is the first step towards rolling.

Secondly, experiments under shear stress conditions help us to understand the mechanisms that regulate leukocyte adhesion to the endothelium. In vivo, wall shear rates are around or below 1000 s⁻¹ in venules and up to 5000 s⁻¹ in arterioles (Ley and Gaehlens, 1991). According to Lawrence et al. (1990), using two mAbs, one against CD18 (TS1/18) and the other against ICAM-1 (R.6.5.D6), above wall shear stresses of 0.5 dyne/cm² (corresponding to a wall shear rate of 70 s⁻¹), neutrophil adherence and rolling appear to be principally CD18/ICAM-1-independent adhesive interactions. At shear stresses of 0.5 dyne/cm² and below, attachment to endothelial cells can be mediated by CD18.

Since it has been well established that adhesion to endothelial cells can be significantly, but not completely, inhibited by mAbs to the CD11/18 family of glycoproteins (Pohlmn et al., 1986; Smith et al., 1988; Zimmerman and McIntyre, 1988), other mechanisms of neutrophil adhesion should exist, as demonstrated by Lawrence et al. (1990). Finally, the adhesion process appears to be complicated, involving various mechanisms, each with a defined function and specificity. Consequently, in flow conditions the adhesive interactions can be separated into CD18/ICAM-1-dependent and -independent components. Furthermore, local flow rates in the vasculature could play an important role, in conjunction with the expression of cell surface molecules, in regulating the margination and initial attachment of leukocytes to the blood vessel wall.

**Rolling**

The earliest visible leukocyte-endothelial cell interaction in vivo involves slow rolling of leukocytes along the wall of postcapillary and collective venules, resulting from a balance of adhesive forces between leukocytes and endothelium, and shear stresses exerted by the flowing blood. Leukocytes roll along venular, but not arteriole walls, at a velocity clearly lower than that of the other blood cells, before they firmly attach to and migrate across the vascular endothelium. The velocity of rolling leukocytes has been observed to range around or below 50 m/s (Firell and Lipowsky, 1989; Ley and Gaehlens, 1991; Tözeren and Ley, 1992), a value far lower than blood flow velocities.

The induction of leukocyte rolling involves several steps. First, the cells need to acquire a marginal position in the flowing blood. Mechanisms of leukocyte margination are not sufficient to explain the leukocyte rolling phenomenon. Experimental results show the specificity of the venular endothelium. Leukocyte rolling is commonly observed in the venules of most tissues exposed for intravital microscopy (Firell and Lipowsky, 1989; House and Lipowsky, 1987, Mayrovitz et al., 1987). By contrast, leukocyte rolling in arterioles is rarely reported and appears to occur only after considerable trauma (Mayrovitz et al., 1980). Some authors supported the idea that differences in hydrodynamic flow conditions may be responsible for preferential leukocyte rolling in venules (Nobis et al., 1985) and demonstrated that the reduction of blood flow and, hence, wall shear rate increases the fraction of rolling leukocytes in venules of the rat mesentery (Firell and Lipowsky, 1989). However, by studying extensively various wall shear rates in both arterioles and venules, recent investigations in the rat mesentery showed that the marked difference between arterioles and venules persists even at identical mean shear rates in both vessel categories (Ley and Gaehlens, 1991). These results indicate that hemodynamic differences between arterioles and venules are not the major cause of the restriction of leukocyte rolling to venules and that the venular endothelium may be specialized to support leukocyte rolling and adhesion in vivo. One question remains unanswered, however: could the pulsatile flow in arterioles inhibit rolling? It would be of interest to examine the effect of pulsatile flow on leukocyte velocities in vivo, and to simulate a pulsatile flow in venules for comparison. Pulsatile flow could also have an effect on the endothelium, where the expression or activation of some adhesion molecules could be modulated in pulsatile conditions of flow.

While mAb 60.3, a monoclonal antibody recognizing an epitope on the leukocyte adhesion glycoprotein CD18, almost completely abolishes leukocyte adhesion in vivo and in vitro (Price et al., 1987; Vedder et al., 1988; Wallis et al., 1986), it has no effect on the rolling phenomenon. This indicates that leukocyte rolling is a distinct phenomenon with a different underlying mechanism and does not directly influence the epitope on the CD18 glycoprotein recognized by mAb 60.3.

