REVIEW

The microcirculation and inflammation: modulation of leukocyte-endothelial cell adhesion

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Abstract: The accumulation of leukocytes in inflamed tissue results from adhesive interactions between leukocytes and endothelial cells within the microcirculation. These adhesive interactions and the excessive filtration of fluid and protein that accompanies an inflammatory response are largely confined to one region of the microvasculature: postcapillary venules. The nature and magnitude of the leukocyte-endothelial cell adhesive interactions that take place within postcapillary venules are determined by a variety of factors, including expression of adhesion molecules on leukocytes and/or endothelial cells, products of leukocyte (superoxide) and endothelial cell (nitric oxide) activation, and the physical forces generated by the movement of blood along the vessel wall. The contribution of different adhesion molecules to leukocyte rolling, adherence, and emigration in venules is discussed. Emerging views on potential endogenous antiadhesion molecules produced by endothelial cells as well as the influence of alterations in shear rate on leukocyte adhesion are addressed. Finally, the pathophysiological significance of the microvascular responses to inflammation are discussed in terms of adhesion-directed strategies for the treatment of different cardiovascular diseases and circulatory disorders. *J. Leukoc. Biol.* 55: 662-675; 1994.

Key Words: *leukocyte rolling • nitric oxide • platelet-activating factor • shear rate • selectins • superoxide • histamine • prostacyclin • β2 integrins • leukotrienes • adenosine • ischemia • reperfusion*

INTRODUCTION

"Next to leukocytes, the vessels and their endothelial lining play the most important role in inflammation."—Elie Metchnikoff, 1883

For well over a century, there has been an appreciation for the contribution of the microcirculation to the inflammatory process [1]. While the microvascular response to inflammation has been a major focal point of investigation since the early descriptions of leukocyte behavior in microvessels, the interest and effort of researchers in recent years have been directed at defining the cellular and molecular mechanisms that underlie the interactions between leukocytes and microvascular endothelium in inflamed tissue. This new emphasis on mechanisms was made possible by technological advances that allowed investigators to grow endothelial cells as monolayers to mimic their spatial symmetry in the vasculature, as well as the production of monoclonal antibodies that bind to and functionally inactivate adhesion receptors on the surface of leukocytes and endothelial cells. The information gained from these in vitro studies of leukocyte-endothelial cell adhesion has greatly extended our understanding of the fundamental principles that govern the targeting of circulating leukocytes to inflamed regions within tissues. Nonetheless, the concern is occasionally raised that in vitro models of leukocyte-endothelial cell adhesion may not adequately reproduce the more complex in vivo situation wherein these interactions occur in the face of a flowing blood stream and the nature and level of adhesion receptor expression may differ from those exhibited on isolated leukocytes and cultured endothelial cells [2].

Refinements in the technique of intravital videomicroscopy now allow investigators to monitor and quantitate the adhesive interactions between different circulating cells and the blood vessel wall (**Fig. 1**). The application of this technology, coupled with the development and availability of significant quantities of monoclonal antibodies that react with adhesion receptors in different animal models, has led to the recent explosion in the literature on modulation of leukocyte-endothelial cell adhesion within the microcirculation. In this review, we summarize this rapidly growing body of information and critically address some of the evolving views that attempt to integrate concepts derived from studies of the cellular and molecular biology of adhesion receptors with physiological and mechanical events taking place within the microcirculation.

LEUKOCYTE ROLLING

As leukocytes exit capillaries, hemodynamic forces give rise to an outward radial movement of leukocytes toward the endothelium. This margination process is generally attributed to red blood cells (which normally pile up behind the larger leukocyte in capillaries) that overtake the leukocyte and tend to push it toward the venular wall once the vessel diameter increases to a size that is about 50% larger than the diameter of the white cell [3]. The importance of red cells in initiating the radial movement of leukocytes toward the periphery of postcapillary venules is exemplified by the observation that very little leukocyte margination occurs in rat mesenteric venules perfused with leukocyte suspensions devoid of red cells [4]. The physical and chemical basis for the transition from margination to actual rolling of leukocytes in postcapillary venules is poorly understood, but the process does appear to result from increased strength of the adhesive interaction between leukocytes and endothelium.

Abbreviations: fMLP, *N*-formylmethionyl-leucyl-phenylalanine; ICAM-1, intercellular adhesion molecule 1; IL-1, interleukin-1; LAD, leukocyte adhesion deficiency; LTB_4, leukotriene B_4; mAb, monoclonal antibody; MTX, methotrexate; NO, nitric oxide; PAF, platelet-activating factor; PGI_2, prostacyclin; SLe^x, sialyl-Lewis x.

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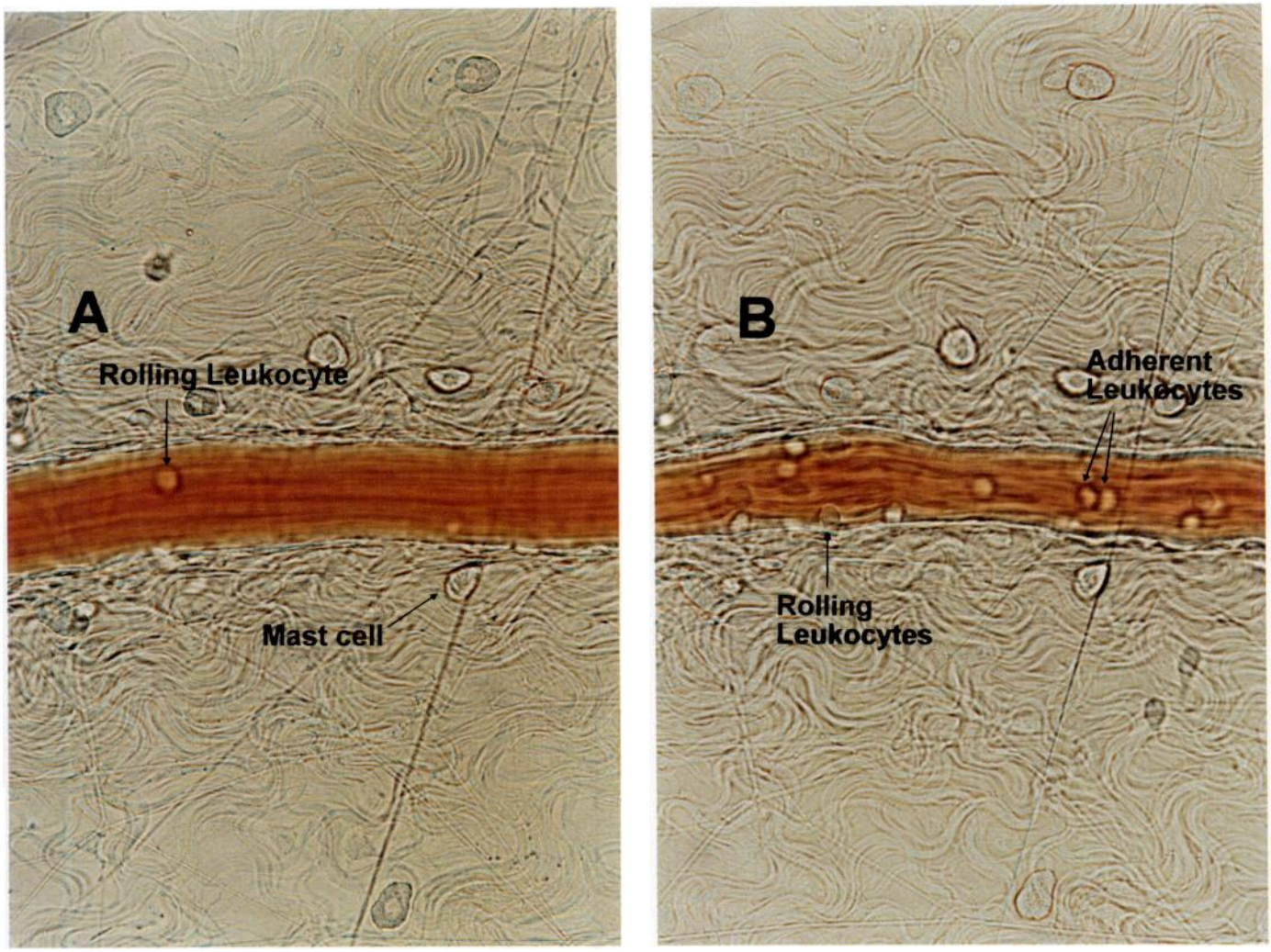

