Transepithelial Migration of Neutrophils
Mechanisms and Implications for Acute Lung Injury

Rachel L. Zemans[1,2], Sean P. Colgan[3,4], and Gregory P. Downey[1,2,5]

[1]Division of Pulmonary and Critical Care Medicine, Department of Medicine, National Jewish Health, Denver; [2]Division of Pulmonary Sciences and Critical Care Medicine; [3]Mucosal Inflammation Program; [4]Division of Gastroenterology, Department of Medicine; and [5]Integrated Department of Immunology, University of Colorado Denver, Aurora, Colorado

The primary function of neutrophils in host defense is to contain and eradicate invading microbial pathogens. This is achieved through a series of swift and highly coordinated responses culminating in ingestion (phagocytosis) and killing of invading microbes. While these tasks are usually performed without injury to host tissues, in pathologic circumstances such as sepsis, potent antimicrobial compounds can be released extracellularly, inducing a spectrum of responses in host cells ranging from activation to injury and death. In the lung, such inflammatory damage is believed to contribute to the pathogenesis of diverse lung diseases, including acute lung injury and the acute respiratory distress syndrome, chronic obstructive lung disease, and cystic fibrosis. In these disorders, epithelial cells are targets of leukocyte-derived antimicrobial products, including proteinases and oxidants. Herein, we review the mechanisms involved in the physiologic process of neutrophil transepithelial migration, including the role of specific adhesion molecules on the leukocyte and epithelial cells. We examine the responses of the epithelial cells to the itinerant leukocytes and their cytotoxic products and the consequences of this for lung injury and repair. This paradigm has important clinical implications because of the potential for selective blockade of these pathways to prevent or attenuate lung injury.

Keywords: inflammation; acute lung injury; tight junctions; adherens junctions; proteolytic enzymes

CLINICAL RELEVANCE

This article reviews the mechanisms involved in transmigration of neutrophils through lung epithelial cells during inflammation. Identification of the signaling pathways involved will help identify novel targets to prevent or attenuate lung injury.

In their primary function in host defense, neutrophils have been likened to a night watchman because they continuously patrol the distant reaches of the lung and other organs, searching for invading microbial pathogens that they hunt down, ingest (phagocytose), and destroy (1, 2). To achieve this purpose, neutrophils possess a potent antimicrobial arsenal that includes oxidants, proteinases, and cationic peptides (3). Oxidants such as O_2^- and H_2O_2 are produced by the phagocyte NADPH oxidase and are potently microbicidal (4). Granules within the cytoplasm of neutrophils contain powerful proteolytic enzymes and cationic proteins that can digest a variety of microbial substrates. When neutrophils internalize microbial pathogens, these cytotoxic compounds are typically released directly into the phagosome, compartmentalizing both the pathogen and the cytotoxic products. However, under pathologic circumstances, these compounds are released into the extracellular space and can damage host tissues. Importantly, neutrophils and their responses in the context of an inflammatory response are inherently beneficial; only when their responses become excessive or unregulated does injury to host tissues ensue (1).

Integral to the effective functioning of neutrophils in host defense in the lung and other organs is their ability to egress from the vasculature and migrate through the tissues to the site of infection. During this journey into the lung, neutrophils pass through the endothelium, interstitial tissues, and epithelium before ending up in the airspaces. It is also during this time that unrestrained activation of neutrophils in response to microbial or host-derived stimuli may result in release of cytotoxic compounds that can injure vicinal host cells. While it is clear that neutrophils can emigrate from the vasculature into the airspaces without causing injury (5–7), there is compelling evidence from observations in humans and in experimental models that in pathologic circumstances, neutrophils are primary perpetrators of inflammatory injury to the lung and other organs. For example, neutrophil influx into the alveolar space correlates with lung injury as manifest by an increase in permeability of the alveolo-capillary membrane (8). Further, in some (but not all) animal models of acute lung injury, neutrophil depletion is protective (9, 10). It is believed that during the translocation of neutrophils from the vasculature to the airspaces, activation may be excessive and/or prolonged, leading to extracellular release of cytotoxic compounds that can induce a spectrum of responses in neighboring cells ranging from activation to injury and death. To understand what goes awry in pathologic conditions, it is necessary to review the physiologic processes involved in neutrophil transmigration into the lung.

NEUTROPHIL TRANSENDOTHELIAL MIGRATION

Neutrophils exit the circulation by a well-characterized series of events involving adhesion to and transmigration across the vascular endothelium into the interstitial space (11). A detailed review of these events is beyond the scope of this manuscript, but several key points that are relevant to transepithelial migration

(*Received in final form September 9, 2008*)

This work was supported by grant #HL090669 from the National Institutes of Health to G.P.D. and HL60569, DK50189, and DE13499 to S.P.C.