Furthermore, leukocyte rolling is inhibited in vivo and in
vitro by dextran sulphate and by other polysaccharides like heparin (Fiebig et al., 1991; Ley and Arfors, 1989; Ley et al., 1991; Rosenberg et al., 1989; Tangelder and Arfors, 1987). Some authors have demonstrated that inhibition of neutrophil adhesion by dextran sulphate in vitro is dependent on shear stress and occurs only when shear stress is present during the adhesion process. In addition, dextran sulphate does not remove adherent neutrophils even by subsequent application of shear stress, indicating that dextran sulphate does not weaken the adhesive forces between neutrophils and endothelial cells once attachment is established (Ley et al., 1989). Dextran sulfate would thus appear to inhibit a primary step in shear conditions. This primary step appears to be necessary for, or at least facilitates, firm attachment of neutrophils.

L- and P-selectin appear to play an important role in the rolling phenomenon. L-selectin is rapidly shed from the neutrophil surface in response to several cytokines in vivo (Jutila et al., 1989, 1991) and in vitro (Jutila et al., 1990b; Kishimoto et al., 1989); there is also a parallel increase in $\beta_2$-integrins, and this has led to the hypothesis that L-selectin might mediate early interactions of the neutrophil with the vascular endothelium, preceding the role of activation-triggered $\beta_2$-integrins (Jutila et al., 1989, 1990b, 1991; Kishimoto et al., 1989). Such early interactions might be involved in leukocyte rolling. This hypothesis has been confirmed by other studies: anti-L-selectin mAb inhibits initial reversible leukocyte rolling along the vascular wall, while anti-CD18 mAb has no effect on rolling but prevents firm attachment of leukocytes to venular endothelium. These in vivo findings suggest that L-selectin and leukocyte $\beta_2$-integrins play two different roles: reversible rolling is mediated in part by L-selectin and $\beta_2$-integrins contribute to activation-dependent firm attachment (Von Adrian et al., 1991).

Some experiments have also recently shown that, in vitro, leukocytes bind to and roll on P-selectin in flow conditions similar to those found in postcapillary venules. They established that there are qualitative differences between selectin and integrin adhesion mechanisms, and that the two mechanisms are cooperative (Lawrence and Springer, 1991). At physiological shear stress, rolling on a selectin is a prerequisite for activation-induced adhesion strengthening by means of integrins. Neutrophil interaction with selectins may promote close physical interactions between integrins; in addition, the slow velocity of rolling raises the chance of enough bonds being formed for the rolling adhesion and permits stationary adhesion with the contribution of ICAM/CD18 binding.

Additional results (Tissot et al., 1992) suggest that, in vitro, leukocytes may be in contact with substrata over very limited areas (corresponding to the tip of microvilli) and that a single bond might be responsible for initial cell arrest. Selectins should be present in this area and it would be interesting to determine how many bonds are responsible for initial attachment and how L-selectin and P-selectin compete in this attachment.

In the inflammatory process, the number of rolling leukocytes increases and the leukocyte rolling velocity decreases. After local tissue injury, nearby blood vessels undergo rapid dilation, leading to a slower blood flow and lower fluid shear stresses. In consequence, a large proportion of rolling leukocytes becomes stationary (adherent). At this point, inflammatory agents such as TNF-α could activate the expression of adhesion molecules or receptors (like E-selectin and ICAM-2) and increase leukocyte adhesion to the endothelium. A significant proportion of these adherent leukocytes migrate across the endothelium.

**Promoting adhesion, migration and permeability**

Leukocytes, especially neutrophils, are involved in the increased vascular permeability to macromolecules associated with acute inflammation or ischemia-reperfusion. For example, leukocytes play a role in the gastrointestinal mucosal injury associated with ischemia-reperfusion (Hernandez et al., 1987) and induce oedema in different inflammatory models (Issekutz, 1981; Wedmore and Williams, 1981).

What we know of the role of leukocytes is largely based on in vivo observations and it is generally assumed that the adhesion and migration of leukocytes in postcapillary venules are responsible for the vascular dysfunction (Granger et al., 1989; Hernandez et al., 1987; Messmer et al., 1988). Neutrophils appear to be the predominant leukocytes that adhere and emigrate in post-ischemic venules (Oliver et al., 1991). Electron microscopic examination following reperfusion demonstrated that neutrophils exit the venular lumen by migrating between endothelial cells, resulting in increased neutrophil traffic through disrupted endothelial cell tight junctions. This migration has been observed by Marchesi (1961) and recently described by Oliver et al. (1991).