Fig. 1. (A) Postcapillary venule (35 μm in diameter) in rat mesentery superfused with bicarbonate-buffered saline and observed by intravital videomicroscopy. Rolling, but not firmly adherent, leukocytes are noted during this basal state. (B) Effect of reducing shear rate (hemorrhage) on leukocyte–endothelial cell adhesion. The reduced shear rate elicited the recruitment of both rolling and firmly adherent leukocytes.

Leukocyte rolling can be described as a low-affinity adhesive interaction between leukocytes and vascular endothelium whereby the force of blood flow acts on the leukocyte to induce a rotational motion. However, rolling leukocytes are generally defined as white cells that move through microvessels at a rate that is lower than that of red blood cells. In 30-μm-diameter postcapillary venules, the red blood cell velocity is usually 1–3 mm/s, whereas leukocytes roll at velocities ranging between 5 and 300 μm/s, with the most frequently observed rolling velocities lying between 20 and 60 μm/s. Rolling leukocytes are not always committed to either firmly adhering to the vessel wall or rolling along the entire vessel length; rolling leukocytes frequently detach and return to the mainstream of flowing blood. Studies employing fluorescently labeled leukocytes have led to the recognition that about 40% (20–70%) of the leukocytes entering a venule will exhibit rolling behavior [5, 6].

It remains unclear whether leukocyte rolling is normally present in postcapillary venules in the absence of experimentally induced trauma. Some investigators contend that the basal rolling of leukocytes observed in preparations that involve exteriorization of internal organs (e.g., mesentery) results from the surgical procedure and accompanying trauma to the isolated microvascular bed [7]. Others, however, have demonstrated that leukocyte rolling is prominent in dermal venules even without prior stimulation or surgical trauma [8, 9]. The latter possibility appears to provide a tenable explanation for the sustained resident population of interstitial granulocytes in various tissues including the intestinal mucosa, despite a significant turnover rate of these resident cells [10]; that is, leukocyte rolling and the subsequent adherence or emigration must occur continuously in order to maintain a constant resident cell population. Indeed, leukocyte rolling is likely to occur under normal physiological conditions in all tissues (gastrointestinal mucosa, skin, lung) that are continually exposed to external inflammatory stimuli that are physical and/or chemical in nature.

The leukocyte–endothelial cell adhesion that is manifested as rolling can be elicited by a number of agents and experimental conditions (**Table 1**). Some agents or conditions elicit recruitment of rolling leukocytes within minutes (histamine), whereas others (interleukin-1) require hours to produce the same response. To date, it appears that the leukocyte rolling induced by all known stimuli is mediated by unique glycoproteins that belong to the selectin family of cell adhesion receptors. These include L-selectin, which is constitutively expressed on the surface of leukocytes; E-selectin, which is found on cytokine-activated endothelium; and P-selectin, which is expressed on activated endothelium and platelets [11]. There is striking homology between the cDNA

TABLE 1. Agents or Conditions That Promote Leukocyte Rolling and/or Adherence in Postcapillary Venules

Agent or condition	References
Stimulants for rolling	
Superoxide	22, 23, 33
Lactoferrin	24
Histamine	25, 34
A23187 (calcium ionophore)	26
Interleukin-1	28
Hydrogen peroxide	26
Compound 48/80	35
Indomethacin	20
Ischemia-reperfusion	36
Low shear rates	6, 19
Stimulants for adherence	
C5a	37
Platelet-activating factor	38-40
Leukotriene B_4	41-43
N-Formylmethionyl-leucyl-phenylalanine (fMLP)	44, 45
Hydrogen peroxide	46
Oxidized low-density lipoprotein	47
Cigarette smoke	48
Aspirin	49
Indomethacin	20, 50
Nitric oxide synthesis inhibitors	51, 52
Compound 48/80	53, 54
Ischemia-reperfusion	55, 56
Endotoxin	57
Monochloramine	46
Acute systemic hypoxia	58

sequences of these lectin-like molecules, suggesting a common functional purpose within the vasculature. The ligands for these adhesion molecules have not been clearly defined, but sialyl-Lewis x (SLe^x) and other fucosylated carbohydrates have been postulated as potential counterreceptors [12]. A direct interaction between L-selectin on leukocytes and P- or E- selectin on endothelial cells has also been postulated [13]. During the past 3 years a rapidly growing body of evidence has implicated the selectins as mediators of leukocyte rolling in postcapillary venules.

L-selectin

Several experimental strategies have been used to assess the contribution of L-selectin to leukocyte rolling in postcapillary venules [14, 15]. A monoclonal antibody (mAb) directed against L-selectin (DREG-200) dramatically reduced the number of spontaneously rolling leukocytes in rabbit mesenteric venules. Moreover, microinfusion of either soluble recombinant L-selectin immunoglobulin G chimera or polyclonal antiserum to L-selectin into venules of rat mesentery reduced leukocyte rolling [14]. Other studies have capitalized on the fact that L-selectin is rapidly shed from the surface of leukocytes that are either activated with an inflammatory stimulus or exposed to chymotrypsin [16]. Normally, isolated and fluorescently labeled human neutrophils exhibit significant rolling behavior when injected into a rabbit mesenteric preparation; however, a much smaller fraction of these cells exhibit rolling if L-selectin is shed prior to intravascular administration.

Additional support for a contribution of L-selectin to leukocyte rolling is provided by data derived from studies employing cell lines (300.19) that display stable surface expression of L-selectin following transfection with human L-selectin cDNA [17]. Although control 300.19 cells do not roll in rat mesenteric venules, approximately 17% of their L-selectin-transfected counterparts exhibit rolling behavior, which is completely inhibited by administration of monoclonal antibodies directed against L-selectin. The construction of mutant L-selectin molecules in these transfected cell lines has also allowed assessment of the contribution of specific domains of the selectin to leukocyte rolling in vivo [17]. This approach has yielded data suggesting that a mutant form of the cytoplasmic domain of L-selectin does not support leukocyte rolling in postcapillary venules, despite the fact that the structural and functional features of the extracellular portion of L-selectin remained intact. This observation suggests that the intracellular portion (cytoplasmic tail) of L-selectin is a critical determinant of the activation state of this leukocyte rolling receptor.