Correspondence and requests for reprints should be addressed to Gregory P. Downey, M.D., Drs Harold and Mary Zirin Chair in Pulmonary Biology, K701b, National Jewish Medical and Research Center, 1400 Jackson Street, Denver, CO 80206. E-mail: downeyg@njhealth.org

Am J Respir Cell Mol Biol Vol 40. pp 519–535, 2009
Originally Published in Press as DOI: 10.1165/rcmb.2008-0348TR on October 31, 2008
Internet address: www.atsjournals.org

will be briefly summarized. The first step in neutrophil emigration from the circulation involves adhesion to the vascular endothelial cells. Traditionally, these events have been viewed as involving three discrete phases: rolling, activation, and firm adhesion (11, 12). However, recent studies have added additional events to this sequence including tethering, slow rolling, modulation of adhesion strength, intraluminal crawling, and finally transcellular and paracellular migration (11). The initial step in leukocyte adhesion is capture (or tethering) mediated by interactions between L, E, and P-selectins and P-selectin glycoprotein ligand (PSGL1) and $\alpha4\beta1$ (VLA4) integrin. L-selectin is expressed by leukocytes, P-selectin is expressed by inflamed endothelium and platelets, E-selectin is expressed by inflamed endothelium, and PSGL1 is expressed by endothelium and some leukocytes. The subsequent rolling step is mediated by interactions between selectins and PSGL-1 and other glycosylated ligands. It is noteworthy that L-selectin and P-selectin require shear stress to support adhesion (13), and this may explain in part why selectins are not involved in neutrophil–epithelial adhesion and transmigration. Subsequent to rolling is slow rolling (mediated by selectin-triggered signaling), followed by arrest of the neutrophils on the endothelial surface involving β_1 and β_2 integrins and their cognate binding partners. Integrins are transmembrane glycoproteins expressed on leukocytes that are composed of α and β chains. In the case of β_2 integrins, there is a common β chain (CD18) and a variable α chain (CD11a, b, c, or d). The phase of leukocyte arrest is mediated by interactions between the β_2 integrin CD11a/CD18 (LFA-1) and intercellular adhesion molecule (ICAM)1, $\alpha4\beta1$ (VLA-4) and vascular cell adhesion molecule (VCAM)1, and $\alpha4\beta7$ and MADCAM1. The next phase involves adhesion strengthening and spreading that is mediated by activation of outside-in and inside-out intracellular signaling pathways. The phase of intravascular crawling that follows is mediated by interactions between CD11b/CD18 ($\alpha m\beta2$ or Mac-1) and ICAM1. Leukocytes then transmigrate across the endothelium, taking either a paracellular route involving platelet endothelial cell adhesion molecule (PECAM)1, CD99, junctional adhesion molecules (JAMs), and ESAM or a transcellular route involving ICAM1, PECAM1, and caveolins.

NEUTROPHIL TRANSEPITHELIAL MIGRATION

Analogous to the endothelial events, neutrophil migration across epithelia can also be considered in three sequential stages: adhesion, migration, and postmigration events (14) (Figure 1). The initial stage of neutrophil transepithelial migration is characterized by adhesion of the neutrophils to the epithelial membrane. However, distinct from interactions with the endothelium, neutrophil adhesion to the epithelium occurs on the basolateral as opposed to the apical surface. Further, the neutrophils that arrive at the epithelium have a "prior history" inasmuch as they have just crossed the endothelium and migrated through interstitial tissues before arriving at the base of the epithelium. These prior adhesive and migratory events, in conjunction with exposure to the chemoattractants that have spurred this movement, undoubtedly result in an alteration in the state of activation ("priming") of the migrating neutrophils. The neutrophils subsequently travel between adjacent epithelial cells (the interepithelial "migration tunnel"), crossing interepithelial junctions. Of importance, the epithelium and thus the interepithelial migration tunnel through which the neutrophils crawl is significantly longer (20 μm in length or more) than the equivalent structure between endothelial cells (a few micrometers at most) (15). In addition, there is no evidence that neutrophils take a transcellular route through epithelial cells unlike transendothelial migration. Finally, after traversing the epithelial barrier, neutrophils emerge on the apical surface of the epithelium and adhere to this surface for varying periods of time. During these events, multiple, sequential adhesive interactions must occur in order for the neutrophils to completely traverse the epithelial monolayer.

Our understanding of transepithelial neutrophil migration is less comprehensive than that of transendothelial migration. Further, much of our knowledge about transepithelial neutrophil migration derives from *in vitro* studies with intestinal epithelium, with relatively fewer studies using lung and other types of epithelial cells. In this review, we will discuss in detail the current knowledge of the adhesive interactions during each of these three stages of transepithelial migration. As mentioned above, we will compare and contrast the steps involved in transepithelial neutrophil migration in different types of epithelia and with those involved in transendothelial migration (Tables 1 and 2).