**Role of PAF and LTB4**

Two proinflammatory agents appear to play an important role in leukocyte accumulation, adhesion, migration and vascular dysfunction: platelet-activating factor (PAF) and leukotriene B4 (LTB4) (Kubes et al., 1990a,b; Zimmerman et al., 1990a). Leukotrienes are formed in the 5-lipoxygenase pathway from arachidonic acid. LTB4, one of the most powerful chemotactic substances known, stimulates the adhesion of leukocytes to endothelial cells. LTB4 induces leukocyte adhesion to the endothelium through the upregulation of Mac-1 (CD11b/CD18) adherence receptors on the leukocyte surface (Lindström et al., 1990).

PAF is a biologically active phospholipid synthesized within minutes by endothelial cells after appropriate stimulation by histamine, thrombin and other agonists. A portion of synthesized PAF is then expressed on the endothelial cell surface, where it is recognized by the neutrophil receptor for PAF (Zimmerman et al., 1990b). Ligation of this receptor by PAF serves as a signal for activation-dependent changes in CD11/CD18 integrins (LFA-1 and Mac-1) on the neutrophil plasma membrane that makes neutrophils competent to bind to counter-receptors on endothelial cells.

Furthermore, PAF and P-selectin act in a cooperative fashion for neutrophil adhesion. The tethering effect mediated by P-selectin facilitates PAF interaction with its receptor on the neutrophil and enhances PAF-stimulated adhesiveness (Lorant et al., 1991). It is conceivable that binding of P-selectin to its receptor on the neutrophil brings local domains of the neutrophil plasma membrane into close contact with the endothelium, thus facilitating firm adhesion of leukocytes.
proximity with endothelial cell-associated PAF and thus facilitates receptor-ligand interaction.

However, the P-selectin- and CD11/CD18-dependent mechanisms may be influenced by different conditions of flow and stasis. Effectively, Lawrence et al. (1990), as described above, predicted a CD11/CD18-independent adhesion interaction above wall shear stresses of 0.5 dyne/cm².

Some authors (Kubes et al., 1991a) have developed an in vivo model for measurement of microvascular protein exchanges as well as leukocyte adhesion and migration in the mesenteric microcirculation during an acute inflammatory response. These authors observed neutrophil adherence and migration when exposed to platelet-activating factor (PAF) and leukotriene B⁴ (LTB⁴). They demonstrated that PAF induces migration of leukocytes and increases vascular protein leakage. Since mAb IB₄, a monoclonal antibody against the common β-subunit of the adhesive glycoprotein complex CD11/CD18, was shown to effectively reduce PAF-induced vascular protein leakage, PAF-mediated vascular dysfunction is dependent on leukocyte adhesion. It was also observed that LTB⁴-induced leukocyte adherence was not accompanied by increased vascular leakage.

However, Björk et al. (1982) demonstrated that superfusion of the hamster cheek pouch with LTB₄ results in increased leakage of fluorescent dextran from postcapillary venules. In fact, when LTB₄ is superfused on tissue so that its highest concentration is outside the vasculature, leukocyte migration occurs (Asako et al., 1991), together with an increase in vascular protein leakage (Dahlen et al., 1981). Thus, in vitro and in vivo experiments have shown that an LTB₄ concentration gradient appears essential for leukocyte migration and vascular permeability (Rosengren et al., 1991).

This phenomenon was not observed with PAF: although an unfavourable chemotactic gradient for migration occurs during intra-arterial PAF infusion, a large proportion of adherent leukocytes migrate across postcapillary venules. Thus, Kubes et al. (1991a) speculated that PAF may release another chemotactic substance from cells outside the vasculature, which, in turn, would provide the favourable gradient necessary for leukocyte migration.