P-selectin

Only recently has the contribution of P-selectin to the modulation of leukocyte rolling in postcapillary venules come to light. Two different experimental strategies have been employed to assess the significance of P-selectin to leukocyte rolling in vivo: (1) immunoneutralization with P-selectin mAbs and (2) P-selectin "knockout" mice. In dogs, administration of a P-selectin-specific mAb reduced the flux of spontaneously rolling leukocytes in mesenteric venules by greater than 90% [18]. However, this reduction in the flux of rolling leukocytes returned to basal levels within 20 min after mAb administration. The transient inhibitory effect of the P-selectin mAb was attributed to internalization of the antibody that was bound to initially expressed P-selectin, with subsequent mobilization of new P-selectin from its normal endothelial cell storage site, the Weibel-Palade body [18]. P-selectin-specific mAbs have not been effective in reducing the spontaneous rolling observed in mesenteric venules of species other than the dog (cat and rat), despite the fact that the same mAbs are very effective in abolishing the recruitment of rolling leukocytes elicited by different agents [19-21]. An explanation for this discrepancy is not readily available; however, it may result from species-related differences in the expression of rolling receptors elicited by initial surgical manipulation and exposure of tissues to be examined by intravital microscopy. This possibility is supported by the higher basal leukocyte rolling flux observed in dog mesentery [18]. One approach that can be used to obviate this inherent complication of intravital microscopy is to allow longer stabilization periods after isolation of the tissue. In the rat, for example, allowing the mesentery to stabilize for approximately 60-90 min results in approximately 50% reduction in leukocyte rolling [22].

P-selectin monoclonal antibodies have also been used in studies designed to determine the contribution of different adhesion molecules to the recruitment of rolling leukocytes observed in feline mesenteric venules that are exposed to low shear rates [19]. Although mAbs against CD11/CD18 and ICAM-1 were ineffective in attenuating the recruitment of rolling leukocytes, a P-selectin mAb inhibited the shear rate-dependent response by about 50%. P-selectin specific mAbs have proved to be even more effective in blunting the recruitment of rolling leukocytes in rat mesenteric venules elicited by products of neutrophil activation, that is, superoxide [23] or lactoferrin [24]. Superfusion of the mesentery with the superoxide-generating system hypoxanthine-xanthine oxidase elicits approximately a threefold increase in the number of rolling leukocytes above that observed in untreated controls. However, pretreatment with a P-selectin mAb (PB1.3) prior to superoxide exposure completely abolished the recruitment of rolling leukocytes. The same P-selectin mAb is similarly effective in abolishing the

recruitment of rolling leukocytes induced by local intra-arterial infusion of lactoferrin [24].

Histamine, which is an effective stimulant of P-selectin expression on monolayers of cultured endothelial cells [11], may play an important role in the recruitment of rolling leukocytes in the initial stages of an inflammatory response. Superfusion of the rat mesentery with histamine results in a dose-related increase in the number of rolling leukocytes, a reduction in rolling velocity, and a small but significant increase in the number of firmly adherent leukocytes [25]. The histamine-induced recruitment of rolling leukocytes is prevented by histamine H_1 (hydroxyzine, diphenhydramine) but not H_2 (cimetidine) receptor antagonists. Pretreatment with either a P-selectin mAb or soluble sialyl-Lewis x oligosaccharide (a putative carbohydrate ligand to P-selectin) also effectively prevents histamine-induced recruitment of rolling leukocytes. These observations suggest that histamine released from degranulated mast cells (or other cellular sources) can promote leukocyte rolling in postcapillary venules through engagement of H_1 receptors, presumably located on endothelial cells. The proadhesive effects of histamine appear to be largely mediated by an interaction between P-selectin on endothelial cells and a ligand containing SLe^x on leukocytes [25].

Histamine-induced, P-selectin-mediated adhesion appears to contribute to the recruitment of rolling leukocytes elicited by lactoferrin [24]. A P-selectin mAb completely prevents lactoferrin-induced leukocyte rolling, whereas an E-selectin mAb has no effect. Diamine oxidase (histaminase) and an H_1 (but not the H_2) receptor antagonist are also effective in attenuating the recruitment of rolling leukocytes elicited by lactoferrin. Furthermore, lactoferrin causes significant degranulation of mast cells that surround postcapillary venules in rat mesentery. Collectively, these observations suggest that neutrophil-derived lactoferrin can amplify the process of leukocyte recruitment in inflamed tissue via a P-selectin-dependent mechanism that involves the engagement of mast cell-derived histamine with H_1 receptors on endothelial cells in postcapillary venules.

Another approach that has been used to assess the contribution of P-selectin to leukocyte rolling is the application of intravital microscopy to mesenteric microvessels in P-selectin-deficient mice [26]. The exteriorized mesentery in these P-selectin knockout mice revealed complete impairment of baseline leukocyte rolling, whereas venules in wild-type mice had a significant number of rolling leukocytes under baseline conditions. Although exposure of mesenteric venules to either hydrogen peroxide or A23187 (calcium ionophore) in wild-type mice promoted leukocyte rolling, such a response was not observed in the P-selectin-deficient mice. These genetically engineered animal models represent a powerful tool for future work directed at elucidating the contribution of adhesion glycoproteins to different components of the inflammatory response.

E-selectin

Much of the evidence that implicates E-selectin as a mediator of leukocyte rolling in inflamed microvessels is inferred from in vitro studies [27]. The dearth of information regarding the contribution of E-selectin to leukocyte-endothelial cell adhesion in the microcirculation probably results from the short-term experimental protocols that have dominated published work employing intravital videomicroscopy. For example, it has been shown that an E-selectin mAb does not attenuate the recruitment of rolling leukocytes observed within 30 min of exposure of postcapillary venules to lactoferrin [24]. Under such circumstances, the ineffectiveness of an E-selectin mAb could be attributed to absence of constitutively expressed E-selectin as well as the relatively long (hours) time required for significant E-selectin expression on the surface of activated endothelial cells [11]. However, it was recently reported [28] that a blocking monoclonal antibody directed against E-selectin will prevent granulocyte rolling in rabbit mesenteric venules exposed to IL-1 for 4 h before microscopic examination. Another interesting observation that implicates, albeit indirectly, E-selectin as a modulator of leukocyte rolling is that the recruitment of leukocytes to a site of inflammation becomes apparent 2-4 h after initiation of an inflammatory reaction in P-selectin-deficient mice, which do not display leukocyte rolling under basal conditions [26]. An explanation offered for these observations by the investigators is that E-selectin expression at 2-4 h allows the P-selectin-deficient venules to sustain leukocyte rolling. Direct experimental evidence to support this likely scenario was not provided.

Carbohydrate ligands of selectins

It is now well recognized that the selectins bind to specific carbohydrate structures composed of sialic acid, fucose, galactose, and *N*-acetylgalactosamine. SLe^x, a sialylated and fucosylated structure on carbohydrate groups of both glycoproteins and glycolipids, appears to exhibit binding, with variable degrees of affinity, to E-, P-, and L-selectin [29]. SLe^x and other closely related ligands (e.g., sialyl-Lewis a) lose binding activity upon removal of sialic acid with neuraminidase or in the absence of fucose incorporation [30]. Consequently, it is not unexpected that neuraminidase treatment of isolated granulocytes results in a 50-60% reduction in leukocyte rolling in rabbit mesenteric venules exposed to IL-1 [5]. The importance of SLe^x ligand recognition by selectins in mediating leukocyte rolling has been confirmed by the discovery of a human leukocyte adhesion deficiency (LAD II) resulting from absence of SLe^x on neutrophils [31] and the demonstration that neutrophils isolated from LAD II patients roll poorly and fail to adhere firmly in flowing postcapillary venules of rabbit mesentery [32]. Additional support for a contribution of SLe^x to leukocyte rolling in vivo is derived from the observation that a synthetic analogue of SLe^x-oligosaccharide, but not a control oligosaccharide missing the fucose residue, effectively prevents the recruitment of rolling leukocytes and reduction in leukocyte rolling velocity normally elicited in rat mesenteric venules by histamine [25]. The SLe^x oligosaccharide was as effective as a P-selectin mAb in abolishing histamine-induced rolling, suggesting that the SLe^x-oligosaccharide acts as an inhibitor of P-selectin in this experimental model. Finally, it has been shown that monoclonal antibodies to SLe^x attenuate the leukocyte rolling observed in IL-1-treated mesentery of the rabbit [5].