NEUTROPHIL LIGANDS

Leukocyte β_2 Integrins

Much of our knowledge of the mechanisms of transepithelial neutrophil migration derives from *in vitro* studies of neutrophil transmigration across cultured epithelial cells, although a few studies have examined these events *in vivo* (16). While earlier studies employed epithelial cells cultured on the upper surface of tissue culture inserts (17, 18) with neutrophil transmigration proceeding in a (nonphysiologic) apical to basolateral direction, more recent studies have employed a more physiologic arrangement in which epithelial cells are cultured on the underside of inverted tissue culture (e.g., Transwell) chambers (19–21). After allowing epithelial cell attachment, the chambers are then restored to the upright position and allowed to grow and attain confluence. Neutrophils are subsequently added to the upper chamber (basolateral surface of the epithelia) with a chemoattractant added to the lower chamber (apical surface of the epithelia) such that neutrophils are induced to migrate in the physiologic basolateral-to-apical direction (Figure 2). Some investigators have developed even more elaborate systems in which endothelial and epithelial cells are grown on opposite sides of a semipermeable membrane and neutrophils are induced to migrate sequentially across the endothelial and then the epithelial layer (22, 23).

In the first stage of transepithelial migration *in vivo*, neutrophils adhere to the basolateral epithelial surface (24) via β_2 integrins (19). In most epithelial cell types, CD11b/CD18 is the critical molecule involved in the initial adhesion of neutrophils to the basolateral surface (14, 19, 25). This is in contrast to neutrophil–endothelial adhesion, which is mediated by the combined effects of CD11a, b, and c (26, 27). In intestinal and urinary epithelium, neutrophil adhesion is mediated almost exclusively by CD11b/CD18 while CD11a and CD11c appear to have little or no role (19, 28). In the bronchial and alveolar epithelium, β_2 integrins are similarly critical for neutrophil adhesion (29, 30), with CD11b/CD18 playing the predominant role (31, 32), although CD11a and CD11c appear to participate under certain circumstances (21, 33).

In the presence of inflammatory stimuli, neutrophil–epithelial adhesion is increased in a β_2 integrin–dependent manner (34, 35). Moreover, β_2 integrin binding is a prerequisite to neutrophil migration across both the intestinal and airway epithelium, as blocking antibodies against either subunit almost completely prevent neutrophil migration (19–21). Further confirming the critical role of CD11b/CD18 in transepithelial neutrophil migration, neutrophils from patients with leukocyte adherence deficiency (LAD), a genetic disorder characterized by the