Two findings suggest that further investigations are required: (i) evidence that LTB₄ increases leukocyte adhesion to the endothelium by a mechanism dependent on the expression of CD11/CD18 (Mac-1) glycoprotein family (Lindström et al., 1990; Price et al., 1987); (ii) LTB₄ promotes leukocyte chemotaxis and adhesion to the endothelium of postcapillary venules, while the cysteiny1 leukotrienes (LT₄C₄, LTD₄ and LTE₄) mediate macromolecular leakage (Lehr et al., 1991a).

Furthermore, as colchicine and methotrexate, drugs used in the treatment of gout and rheumatoid arthritis, respectively, attenuate in vivo the adhesive interactions between leukocytes and endothelial cells induced by PAF and LTB₄ (Asako et al., 1992), they should be considered for future studies on the effect of PAF and LTB₄. Colchicine has been used in the treatment of gouty arthritis for centuries, while methotrexate has only recently been used to treat patients with rheumatoid arthritis. A common action shared by colchicine and methotrexate is their inhibition of leukocyte chemotaxis in vitro (see Caner, 1965; Kerwar and Oronsky, 1989; Suarez et al., 1987). Methotrexate is also known for its immunosuppressive effects. Since complications of immunosuppression are thought to be rare with low-dose-pulsed methotrexate (Shiroky et al., 1991), the use of this drug in the treatment of leukocyte-induced microvascular dysfunction might be worthy of attention.

**Effect of essential fatty acids**

Dietary fish oil appears to exert its protective effects on experimental and clinical atherogenesis through inhibition of leukocyte/endothelium interaction (Lehr et al., 1991b). Furthermore, a high dietary intake of n-3 fatty acids, especially the major fatty acids contained in fish oil (eicosapentaenoic acid and docosahexaenoic acid), exerts a protective effect on the microvascular manifestations of ischemia-reperfusion injury and can block these manifestations by inhibition of LT biosynthesis. Dietary fish oil reduces the adherence of leukocytes induced by LTB₄, presumably through effects on LTB₄ receptor expression (Georgilis and Klampner, 1988). However, fish oil may rather affect endothelium-leukocyte interactions through the inhibition of LT biosynthesis than through inhibition of LT action (Lehr et al., 1991c), which in turn inhibits LT-induced leukocyte interactions mediated by CD11/CD18. The in vivo effects of essential fatty acid (EFA) deficiency is more marked than that of dietary n-3 fatty acid supplementation in acute inflammation (Letkowith et al., 1990). This difference in anti-inflammatory potential appears to be due either to the greater effect of EFA deficiency in suppressing in vivo generation of LTB₄ or to a decrease in the number of resident macrophages that may be responsible for a further decrease in the synthesis of pro-inflammatory mediators.

All of these hypotheses show that it is necessary to determine the exact functions of these two inflammatory agents in adherence, migration and microvascular permeability, as well as the indirect effects of the essential fatty acids on CD18-mediated leukocyte adhesion, especially neutrophil adhesion. In addition, fatty acids may attenuate leukocyte/endothelium interactions not only through the inhibition of adhesion-promoting mediators but also through enhanced generation of other antiadhesive agents. Among these, prostacyclin PGI₂, the formation of which is increased by dietary fish oil supplementation, reduces leukocyte adhesion to the endothelium. The effects of dietary fish oil on endothelial cells must also be considered. For example, it may enhance production of endothelium-derived relaxing factor (nitric oxide).

These observations demonstrate that leukocyte adhesion does not always result in microvascular dysfunction and suggest that neutrophil adherence and migration are not the sole determinants of this dysfunction. Therefore, leukocyte adhesion may not be the most critical step in the microvascular dysfunction associated with acute inflammation. Some reports suggest a role of oxidants, in part produced by activated neutrophils, in increased vascular permeability (Granger, 1988; Weiss, 1989).

**Role of oxygen-derived oxidants in leukocyte adhesion and vascular permeability**

There are substantial experimental data indicating that oxygen-derived oxidants, like superoxide anion and hydro-
gen peroxyde, are mediators of the microvascular dysfunction induced by ischemia-reperfusion and inflammatory injury (Granger, 1988; Parks et al., 1982). These oxygen species react with cell-membrane polyunsaturated fatty acids, and induce lipid peroxidation, leading to cell disintegration and irreversible tissue damage. They also induce accumulation of neutrophils in tissues (Schoenberg et al., 1991; Slater, 1984). In addition, the radical reactions may result in microvascular injury leading to increased permeability and extravasation of plasma and even erythrocytes (Del Maestro et al., 1982).