LEUKOCYTE ADHERENCE

In inflamed tissue, leukocyte rolling frequently (but not always) leads to a stationary state in which the leukocyte remains firmly attached to the endothelial cell surface, without rotational motion. This strong (high-affinity) adhesive interaction is often referred to as leukocyte sticking, firm adhesion, or adherence, terms that denote the absence of movement of the leukocyte along the length of the venule. The criteria used to identify an adherent leukocyte differ among laboratories, with periods of the stationary state ranging between 1 and 60 s. Cells exhibiting saltation (transient stoppage of a rolling leukocyte) are generally defined as rolling

leukocytes or treated as a separate population. The most widely employed definition of an adherent leukocyte in postcapillary venule is one that remains stationary for ≥30 s [55]. This duration of firm adhesion, although arbitrary, agrees with the definition used by investigators employing flow chambers to quantitate neutrophil adherence to endothelial cell monolayers exposed to shear [59]. Furthermore, it appears to represent the minimal duration of firm adhesion that is needed as a prelude for emigration (diapedesis) of the leukocyte during an inflammatory response [60]. Because accurate automated procedures for quantitation of leukocyte adherence are not available and playback of videotaped images is a labor-intensive procedure for quantitating firm adhesion, there are few reports that provide the frequency of duration of leukocyte adherence in inflamed postcapillary venules. In one such study, it was noted that as the intensity of the inflammatory stimulus was increased, the proportion of leukocytes that remained firmly adherent for short durations (1–10 s) decreased while the proportion of cells that were adherent for prolonged periods (≥30 s) increased [60].

Induction of leukocyte adherence in postcapillary venules has been described in numerous reports over the past decade. Topical (superfusion) or intravascular (intra-arterial or intravenous infusion) exposure of a tissue to an inflammatory mediator such as leukotriene B_4 (LTB_4) or platelet-activating factor (PAF) elicits recruitment of adherent leukocytes within 10 min, reaching peak values in 30–60 min [40, 41]. Bacterial products such as formylated peptide (fMLP) and a variety of other agents or conditions (Table 1) promote an adherence response with very similar kinetics of leukocyte accumulation. Most of the agents that elicit recruitment of firmly adherent leukocytes in postcapillary venules also have been shown to promote adherence of isolated neutrophils to endothelial cell monolayers [2], suggesting that most of the stimuli studied exert a direct effect on leukocytes and/or endothelial cells, rather than acting through a third cell type (e.g., platelets, mast cells).

Dependence of firm adherence on leukocyte rolling

Many of the agents and conditions that elicit firm adherence of leukocytes in postcapillary venules also elicit the recruitment of rolling leukocytes. This association between the appearance of firmly adherent and rolling leukocytes has led to the widely held view that rolling is a prerequisite for firm adherence of leukocytes in postcapillary venules. Because rolling leads to intimate contact between leukocytes and endothelial cells, this process should allow leukocytes to become activated by agents expressed on the endothelial cell surface (e.g., PAF) or by substances released from cells lying immediately outside the vasculature. Some evidence in the literature supports the view that rolling is required for firm adhesion of leukocytes. First, a positive linear correlation has been demonstrated between the extent of leukocyte rolling and the subsequent firm adhesion of leukocytes in rat mesenteric venules superfused with fMLP [61]. Second, selectin-specific monoclonal antibodies that interfere with leukocyte rolling also attenuate the recruitment of adherent leukocytes in different models of inflammation [62–65]. More definitive support is provided by observations [14–16, 61] that impairment of L-selectin function with mAbs or inhibitors (fucoidin) or by proteolytic cleavage of L-selectin from the leukocyte surface prevents firm adhesion in postcapillary venules exposed to a chemotactic agent. However, when the vessels were occluded to induce stasis, the L-selectin–impaired leukocytes were able to adhere and emigrate.

Role of β_2 integrins in mediating leukocyte adherence

Monoclonal antibodies directed against the β_2 integrins have proved very effective in reducing or preventing firm adhesion of leukocytes in postcapillary venules. The adhesion glycoprotein complex termed CD11/CD18 is found on most leukocytes and is composed of three structurally and functionally related glycoprotein heterodimers, each consisting of an immunologically distinct α subunit (CD11a, CD11b, CD11c) that is noncovalently associated with a common β subunit (CD18) [11, 66]. CD11a/CD18 is expressed on virtually all immunocytes, whereas CD11b/CD18 is expressed predominately on monocytes, macrophages, and granulocytes. Both CD11a/CD18 and CD11b/CD18 are constitutively expressed on the surface of quiescent (nonactivated) leukocytes. After exposure of the cell to phorbol esters or inflammatory mediators, these receptors are rapidly induced into a high-avidity (active) state that promotes firm adhesion. In addition, the same stimuli can mobilize CD11b/CD18 from preformed intracellular pools, thereby increasing surface expression severalfold.

Pretreatment of animals with CD18-specific mAbs, which should effectively immunoneutralize all three heterodimers of CD11/CD18, dramatically reduces or completely prevents the recruitment of adherent leukocytes in postcapillary venules induced by C5a [37], zymosan-activated serum [37], PAF [38–40, 67], LTB_4 [41–43, 67], H_2O_2 [46], monochloramine [46], ischemia-reperfusion [68–70], and hemorrhagic shock [71]. The findings with C5a, PAF, and LTB_4 are consistent with reports that demonstrate the ability of these inflammatory mediators to increase surface expression of CD11/CD18 on isolated neutrophils [66]. The inhibitory action of CD18-specific mAbs continues for several hours after a single injection of the immunoglobulin [72], with multiple injections over a period of 2–3 days resulting in the disappearance of granulocytes in tissues (intestinal mucosa) with a significant population of resident phagocytic cells [10].

CD18-specific mAbs are also effective in reducing leukocyte–endothelial cell adhesion in vivo when administered after the initiation of leukocyte adherence [38, 43]. For example, in experiments involving superfusion of cat mesentery with PAF, administration of a CD18-specific mAb reduced the number of firmly adherent leukocytes in postcapillary venules. In addition to preventing the recruitment of additional adherent leukocytes, the CD18-specific mAb caused approximately 50% of preexisting adherent leukocytes to lose contact with (detach from) venular endothelium, while the remaining adherent cells emigrated out of the vasculature [43]. The net effect at 60 min of PAF superfusion was that the vessel under observation no longer sustained leukocyte adherence. This observation suggests that exclusionary microenvironments between adherent leukocytes and endothelial cells do not prevent access of mAbs to the CD11/CD18 expressed on the surface of leukocytes.