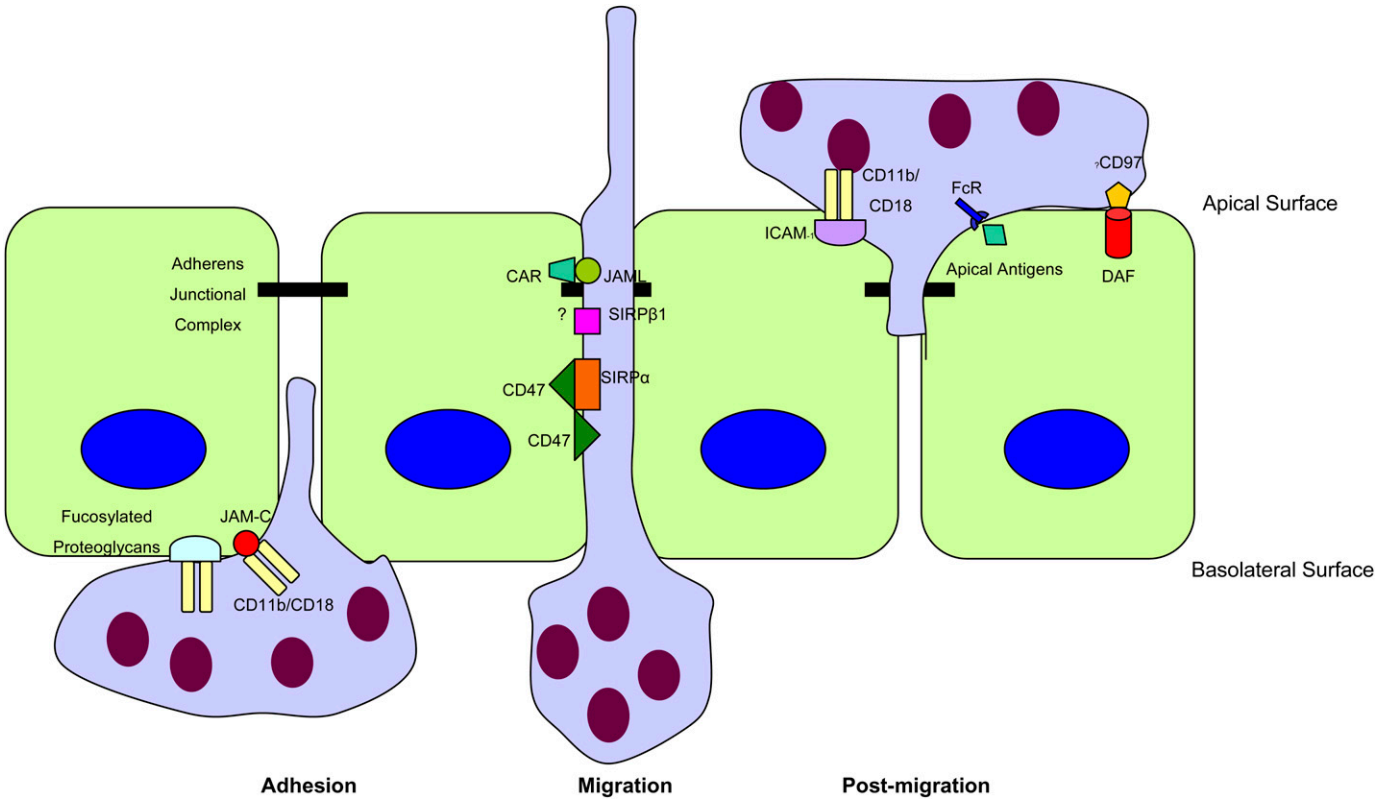

Figure 1. Neutrophil migration across epithelia can be considered in three sequential stages: adhesion, migration, and post-migration events. The initial stage of neutrophil transepithelial migration is characterized by adhesion of the neutrophils to the basolateral epithelial membrane. Adhesion is mediated by ligation of CD11b/CD18 on the neutrophil surface to several molecules on the epithelial surface, including fucosylated glycoproteins, JAM-C, and likely other as yet unidentified molecules. After initial adhesion, neutrophils crawl along the epithelial cell membrane via sequential binding to a number of epithelial cell surface molecules. Both epithelial and neutrophil CD47 are involved during this stage, and CD47 on both cell types may bind to and signal through SIRPα. In addition, SIRPα likely signals via pathways independent of CD47 during neutrophil transepithelial migration. Finally, SIRPβ1 also regulates neutrophil transmigration probably in an inhibitory fashion via signaling through an unidentified adaptor protein. At the level of the tight junction, neutrophil JAML binds epithelial CAR. Once neutrophils have completely traversed the epithelial monolayer, they adhere to the apical epithelial surface, where they resist fluid flow and mechanical forces and constitute a defense barrier against invading microorganisms. Important adhesive interactions on the apical surface include binding of the FcR to apical antigens, binding of CD11b/CD18 to ICAM-1, and likely binding of DAF to CD97. Adapted from Zen and Parkos (14).

absence of CD11/CD18 expression on the neutrophil cell surface, fail to migrate across a cultured GI epithelial monolayer (19). Because of this defect in neutrophil migration, patients with LAD suffer from recurrent mucosal infections (36).

Despite the overwhelming dependence of neutrophil migration on the β_2 integrins, CD18-independent transmigration can occur in some situations (37–39). In the intestine, β_1 and β_3 integrins play no role in neutrophil transepithelial migration (15). Further, there are reports of CD18-independent neutrophil migration in the lung (36, 40–43). Because neutrophils emigrate primarily through capillaries in the lung (44) (in contrast to postcapillary venules in the systemic circulation), and because the pulmonary circulation is characterized by lower pressures and close proximity to the epithelium, it is perhaps not surprising that neutrophil migration into the lung involves distinct mechanisms compared with neutrophil transmigration in other organs such as the intestine and kidney.

Alternate Neutrophil Adhesion Molecules

CD29 (β_1 integrin) appears to be involved in neutrophil transmigration in the lung, although its mechanisms of action may involve adhesion to fibroblasts or interstitial matrix rather than epithelial cells (39). Neutrophil CD44, a glycosylated membrane receptor implicated in a variety of cell adhesion events including neutrophil adhesion to endothelium (45), also plays a role in transepithelial migration, where its activation negatively regulates migration (46, 47). While selectins mediate neutrophil adhesion to the endothelium (11), they do not appear to be involved in adhesion to or transmigration through the epithelium (15, 48, 49).

The differences in cell surface molecules involved in initial adhesion of neutrophils to the epithelium versus endothelium are perhaps to be expected, given that transepithelial migration proceeds in the basolateral-to-apical direction, while transendothelial migration proceeds in the apical-to-basolateral direction (15). Furthermore, because neutrophil adhesion to the endothelium but not the epithelium occurs in an environment of shear force due to blood flow, distinct mechanisms are required (15, 50).

EPITHELIAL LIGANDS

ICAM-1

It has long been known that neutrophil CD11b/CD18 binding to epithelial surfaces is critical to neutrophil–epithelial adhesion and subsequent transmigration, and epithelia clearly express CD11b/CD18 ligands on their basolateral surface (51). However, the search for the epithelial counter-receptor for CD11b/CD18 has