Endothelial cells exposed to oxidants like H$_2$O$_2$ express P-selectin on their surface and a mAb against P-selectin completely blocks neutrophil adherence mediated by P-selectin (Patel et al., 1991). Thus, the mechanism responsible for the adherence of neutrophils to endothelial cells treated with oxidants for long periods is the prolonged expression of P-selectin on the cell surface. This expression of P-selectin after treatment is completely different to the P-selectin expression in response to activation of endothelial cells by histamine or thrombin. The reason for this difference is not clear, but it is possible that the mechanisms responsible for reinternalization of P-selectin were not induced by the oxidants or that the latter altered these mechanisms.

Two possible disease mechanisms have been suggested to explain the production of oxygen radicals. The enzyme xanthine oxidase is the primary source of reactive oxygen metabolites. Xanthine oxidase is capable of reducing molecular oxygen to both superoxide and hydrogen peroxide at cytotoxic rates. The second potential source of oxygen radicals are neutrophils that accumulate in the capillaries and venules of tissues. This has been well studied by Klebanoff (1988) and, to a lesser extent, by Schmid-Schönbein (1987).

**Xanthine oxidase**

Xanthine oxidase is generally found in the intestinal mucosa, especially in endothelial cells (Parks et al., 1986). Xanthine oxidase exists in healthy cells predominantly as NAD$^+$ reducing xanthine oxidase (xanthine dehydrogenase). An understanding of the kinetics of the dehydrogenase to oxidase conversion in endothelial cells may be of importance in leukocyte interactions disorders, but relatively little is still known about the mechanisms and kinetics of xanthine dehydrogenase and xanthine oxidase conversion, although some authors have attempted to calculate the rate of conversion (Parks et al., 1988; Roy and McCord, 1983).

Intra-arterial infusion of xanthine oxidase in nonischemic intestines produces an increase in microvascular permeability that is comparable to that observed after ischemia-reperfusion (Parks et al., 1984). This latter study demonstrated that xanthine oxidase plays a role in vascular permeability. The increase in permeability was largely prevented by superoxide dismutase and dimethylsulfoxide, a hydroxyl radical scavenger. In addition, experimental studies show that inhibition or inactivation of xanthine oxidase protects the intestinal mucosa from post-ischemic damage (Granger et al., 1986; Parks and Granger, 1983; Schoenberg et al., 1985).

Recent studies have also demonstrated that the xanthine oxidase inhibitor oxypurinol reduces leukocyte adhesion in postcapillary venules if it is administered one hour after reperfusion, i.e. after the adhesive interactions between leukocytes and endothelial cells have been elicited (Suzuki et al., 1991b). These authors suggested that the effect of oxypurinol is related to its ability to inhibit superoxide and hydrogen peroxide production by xanthine oxidase (Suzuki et al., 1989), and that xanthine oxidase inhibitor does not exert a direct anti-adhesive action on leukocytes. One possible ability is that xanthine oxidase-derived oxidants, produced at the time of reperfusion in ischemia-reperfusion injury, could lead to the formation of proinflammatory substances (e.g. LTB$_4$ and PAF) that promote the activation, adhesion and infiltration of neutrophils into the post-ischemic tissue (Granger, 1988).

**Neutrophils**

Neutrophil-derived oxidants promote leukocyte adhesion to vascular endothelium. Studies in vitro suggest an interaction between leukocytes and reactive oxygen metabolites and have demonstrated that H$_2$O$_2$ promotes neutrophil adhesion to monolayers of endothelial cells (Lewis et al., 1988). Recently, Shappell et al. (1990) also demonstrated that neutrophil adhesion to vascular endothelium via Mac-l integrin mediates H$_2$O$_2$ production by human and canine neutrophils. It has been shown (Grisham et al., 1990) that activated neutrophils generate monochloramine (NH$_2$Cl), a myeloperoxidase-derived oxidant, which, at physiological concentrations, leads to significant leukocyte adhesion in postcapillary venules of cat mesentery (Suzuki et al., 1991a).