The ability of CD18-specific mAbs to reduce firm adhesion of leukocytes cannot be explained by an effect on leukocyte rolling. The data in the literature indicate that leukocyte rolling velocity and the number of rolling leukocytes in inflamed microvessels are unaffected by mAbs against CD18 [15]. There is some evidence, however, that CD18 may contribute to the leukocyte rolling observed at low shear rates [73]. It is clear, nonetheless, that mAbs directed against CD18 are very effective in reducing leukocyte adherence in venules exposed to both normal (15–20 dyne/cm^2) and low (<3 dyne/cm^2) shear stresses [69, 70, 73]. The effectiveness of CD18-specific mAbs in reducing leukocyte adherence over a wide range of venular wall shear stresses is inconsistent

with reports demonstrating that CD18-specific antibodies significantly reduce adherence of neutrophils to endothelial cell monolayers that are exposed to a shear stress of 0.5 dyne/cm^2; however, when shear stress is increased to 2–3 dyne/cm^2, the CD18 antibodies do not affect adherence [74]. An explanation for the discrepancy is not readily available, but it may reflect in vivo versus in vitro differences in the density of expression of either CD11/CD18 on leukocytes or its ligand(s) on endothelial cells [11].

The potency of an anti-CD18 antibody in reducing leukocyte adherence is due, in large part, to the fact that it is directed against an epitope found on each of the three heterodimers (CD11a, CD11b, CD11c) of the CD11/CD18 glycoprotein complex. However, a limitation of antibodies to the common β subunit is that they do not reveal which of the three heterodimers are involved in the leukocyte adherence elicited by a specific proinflammatory stimulus. This limitation is not inconsequential, as a number of in vitro studies indicate that each of the three heterodimers may play a unique role in different models of inflammation [66]. Relatively few studies have examined the contribution of specific α subunits of CD11/CD18 to the recruitment of adherent leukocytes in postcapillary venules, presumably because of limited availability of the reagents (mAbs) needed for such an assessment. Monoclonal antibodies against CD11a and CD11b have been applied to a rabbit model of C5a-induced leukocyte adherence in mesenteric venules [37]. The mAb directed against CD11a was as effective as a CD18-specific mAb in preventing C5a-induced leukocyte adherence, whereas a CD11b-directed mAb attenuated leukocyte adherence in a variable fashion and by less than 50%. These findings suggest that CD11a/CD18 expression and/or activation is essential for C5a-induced leukocyte adherence in rabbit mesenteric venules, with CD11b/CD18 contributing less to this adhesion process. However, CD11b/CD18 appears to have a more prominant role in mediating the leukocyte adherence in venules exposed to LTB_4 [67], PAF [67], or ischemia-reperfusion [70]. In experiments employing these inflammatory stimuli, a CD11b mAb was as effective as a CD18 mAb in abolishing the recruitment of adherent leukocytes. Collectively, the limited data obtained with antibodies to CD11a and CD11b suggest that the relative contribution of each α subunit of CD11/CD18 to a leukocyte adherence response is stimulus dependent. Little or no information is available concerning the importance of the CD11c/CD18 heterodimer in modulating leukocyte adherence in vivo. Similarly, the CD11/CD18 mAbs tested to date do not allow an assessment of the relative importance of preexisting surface receptors (activation) versus an increased number of surface receptors (up-regulation) in CD11b-mediated leukocyte adherence responses.

Role of intercellular adhesion molecule 1 in leukocyte adherence

ICAM-1 has been implicated as a ligand for CD11a as well as CD11b, although the two α subunits of CD11/CD18 bind to different domains on ICAM-1. ICAM-1 is widely distributed on endothelial cells and various parenchymal cells in a functionally active form, yet its surface expression can be increased by exposure of endothelial cells to various cytokines or endotoxin. Cytokine-induced expression of ICAM-1 requires de novo protein synthesis, thereby increasing the adhesive potential of endothelial cells over a period of hours [66]. Monoclonal antibodies directed against ICAM-1 have been shown to attenuate the recruitment of adherent leukocytes in postcapillary venules exposed to zymosan-activated serum [37], PAF [67], LTB_4 [67], ischemia-reperfusion [70], nitric oxide synthase inhibitors [75], or indomethacin [20]. In some models of inflammation (C5a or PAF) an ICAM-1 mAb is as effective as a CD18-specific mAb in reducing the number of adherent leukocytes, but in most experimental models the ICAM-1 mAb is less effective. This observation suggests that CD11/CD18 binds to receptors (or substrates) other than ICAM-1 on venular endothelial cells. All of the intravital microscopy studies employing ICAM-1 mAbs to date involve protocols that last less than 1–2 h; consequently, it appears likely that the mAbs are acting on constitutively expressed, rather than induced, ICAM-1. Thus, mAbs directed against ICAM-1 are more likely to exert an effect comparable to that of CD18-specific mAbs under conditions characterized by increased expression of ICAM-1 on endothelial cells.

An interesting and potentially important difference between the actions of CD18 and ICAM-1 mAbs is their ability to reverse an existing leukocyte adherence response. ICAM-1 mAbs (unlike CD18 mAbs) are unable to free (detach) already adherent leukocytes from venular endothelium and appear incapable of preventing subsequent leukocyte adhesion, when administered after exposure of the microvessels to an inflammatory stimulus [37]. A definitive explanation for this discrepancy is not available, but it may be related to the ability of some inflammatory stimuli to mobilize soluble ICAM-1 into the blood stream, where it binds to the mAb and renders it nonfunctional [76]. The physiological significance of circulating soluble endothelial cell adhesion molecules in modulating leukocyte–endothelial cell adhesion in postcapillary venules remains to be determined.

LEUKOCYTE EMIGRATION

Following a period of stationary adhesion, a leukocyte may leave the postcapillary venule by extending pseudopodia between apposing endothelial cells and pulling itself into the subendothelial space and the adjacent interstitial compartment. This complex event, which is often termed leukocyte extravasation, emigration, or diapedesis, is dependent on an array of cellular processes including adhesion molecule expression and activation, cytoskeletal reorganization, and alterations in membrane fluidity. The actual process of leukocyte emigration across postcapillary venules is difficult to visualize with intravital microscopy because the leukocyte undergoes considerable deformation, characterized by a spreading or flattening of the cell before its disappearance from the blood stream and subsequent emergence in the subendothelial space. Intravital microscopy does, however, provide a means for quantitating the number of leukocytes that appear in the perivenular interstitium during an inflammatory reaction. This approach has been used to demonstrate that a variety of agents and experimental conditions can elicit the emigration of leukocytes from postcapillary venules [43, 45, 55, 67].

Superfusion of postcapillary venules with neutrophil chemoattractants such as PAF, LTB_4, or fMLP elicits profound leukocyte emigration within 30–60 min [45, 77]. If a chemotactic agent such as LTB_4 is administered intravascularly (local intra-arterial infusion) a similar level of leukocyte adherence can be achieved; however, these cells do not emigrate from the venules [43]. These observations suggest that leukocytes emigrate only if a favorable chemotactic gradient exists across the vessel wall. Additional support for this view is provided by studies employing compound 48/80, a selective activator-degranulator of connective tissue mast cells

[54]. Upon degranulation, mast cells release histamine, PAF, leukotrienes, and other proinflammatory molecules that elicit adhesion of leukocytes in postcapillary venules of hamster cheek pouch [54]. Compound 48/80 caused leukocytes to emigrate from the vasculature and migrate toward the arterioles, where most of the degranulating mast cells were located. Other agents such as PAF elicit a similar leukocyte emigration response irrespective of whether they are administered intravascularly (intra-arterial infusion) or extravascularly (superfusion); this suggests that a chemotactic gradient is not necessary for PAF to mediate leukocyte emigration [43, 77]. Indeed, it has been proposed that PAF may act as a cell-associated signal for leukocyte emigration [78] because in cat mesenteric venules exposed to ischemia-reperfusion, a PAF receptor antagonist (WEB 2086) is much more effective at reducing leukocyte emigration (>80%) than leukocyte adherence (~40%) [78].