TABLE 1. ADHESION MOLECULE EXPRESSION BY LEUKOCYTES AND ENDOTHELIAL AND EPITHELIAL CELLS

Adhesion Molecule	Subclasses	Cell Types	Ligands
Integrins	β_1 integrins:	Leukocytes, epithelial cells, endothelial cells, fibroblasts	Diverse ECM ligands depending on α and β subunits
	$\alpha4\beta1$, $\alpha6\beta1$		
	β_2 integrins:	Leukocytes	ICAM1/2
	$\alpha L\beta_2$ (CD11a/CD18, LFA-1)		
	$\alpha M\beta_2$ (CD11b/CD18, Mac-1)		
	$\alpha x\beta_2$ (CD11c/CD18)		
	$\alpha x\beta_2$ (CD11d/CD18)		
	Other integrins	Diverse cells	
	$\beta_{3,\,4,\,5,\,6,\,8}$		
Selectins	L-selectin	Lymphocytes, monocytes, neutrophils	PSGL-1
	P-selectin	Platelets, Endothelium	
	E-selectin	Endothelium	
Glycosylated molecules	PSGL-1	Leukocytes	Selectins
Other Adhesion molecules	JAM-A	Leukocytes, endothelial cells, epithelial cells	CD11a/CD18, Homophilic
	JAM-B	Epithelium, Endothelium	
	JAM-C	Epithelium, Endothelium, Platelets, T cells, NK cells, dendritic cells	VLA-4, HomophilicCD11b/CD18, Homophilic
	JAM-L		CAR
	PECAM		Homophilic, heparin, β-catenin, various kinases and phosphatases
IgG Superfamily	ICAM-1/2	Epithelial cells, endothelial cells	β_2 integrins
	CD47	Epithelial cells, neutrophils	CD47, SIRPα
Intercellular (Junctional) Adhesion Molecules	JAM-A,B,C	Endothelial and epithelial cells, neutrophils	See above
	JAM-L	Neutrophils	CAR
	VE-Cadherin	Endothelial cells	Adherens junctions
	E-Cadherin	Epithelial cells	Adherens junctions
	ZO-1	Endothelial and epithelial cells	Tight junctions
	Occludin	Endothelial and epithelial cells	Tight junctions

Definition of abbreviations: CAR, coxsackie and adenovirus receptor; ECM, extracellular matrix; ICAM, intercellular adhesion molecule; JAM, junctional adhesion molecule; PECAM, platelet endothelial cell adhesion molecule; PSGL, P-selectin glycoprotein ligand; ZO-1, zonula occludens-1.

A diverse set of adhesion molecules are expressed on the surfaces of hematopoietic, endothelial, and epithelial cells. Each adhesion molecule has a unique set of ligands to achieve its function during leukocyte migration.

proven elusive, in part because there may be multiple CD11b/CD18 ligands so that standard inhibitory studies are unrevealing (15, 49). One obvious candidate is ICAM-1, a known CD11b/CD18 ligand (52) that is critical for neutrophil adhesion to and migration across the endothelium (53, 54) as well as transepithelial T cell (55) and possibly eosinophil (56) migration. In fact, inflammatory stimuli up-regulate ICAM-1 on the surface of intestinal (25, 57, 58), conjunctival (57), renal, and bladder (59–61), as well as tracheal (32, 33, 62), bronchial (31, 63–65), and alveolar (66, 67) epithelial cells. Furthermore, neutrophil adherence to the apical surface of cultured tracheal (33), bronchial (31, 68), and intestinal (58) epithelial cells is ICAM-1 dependent. Finally, ICAM-1 inhibition may have a protective effect in lung injury (69, 70) and sepsis (71), although there are conflicting

TABLE 2. ADHESION MOLECULE INVOLVED IN NEUTROPHILS TRANSEPITHELIAL AND TRANSENDOTHELIAL MIGRATION

	Endothelium	Intestinal Epithelium	Bronchial Epithelium	Alveolar Epithelium
CD18/CD11a (LFA-1)	+	—	+	+
CD18/CD11b (Mac-1)	+	+	+	+
CD18/CD11c (p150)	+	—	+	?
CD18/CD11d	—	—	—	?
CD18-Independent	+	+/−	+	+
ICAM-1 (CD54)	+	—	+/−	—
PECAM (CD31)	+	—	—	—
CD62L, E, P (Selectins)	+	—	—	—
Carbohydrates	+	+	?	?
JAM-A	+	—	?	?
JAM-C	+	+	?	+
JAML	+	+	?	?
CAR	—	+	?	?
CD47	+	+	?	?

Definition of abbreviations: CAR, coxsackie and adenovirus receptor; ICAM, intercellular adhesion molecule; JAM, junctional adhesion molecule; PECAM, platelet endothelial cell adhesion molecule.

Although there are many similarities, distinct adhesion molecules are often involved in neutrophil migration across the endothelium and epithelium. Moreover, neutrophil migration across intestinal, bronchial, and alveolar epithelium may be mediated by distinct adhesion molecules.