Furthermore, superoxide dismutase (SOD) and other agents, which have been used either to scavenge or to prevent the production of oxygen radicals (i.e. catalase), exert a significant inhibitory influence on the adhesive interactions between leukocytes and venular endothelium. This observation indicates that superoxide is also a proadhesive product of neutrophil activation (Granger et al., 1986; Oliver et al., 1991; Suzuki et al., 1989).

However, the mechanism by which H$_2$O$_2$, NH$_2$Cl and superoxide promote leukocyte adhesion and vascular permeability is unclear. Oxidants may mediate the production of proinflammatory mediators by endothelial cells. It has been suggested that an important physiological action of neutrophil-derived H$_2$O$_2$ is to stimulate PAF production, which, in turn, could promote leukocyte adherence via expression of the leukocyte adhesion glycoprotein CD11/CD18, as well as microvascular permeability (Kubes et al., 1990c; Patel et al., 1991; Suzuki et al., 1991a). It is clear that factors other than PAF may significantly contribute to H$_2$O$_2$-induced leukocyte adhesion, but these remain to be identified. NH$_2$Cl, like H$_2$O$_2$, exerts a direct effect on neutrophils to initiate CD11/CD18 expression, but PAF does not contribute to NH$_2$Cl-mediated expression of CD11/CD18 (Suzuki et al., 1991a). NH$_2$Cl may exert a direct effect on neutrophils to initiate CD11/CD18 expression or cause CD18-dependent leukocyte adherence and permeability by enhancing the formation of an inflammatory mediator like LTB$_4$

The mechanism by which superoxide induces leukocyte adhesion and permeability has not yet been determined.
fully. However, it is conceivable that inactivation of nitric oxide (NO) by superoxide may be a crucial step in leukocyte-dependent microvascular dysfunction. NO is a biologically active compound produced by vascular endothelium and prevents leukocyte adhesion to endothelial cells in postcapillary venules (Kubes et al., 1991b). The precise mechanism by which NO exerts this effect remains to be identified. NO may mediate its antiadhesive effect through the leukocyte adhesion to $\beta_2$-integrins. NO could either interfere with the ability of constitutively expressed $\beta_2$-integrins to form an adhesive bond with the endothelial cell surface or act to suppress $\beta_2$-integrin expression on leukocytes.

It has recently been suggested that continuous release of NO plays an important role in maintaining an intact endothelial cell barrier and that inhibition of NO production by vascular endothelium leads to a reversible increase in microvascular protein leakage that appears to be mediated by both leukocyte-dependent and -independent mechanisms, which remain to be defined (Kubes and Granger, 1992). Two possibilities are tenable: first, inhibition of NO production could allow an accumulation of superoxide and/or H$_2$O$_2$ and; secondly, inhibition of NO may lead to activation of other inflammatory cells, which, in turn, release substances that could increase microvascular permeability. For example, it is known that inhibition of NO synthesis promotes platelet adhesion, and consequently causes endothelial cell injury (Packham et al., 1968). In the view of these studies, NO production/inhibition and modulation of vascular permeability require further investigation. It is also conceivable that neutrophils release other agents that are capable of damaging the microvasculature or preventing injury.

**FUTURE PERSPECTIVES**

In this Commentary, leukocyte/endothelial cell interactions have been described, from the rolling phenomenon to vascular permeability. The mediators of these events have been thoroughly studied at the molecular level. More work on mechanisms is required to determine exactly how these molecules are orchestrated in vivo to regulate the different events. The identification of new receptors may help us to understand and cure leukocyte-induced disorders. This review underlines the key role of PAF and LTB$_4$, two of the most potent chemotactic substances known, and suggests that NO production and the beneficial effects of fatty acids are potential future directions to follow for the treatment of leukocyte disorders. With regard to oxidants, future progress will depend on finding new compounds that selectively interfere with their production or inhibition, together with investigations of the different mechanisms by which oxidants mediate leukocyte adhesion and vascular permeability. Experiments in flow conditions also appear to be important for future research on leukocyte adhesion and detachment mechanisms. In particular, it will be of interest to determine how flow conditions influence and modulate the expression or activation of adhesion molecules. The kinetics of binding formation should also be studied. Finally, even slight changes in shear stress, due to dilation or contraction of the vessel, could cause extensive changes in leukocyte adhesion to endothelial cells.

**REFERENCES**


Neutrophil/endothelium interactions