A limited number of studies have addressed the importance of different adhesion molecules in the emigration of leukocytes from postcapillary venules. In general, monoclonal antibodies that block or attenuate the firm adherence of leukocytes will exert a similar effect on leukocyte emigration [67, 68, 70, 75]. The parallel responses of leukocyte adherence and emigration to mAbs against different adhesion molecules may be related in part to the fact that leukocyte adherence is a prerequisite for leukocyte emigration and any intervention that blocks the former will inevitably affect the latter. However, with some inflammatory mediators (e.g., PAF), ICAM-1 and E-selectin specific mAbs exert a greater inhibitory influence on emigration than on adherence [67].

Some agents, such as adenosine [79] and phalloidin [45], appear to exert a profound influence on leukocyte emigration relative to adherence in the same vessels. These agents may exert their unique effects on leukocyte emigration by acting on endothelial cells to prevent their retraction when leukocytes attempt to extend their pseudopodia into the intercellular junctions. Retraction of endothelial cells appears to be an important component of the emigration process [80]. This contention is supported by the observation that phalloidin, which stabilizes F-actin in endothelial cells and prevents their retraction, does not modify the recruitment of adherent leukocytes normally elicited by PAF, LTB_4, fMLP, or ischemia-reperfusion [45, 70], but it does prevent or greatly attenuate the emigration of leukocytes in the same venules. At the concentrations used, phalloidin did not affect neutrophil functions such as superoxide formation and release of proteases [45]. Nonetheless, agents such as phalloidin and adenosine could also affect various physical properties of the leukocytes that facilitate the emigration process, such as cell deformability.

FLOW DEPENDENCE OF LEUKOCYTE-ENDOTHELIAL CELL ADHESION

It is now well recognized that physical forces (shear rate, shear stress) generated by the movement (flow) of blood within the microcirculation play an important role in the modulation of leukocyte–endothelial cell adhesion in both normal and inflamed tissues. The prevailing wall shear rate in postcapillary venules determines the level of leukocyte rolling and firm adhesion, and it dictates the contact area between rolling leukocytes and the endothelial cell surface [19, 73, 81, 82]. Data for individual venules or for a population of venules that fall within a narrow range of internal diameters suggest that reductions in shear rate over a range of 800–50 s^{-1} lead to (1) an increased flux of rolling leukocytes, (2) a reduction in leukocyte rolling velocity, and (3) a reduction in the contact area between rolling leukocytes and the endothelial cell surface [81]. The recruitment of rolling leukocytes that is observed at low shear rates in noninflamed tissue appears to be mediated, at least in part, by P-selectin [19]. The chemical(s) that elicits the expression of P-selectin in venules exposed to low shear rates remains undefined; however, it is conceivable that shear rate or shear stress may act as a transducer that promotes the mobilization of Weibel-Palade bodies to the endothelial cell surface.

Based on the changes in leukocyte rolling that accompany a decline in wall shear rate, it can be inferred that the number of adherent leukocytes in postcapillary venules also depends strongly on the prevailing wall shear rate. Indeed, even in the absence of an inflammatory stimulus, graded reductions in venular shear rate for a period of 2 min elicit progressive recruitment of firmly adherent leukocytes [19, 73]. It has been demonstrated that leukocytes must slow to a velocity of approximately 50 μm/s before significant adherence can occur. As leukocyte rolling velocity is reduced progressively below 50 μm/s, the rate of leukocyte adherence rises dramatically [73]. At these lower velocities, the contact time between the adhesion molecules on the two cell surfaces is increased. Presumably there is a critical contact time between leukocytes and endothelium that is required before the interaction is strong enough to sustain firm adhesion. It has also been noted that the number of adherent leukocytes that is recruited by an inflammatory mediator such as PAF is far greater at low (<250 s^{-1}) than at normal (≥ 750 s^{-1}) shear rates [83], suggesting that it is easier for leukocytes to create strong adhesive bonds with endothelial cells at low shear rates. Furthermore, it appears that a higher shear rate is required to dislodge an adherent leukocyte in postcapillary venules exposed to PAF [83]. The dependence of leukocyte adherence on shear forces has also been demonstrated in in vitro models employing isolated neutrophils and monolayers of cultured endothelial cells [59, 74]; however, it appears that much lower shear stresses (3 vs. 15 dyne/cm^2) are needed to dislodge an adherent neutrophil in vitro.

Whether or not leukocytes adhere to endothelium depends on the relative magnitude of the chemical forces generated by adhesion molecules on leukocytes and endothelial cells and the physical forces (shear rate) that act to dislodge leukocytes from the vessel wall. The recruitment of adherent leukocytes observed at low shear rates appears to result from changes in chemical as well as physical forces generated on the endothelial cell surface. Monoclonal antibodies directed against either CD18 or ICAM-1, but not P-selectin, largely prevent the recruitment of adherent leukocytes at low shear rates [19], suggesting an interaction between CD11/CD18 on leukocytes with constitutively expressed ICAM-1 on endothelial cells. There are two possible explanations for the participation of CD11/CD18 in the increased leukocyte adherence observed at low shear rates: (1) there is a low level of expression of CD11/CD18 (in a high-avidity state) on normal circulating leukocytes and when shear rate is reduced the binding affinity of this adhesion glycoprotein is sufficient to overcome the lower physical dispersal forces, or (2) with a reduction in shear stress there is a concomitant decline in the washout of endogenous inflammatory mediators that are normally produced by endothelial and/or parenchymal cells. At low shear rates these mediators accumulate within microvessels to elicit increased expression/activation of CD11/CD18 on rolling leukocytes, which ultimately leads to adherence [84]. Support for the latter possibility is provided by data demonstrating that an LTB_4 receptor antagonist or

a 5-lipoxygenase inhibitor largely prevents the recruitment of adherent leukocytes normally observed at low venular shear rates, suggesting that LTB_4 accumulation mediates this phenomenon [84].

Venules generally exhibit lower shear rates than arterioles of comparable internal diameter. Consequently, it is often assumed that this difference in shear rates accounts for the predilection for leukocyte rolling and adherence in venules [85]. Indeed, firm adhesion of leukocytes is rarely observed in arterioles and only 0.6% of all leukocytes passing through arterioles roll, compared with 39% for venules [81, 82]. A growing body of evidence indicates that factors other than physical dispersal forces account for the inability of arterioles to sustain leukocyte-endothelial cell adhesion in normal and inflamed tissue. For example, when shear rate in arterioles is lowered to levels determined in venules, significant rolling and adherence of leukocytes are not observed [73]. Although retrograde perfusion of the mesenteric microcirculation causes a reduction in the flux of rolling leukocytes in venules and increased leukocyte rolling flux in arterioles, the latter was much less than the normograde venular flux of rollers. Furthermore, the venular retrograde flux of rollers was greater than the arteriolar flux under normograde conditions [86]. A more likely explanation for the propensity of leukocytes to roll, adhere, and emigrate in postcapillary venules is the preferential distribution of counterreceptors for leukocyte adhesion molecules in these vessels. Although there are no quantitative data that address such differences in adhesion molecule expression between arterioles and venules, qualitative histochemical analyses support this view [2]. It also remains unclear whether the few experimental conditions that are associated with leukocyte rolling and adherence in arterioles (thermal injury, oxidatively modified low density lipoprotein, and cigarette smoke) are accompanied by increased expression or activation of endothelial cell adhesion molecules.