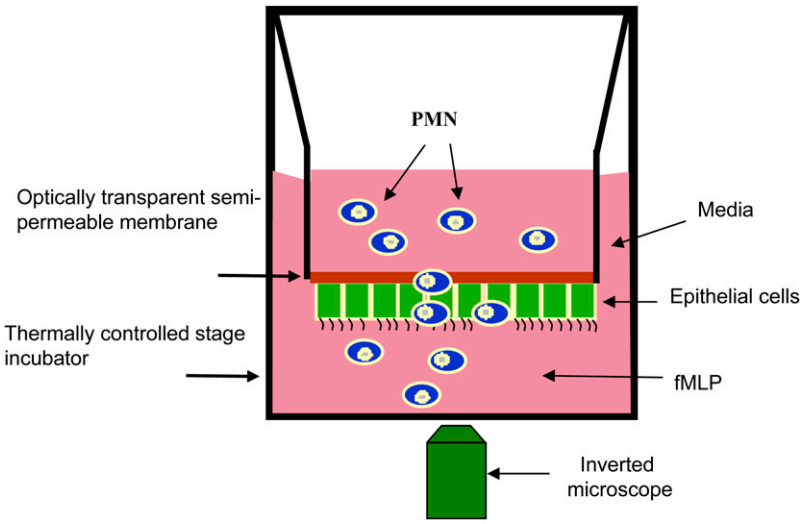

Figure 2. Neutrophil transmigration across cultured epithelia. Much of our knowledge about the mechanisms of transepithelial neutrophil migration is based on *in vitro* studies in which epithelial cells are cultured on the underside of inverted tissue culture (e.g., Transwell) chambers. Neutrophils are then added to the upper chamber (basolateral surface of the epithelia) with a chemoattractant added to the lower chamber (apical surface of the epithelia) such that neutrophils are induced to migrate in the physiologic basolateral-to-apical direction.

reports (70) and these results may be due to effects on endothelial rather than epithelial ICAM-1 (72).

Despite the evidence suggesting that ICAM-1 mediates neutrophil–epithelial adhesion, ICAM-1 is expressed exclusively on the apical epithelial surface of both intestinal (25, 58) and alveolar (66, 67) epithelial cells, thus precluding a role in initial adhesion to the basolateral surface during transepithelial migration. In fact, studies using blocking antibodies have definitively established that ICAM-1 binding is not critical to neutrophil migration across intestinal epithelium (19, 35). Still, ICAM-1 may play a role in neutrophil transmigration across other types of epithelia. In the lung, although ICAM-1 is unlikely to mediate neutrophil transmigration across the alveolar epithelium (67), there is some controversy in the literature regarding the role of ICAM-1 in neutrophil transmigration across the airway epithelium, where ICAM-1 is expressed on the basolateral surface (55). Whereas Liu and colleagues found only a very minimal reduction in neutrophil transmigration across a bronchial epithelial monolayer (20), there are two reports of substantial inhibition of neutrophil transmigration by ICAM-1 antibodies (21, 73). Finally, neutrophil transepithelial migration across uroepithelial cells (28) and possibly the gingival epithelium (74) appears to be mediated by ICAM-1.

VCAM

VCAM-1 expression in bronchial (65) and renal tubular (59) epithelial cells is induced by exposure to inflammatory stimuli in some reports but not in others (31), and there are reports that neutrophil–epithelial adhesion is VCAM-1 dependent (75). However, this interaction has not been shown to be important in neutrophil transepithelial migration.

PECAM

Although PECAM1 mediates transendothelial neutrophil migration, it is not expressed by epithelial cells and does not play a role in transepithelial neutrophil migration (14, 15).

JAMs

Other candidate epithelial ligands for β_2 integrins include the JAMs, transmembrane junctional proteins that are members of the Ig superfamily. JAM-A, a ligand for CD11a/CD18 (76), and JAM-C, a ligand for CD11b/CD18 (77), mediate transendothelial neutrophil migration (76, 78, 79) although there are conflicting reports (80, 81). Since JAM-A (82) and JAM-C (83) are expressed on epithelial cells, it is plausible that they serve as ligands for CD11b/CD18. However, JAM-A–blocking antibodies do not impede neutrophil transmigration across the intestinal epithelium (84), and no role for JAM-A in neutrophil transmigration across the alveolar epithelium has been demonstrated (85). Still, JAM-A is also expressed by neutrophils (84), and while epithelial JAM-A is apparently not involved in transepithelial migration, neutrophil JAM-A may play a role in the final stages of transepithelial migration by mediating neutrophil detachment from the epithelium (86).

By contrast, JAM-C plays a critical role in neutrophil transmigration across epithelial cells (83). Furthermore, epithelial JAM-C binds specifically to neutrophil CD11b/CD18 and mediates neutrophil adhesion to and migration across epithelia via direct ligation of CD11b/CD18 (83). However, JAM-C is expressed at the level of the desmosome and its binding to CD11b/CD18 occurs at sites distal to the initial adhesive interactions with the basolateral membrane of the epithelium (87). Furthermore, JAM-C blockade only partially ($\sim$ 50%) decreases neutrophil transepithelial migration and does not inhibit initial adhesion to the epithelial monolayer, suggesting the existence of other, uncharacterized epithelial ligands that bind CD11b/CD18 before JAM-C. Nonetheless, JAM-C is an important ligand for CD11b/CD18 and is critical to subsequent steps in transepithelial neutrophil migration. Although attributed to its role in transendothelial neutrophil migration, the importance of JAM-C in an *in vivo* murine model of acute pulmonary inflammation (88) may also be related to its role as a CD11b/CD18 ligand during transepithelial neutrophil migration.

Carbohydrate Ligands

Since CD11b/CD18 can also bind carbohydrates (89), which are known to be involved in neutrophil adhesion to the endothelium (12), some have postulated that epithelial cell surface carbohydrates may mediate neutrophil transmigration via binding to CD11b/CD18. Indeed, Colgan and colleagues demonstrated that carbohydrate interactions are involved in neutrophil transepithelial migration, as pretreatment with various polysaccharides inhibited neutrophil transmigration (48). Moreover, several carbohydrates similarly inhibit epithelial cell adhesion to purified CD11b/CD18, suggesting that the mechanism by which soluble carbohydrates inhibit neutrophil transepithelial migration is by interfering with binding of neutrophil CD11b/CD18 to cognate ligands on the epithelial cell surface (90). In addition, of the various carbohydrates tested, fucoidin, which binds relatively specifically to CD11b/CD18, was the

most potent inhibitor of epithelial binding to CD11b/CD18 and fucosidase treatment or inhibition of proteoglycan synthesis of the epithelial cells reduced neutrophil adhesion, suggesting that fucosylated proteoglycans are the epithelial ligands for neutrophil CD11b/CD18. Several fucosylated proteoglycans are expressed on the epithelial cell surface (91), but the identity of the specific molecule(s) that binds to CD11b/CD18 is unknown, although several potential candidates have been identified but not fully characterized (90). Notably, despite their role in transmigration via CD11b/CD18 binding, soluble carbohydrates did not prevent neutrophil adhesion to the epithelium (48). This suggests that the role of carbohydrates in neutrophil transmigration occurs during later stages, after firm adhesion has been established, and that other CD11b/CD18 ligands mediate the initial binding phase.

Nectins are transmembrane proteins involved in epithelial cell adhesion, but whether they serve as ligands for CD18/CD11b or are otherwise involved in epithelial–neutrophil adhesion has not been determined (14).

In summary, CD18/CD11b is clearly the most important neutrophil surface adhesion molecule mediating the initial adhesion of neutrophils to the basolateral surface of the epithelium. There are multiple epithelial ligands for CD18/CD11b, including fucosylated proteoglycans and JAM-C. The role of ICAM-1 in initial adhesive events remains uncertain. Finally, other known CD18/CD11b ligands, including ICAM-2, IC3b, heparin, and fibrinogen, do not appear to be involved in this process (15, 50).

NEUTROPHIL MIGRATION ACROSS THE EPITHELIUM

Once neutrophils have firmly adhered to the basolateral surface of the epithelium, they begin to migrate across the epithelial monolayer through the lateral paracellular space (transmigration tunnel). It is well established that neutrophils transmigrate across the epithelium exclusively via a paracellular rather than transcellular route (24, 50, 92–94). This is in contrast to transendothelial neutrophil migration, which can occur via either paracellular or transcellular routes (95). Interestingly, neutrophils tend to migrate in clusters (19, 85, 96) and preferentially at the tricellular corners, which are favorable for transmigration given the discontinuous nature of the tight junctions at these sites (85). In the lung, neutrophils migrate through tricellular corners located at the junctions of two alveolar type I cells and one alveolar type II cell (97) and may be guided by pulmonary fibroblasts (16, 98).

Role of CD47 and SIRPα

The process of neutrophil transmigration also depends on sequential adhesive interactions as the neutrophils crawl through the "tunnel" between epithelial cells. One of the cell surface molecules involved in this process is CD47, which has a well-characterized role in transendothelial migration (99). CD47 is expressed both on the basolateral surface of epithelia and on neutrophils (100), and its expression in epithelium is upregulated experimentally in response to inflammatory stimuli (101) and in inflammatory bowel disease (IBD) (102). Further implicating its role in transmigration, CD47-deficient mice demonstrate defective neutrophil accumulation in a murine model of *Escherichia coli* peritonitis, resulting in enhanced bacterial growth and increased mortality (103). *In vitro* studies initially showed that blocking antibodies against CD47 inhibited neutrophil transmigration across an intestinal epithelial monolayer (100), but it subsequently became clear that transmigration was delayed rather than fully prevented (101). Interestingly, both epithelial and neutrophil CD47 are involved in the process of transmigration across the intestinal epithelium (100, 101). In the distal lung (alveolar) epithelium, CD47 plays a role in monocyte transmigration (104), but its role in neutrophil transmigration has not yet been demonstrated.

The mechanism by which CD47 mediates transepithelial neutrophil migration has not been fully defined, although CD47 does not appear to be a ligand for CD11b/CD18. Furthermore, blocking antibodies do not interfere with neutrophil adhesion to the epithelial surface (100), and epithelial cells do not adhere to purified CD47 (15), suggesting that the role of CD47 in transepithelial migration occurs subsequent to the initial adhesive events. In fact, blocking antibodies result in accumulation of neutrophils within the epithelial monolayer (in the inter-epithelial tunnel) despite decreased transmigration to the apical surface (100). Therefore, some have suggested that CD47 might function to trigger de-adhesion from CD11b/CD18 (49).