ENDOGENOUS ANTI-INFLAMMATORY MOLECULES

The effects of different drugs on leukocyte adhesion in postcapillary venules have been examined in an effort to identify potential therapeutic agents for chronic inflammatory diseases. These studies have led to the recognition that some classical anti-inflammatory drugs such as indomethacin and aspirin actually promote leukocyte adhesion in venules [20, 49, 50], whereas a variety of other compounds appear to be effective in reducing or abolishing leukocyte-endothelial cell adhesion in different experimental models of inflammation (**Table 2**). With the application of specific biosynthesis inhibitors, receptor agonists and antagonists, and scavenging agents has come an appreciation of the potential importance of endothelial cell-derived substances that may contribute to the modulation of leukocyte adhesion in postcapillary venules. Three molecules that fall in this category are nitric oxide, adenosine, and prostacyclin.

Nitric oxide (NO) is a biologically active gas that is synthesized by a variety of cells, including vascular endothelium, from the guanido group of L-arginine. This reactive nitrogen molecule has been invoked as a mediator of vascular phenomena such as arteriolar dilation, platelet aggregation, and platelet-leukocyte adhesion [87]. Several lines of evidence also implicate NO as an endogenous inhibitor of leukocyte adhesion in venules: (1) NO synthase inhibitors elicit recruitment of adherent leukocytes [51–53, 75], (2) NO donors (nitroprusside, SIN-1) attenuate or prevent the leukocyte adherence induced by different inflammatory stimuli [22], (3) superoxide, which reacts with NO to render it biologically inactive, promotes leukocyte adherence [33], and (4) superoxide dismutase, which scavenges superoxide and limits the inactivation of NO, inhibits leukocyte adhesion [69, 88].

The proadhesive effect of NO synthase inhibitors cannot be explained on the basis of a reduction in venular shear rate [51], but the leukocyte adherence and emigration elicited by these agents can be attenuated by monoclonal antibodies against CD18, ICAM-1, or P-selectin [75]. Direct exposure of isolated neutrophils to NO synthase inhibitors does not induce increased expression/activation of CD11/CD18 or increased adhesion to biologically inert surfaces (plastic), indicating that these agents promote leukocyte adherence through an action on the endothelium or other cell types [51]. The leukocyte-endothelial cell adhesion elicited by NO synthase inhibitors is also blunted by administration of a PAF receptor antagonist and 8-bromo-cGMP [75, 89]. Overall, the available data suggest that any condition that tips the balance between NO production and superoxide generation

TABLE 2. Agents That Reduce Leukocyte Adherence in Postcapillary Venules

Compound	Biological action	References
Adenosine	Endogenous anti-inflammatory agent	79, 92
Prostacyclin (PGI_2)	Endogenous anti-inflammatory agent	49, 50
Iloprost	PGI_2 analogue	96
NO donors	Release nitric oxide	22, 99
8-Bromo-cGMP	Cyclic GMP analogue	75
Superoxide dismutase	Scavenges superoxide	100, 101
Catalase	Detoxifies H_2O_2	88
Quinacrine	Phospholipase A_2 inhibitor	89
WEB2086	PAF antagonist	46, 78, 89
Misoprostol	PGE_2 analogue	49, 50
SC41930	LTB_4 antagonist	50, 97
L663, 536, MK886	Lipoxygenase inhibitors	50, 98
Dietary fish oil	?	56
Colchicine	Antirheumatic	93
Methotrexate	Antirheumatic	92, 93
Ketotifen, cromolyn	Mast cell stabilizers	53
Cyclosporine, L683, 590	Immunosuppressive agents	102, 103
Salicylate	Anti-inflammatory agent	49
Leumedins	Anti-inflammatory agent	104
L658,758, Eglin C	Neutrophilic protease inhibitors	105, 106
Dimethyl sulfoxide	Hydroxyl radical scavenger	107
Verapamil, diltiazem	Calcium antagonists	108

in favor of the latter will elicit recruitment of adherent leukocytes within postcapillary venules. This imbalance between superoxide and nitric oxide is likely to result in (1) a reduction in cellular cGMP levels, (2) enhanced production of PAF (and possibly LTB_4) which activates CD11/CD18-ICAM-1-dependent adhesion mechanisms, and (3) mobilization of P-selectin to the endothelial cell surface [22, 23, 75]. The results of a recent report indicate that mast cell activation may also contribute to the leukocyte adhesion responses elicited by an imbalance between superoxide and nitric oxide [53].

Adenosine exerts a significant inhibitory effect on the function of isolated neutrophils, including superoxide production, chemotaxis, and adherence [90, 91]. This purine metabolite also appears to influence profoundly leukocyte-endothelial cell adhesion in venules, and its enhanced production by endothelial cells may explain the anti-inflammatory properties of methotrexate [92, 93]. Adenosine is very effective in reducing the leukocyte adherence and emigration in mesenteric venules elicited by either ischemia-reperfusion [79] or PAF [92, 93]. These actions of adenosine are mediated through the engagement of A_2, but not A_1, receptors on leukocytes [91, 92]. Although adenosine-mediated inhibition of neutrophilic superoxide production is also mediated through the A_2 receptor [91], this does not appear to provide the basis for adenosine's antiadhesive action because superoxide dismutase is much less effective than adenosine in reducing PAF-induced leukocyte adherence in mesenteric venules [38, 92]. Inasmuch as adenosine levels rise significantly in ischemic tissues, due to limited washout by blood flow, it has been proposed that the purine may minimize leukocyte-endothelial cell adhesion in venules during ischemia [79, 94].

Methotrexate (MTX), which is commonly used in the treatment of rheumatoid arthritis, is also effective in reducing the adhesion of neutrophils to endothelial cell monolayers [95] and the adherence and emigration of leukocytes in postcapillary venules [92, 93]. A role for adenosine in mediating the antiadhesive properties of MTX can be invoked based on reports demonstrating that (1) MTX stimulates adenosine production by monolayers of cultured endothelial cells [95], (2) adenosine deaminase, which metabolizes adenosine, abolishes the antiadhesive effects of MTX [92], and (3) the inhibitory effect of MTX on leukocyte adherence and emigration in venules is blunted by adenosine A_2, but not A_1, receptor antagonists [92]. Although both adenosine and MTX are highly effective in attenuating the leukocyte-endothelial cell adhesion elicited by some inflammatory stimuli (PAF, ischemia-reperfusion), neither agent affects the recruitment of adherent leukocytes elicited by LTB_4 [92].

Prostacyclin (PGI_2), which is known for its ability to inhibit platelet aggregation, also appears to act as an inhibitor of leukocyte-endothelial cell adhesion. The view that PGI_2 affects leukocyte adherence is supported by the observation that prostaglandin synthesis inhibitors (e.g., indomethacin) promote leukocyte adherence in mesenteric venules, an effect that can be reversed with exogenous PGI_2 [50]. Iloprost, a stable prostacyclin analogue, also exerts a profound inhibitory influence on leukocyte adherence in postcapillary venules exposed to ischemia-reperfusion [96].

CRITICAL ROLE OF LEUKOCYTE ADHESION IN INFLAMMATORY DISORDERS

Evidence implicating leukocytes as mediators of tissue injury in different disease processes is accumulating rapidly. Indeed, there has been growing recognition that granulocyte sequestration and activation are key components of the pathophysiology of diseases that have not previously been regarded as "inflammatory." Circulatory disorders such as myocardial infarction, stroke, circulatory shock, and atherosclerosis best exemplify such disease processes. The awareness that leukocytes may contribute to the pathogenesis of these and other diseases is largely a result of efforts to evaluate the effectiveness of monoclonal antibodies directed against leukocyte and endothelial cell adhesion molecules in different experimental animal models. These studies have revealed that prevention or reduction of leukocyte-endothelial cell adhesion often results in a profound attenuation of the microvascular and parenchymal cell dysfunction observed in different animal models of human disease [109-111].

Table 3 summarizes some of the recent literature that demonstrates a beneficial action of antiadhesion therapy in different experimentally induced inflammatory conditions. The protective action of antiadhesion mAbs is often manifested as, or can be explained by, an improvement of microvascular function (perfusion and exchange). Indeed, the microvasculature appears to be particularly vulnerable to the deleterious effects of an inflammatory response. This contention is supported by the fact that most of the conditions outlined in Table 3 are accompanied by one or more of the following microvascular changes: leukocyte adherence and emigration, leukocyte-capillary plugging, platelet-leukocyte aggregation, endothelial cell swelling and inactivation of various surface enzymes and membrane receptors, disruption of the endothelial cell glycocalyx and capillary basement membrane, and reduced sensitivity to vasoactive agents [109]. Important consequences of these leukocyte adhesion-dependent changes include enhanced extravasation of plasma proteins, increased fluid filtration, interstitial edema, and impaired tissue perfusion leading to hypoxia. The excessive leakage of fluid and protein from the vascular compartment is largely confined to the postcapillary venules, the dominant site of leukocyte adherence and emigration. Recent evidence suggests that the emigration (transmigration) of leukocytes, rather than the firm adhesion of leukocytes to the endothelial cell surface, is the limiting stimulus for vascular protein leakage in inflamed tissue [70, 75]. Although inflamed tissues often undergo an intense hyperemic phase (increased blood flow), poor tissue perfusion can result from leukocyte-capillary plugging, platelet-leukocyte aggregation, endothelial cell swelling, and reduced sensitivity of arterioles to endogenous vasodilators. Significant reductions in blood perfusion in inflamed tissue are undesirable because low shear rates further amplify the leukocyte-endothelial cell adhesion and the reduced convective delivery of oxygen may render parenchymal cells more vulnerable to the cytotoxic actions of extravasated granulocytes.

Interpretation of the improved organ responses to monoclonal antibody treatment is made more difficult by the multiple cell-cell interactions that can be mediated by some adhesion glycoproteins. For example, although it is tempting to draw the conclusion that an mAb directed against P-selectin affords protection in a model of inflammation by preventing leukocyte rolling, it is often equally plausible that the mAb exerts its beneficial action through an effect on platelet-leukocyte aggregation or leukocyte-capillary plugging [75, 112]. The remote organ injury observed in the lung following reperfusion of ischemic intestine [113, 114] best exemplifies this problem. While mAbs directed against CD11b/CD18 or P-selectin effectively prevent the pulmonary microvascular dysfunction elicited by reperfusion of a distant ischemic organ, neither mAb attenuated the granulocyte sequestration in lung tissue that normally accompanies this

TABLE 3. Protective Effects of Monoclonal Antibodies Against Adhesion Glycoproteins in Experimental Models of Inflammation

Experimental model	mAb specificity	Attenuated response	References
Ischemia-reperfusion			
Myocardium	CD18, CD11a, CD11b	Tissue necrosis	117, 118
	ICAM-1, P-selectin		119, 120
	CD18, ICAM-1, P-selectin	Endothelium-dependent vascular relaxation	118, 119
			120
Intestine	CD18, ICAM-1, CD11b	Microvascular protein leakage	70, 121, 122
	CD18	Mucosal injury	72
	P-selectin	Pulmonary vascular dysfunction	113
	CD18	Liver dysfunction	123
Skeletal muscle	CD18, P-selectin, ICAM-1	Capillary no-reflow	112, 124
	CD18	Contractile dysfunction	125
	CD18	Intestitial edema	124
Lung	CD18	Capillary fluid filtration	126
	CD18	Reexpansion edema	127
Brain	CD18	Capillary no-reflow	128
Transplantation	ICAM-1	Organ rejection	129, 130
Hemorrhagic shock	CD18	Mortality rate	131-133
		Visceral organ injury	
		Hemorrhagic necrosis	
		Endothelial cell dysfunction	
Sepsis	CD18	Liver dysfunction	134
		Pulmonary vascular injury	135
		Systemic vascular injury	136
Meningitis	CD18	Blood-brain barrier disruption	137
		Cerebral edema	
		Mortality	
Frostbite	CD18	Cellular necrosis	138
Thermal trauma	CD18, ICAM-1	Burn wound extension	139
		Ischemia	
Pulmonary oxygen toxicity	ICAM-1	Endothelial cell injury	140
Immune complex-induced alveolitis	CD18, ICAM-1,	Vascular proten leakage	141-144
	E-selectin, P-selectin		

response. Consequently, it has been proposed that the beneficial action of these reagents in models of distant organ injury to the lung could be related to the modulatory influence of CD11b/CD18 and P-selectin on oxygen radical formation by granulocytes [115, 116]. Despite the need for such caveats in interpreting the experimental findings in this field, the beneficial effects of antiadhesion therapy summarized in Table 3 clearly provide a basis for additional work on the application of this principle in the clinical setting.

FUTURE DIRECTIONS FOR MICROVASCULAR STUDIES OF LEUKOCYTE-ENDOTHELIAL CELL ADHESION

Over the past decade we have witnessed a resurgence of interest in and information on the role of microvascular elements (e.g., endothelial cells) in the initiation and maintenance of an inflammatory response. The discovery of specific adhesion molecules that underlie the phenomena of leukocyte rolling and adherence and the development of blocking antibodies directed against these molecules have provided microvascular biologists of the 1990s with the tools needed to address the mysteries of inflammation that perplexed their predecessors. It is anticipated that modern molecular biology will lead to the identification and characterization of new adhesion glycoproteins as well as surface molecules that contribute to poorly understood leukocyte functions such as spreading (shape change), aggregation, and emigration. Reagents and experimental interventions are also needed to define the contribution of molecules that modulate leukocyte-endothelial cell adhesion in postcapillary venules by modifying cell surface charges. Leukosialin (CD43), a sialic acid-rich molecule found on the surface of most leukocytes and shed during neutrophils activation, may be such a molecule. Perhaps the greatest future advances in this field will come from the application of intravital videomicroscopy to genetically engineered animals that either lack or overexpress putative adhesion molecules or other regulatory factors (e.g., nitric oxide).

There is also a need for improvement of the intravital microscopic techniques that are currently used to monitor leukocyte-endothelial cell adhesion in vivo. Visualization of leukocyte behavior in some organs, including the heart, lung, and kidney, remains difficult. Approaches are needed for study of selected populations of leukocytes without prior isolation and activation of these cells. On-line techniques to observe, quantitate, and correlate the expression of various adhesion molecules simultaneously with leukocyte behavior are still lacking. Finally, there is a need for work that relates quantitative measures of leukocyte activation and adhesion to equally quantitative measures of microvascular dysfunction, such as albumin extravasation or capillary perfusion. These technological advances would go a long way in ensuring that progress made in understanding the microvascular responses to inflammation during the next decade will match the remarkable insights gained during the past few years.

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