Neutrophil CD47 plays a critical role in transepithelial migration, as CD47 is redistributed to the neutrophil cell membrane during transmigration and preincubation of neutrophils with blocking antibodies inhibits transmigration across either epithelial monolayers or cell-free filters (101). It appears that in neutrophils, CD47 ligation triggers downstream signaling cascades including tyrosine kinase-mediated events, leading to enhanced neutrophil migration, presumably via regulation of cytoskeletal reorganization (101). In addition, CD47 on the neutrophil cell surface binds SIRPα in *cis* (i.e., also on the neutrophil cell surface) and regulates neutrophil migration in part via SIRPα-dependent mechanisms (105, 106). Since SIRPα interacts with SHP-1 and SHP-2, it may be that the signal transduction–modulating effects of CD47 are mediated through SIRPα and SHP-1/SHP-2. The role of SIRPα in regulation of neutrophil migration may extend beyond its role as a ligand for CD47, as it appears to regulate transmigration by PI3K-dependent, tyrosine kinase–independent mechanisms, whereas tyrosine kinases but not PI3K appear to play a role in CD47-mediated signaling pathways. Furthermore, antagonism of SIRPα results in inhibition of neutrophil transmigration rather than the delay in transmigration attributable to CD47 (105). In addition, the related protein SIRPβ1, which is not a CD47 ligand, also regulates neutrophil transmigration, probably in an inhibitory fashion via signaling through an unidentified adaptor protein (107). This evidence further suggests that SIRP family members control neutrophil transmigration via CD47-independent mechanisms.

The role of epithelial CD47 in facilitating neutrophil transmigration has not been elucidated, although it is clear that epithelial CD47 is critical, as preincubation of epithelial cells with a CD47-blocking antibody inhibits transmigration (100, 101) and neutrophil transmigration across CD47-deficient epithelia is increased after transfection with CD47 (101). Further, neutrophil migration into the alveolar space and the attendant increase in lung permeability in response to LPS on Gram-negative bacteria is attenuated in CD47-deficient mice, an effect that is attributable to neutrophil (as opposed to endothelial or epithelial) CD47 (280). However, little is known about the mechanisms by which epithelial CD47 mediates neutrophil transmigration. Some hypothesize that neutrophil SIRPα binds epithelial CD47 *in trans*, suggesting that this interaction may initiate functional responses in the epithelium during leukocyte transepithelial migration (14).

In addition to CD47 and SIRPα, neutrophil migration through the epithelial monolayer is mediated by another member of the JAM family, JAM-like protein (JAML), known to be expressed on neutrophils and to mediate neutrophil adhesion to the endothelium (108). It has recently been shown that neutrophil

JAML binds to the coxsackie and adenovirus receptor (CAR), an Ig superfamily receptor expressed at epithelial tight junctions, and that this interaction is important for neutrophil transmigration (109).

In summary, after initial adhesion to the basolateral epithelial surface, neutrophils migrate across the epithelium via the interepithelial tunnel by mechanisms using the cell surface molecules CD47, SIRPα, and SIRPβ, followed by binding of neutrophil JAML to CAR.

POST-MIGRATION EVENTS: ADHESION TO APICAL SURFACE

Once neutrophils have completely traversed the epithelial monolayer, they participate in adhesive interactions with the apical epithelial surface such that they are retained on the lumenal side despite fluid flow (pulmonary edema in the lung and diarrhea in the gut) and mechanical forces (cough in the lung and intestinal peristalsis in the gut). In this location, neutrophils can constitute a defense barrier and perform their essential function of eradicating invading microorganisms (14). In fact, in patients with inflammatory bowel disease, high numbers of neutrophils are present in the lumen bound to the apical surface of the epithelium (102). Further, neutrophil adhesion to the luminal surface may be one reason why bronchoalveolar lavage (BAL) recovers only a small percentage of alveolar neutrophils (44). Binding of neutrophils to the apical epithelium is also mediated by specific adhesion molecules, which have begun to be elucidated. As discussed above, ICAM-1, an adhesion molecule expressed predominantly on the apical surface of epithelial cells (25, 58, 67), is up-regulated in response to inflammatory stimuli (25, 58, 61, 63, 64). Importantly, ICAM-1 is a known ligand of neutrophil CD18/CD11b (52) and mediates neutrophil–epithelial adhesion (58). While ICAM-1 is aptly suited to serve in tethering neutrophils at mucosal surfaces, this remains to be proven (14, 15, 49, 58, 85, 102).

In addition to ICAM-1, neutrophils may be retained at the apical epithelial surface via Fc interactions. Specifically, it is hypothesized that neutrophil Fc receptors bind to soluble antibodies that are in turn bound to specific ligands on the apical epithelial surface. Indeed, the intestines of patients with inflammatory bowel disease are characterized by auto-antibodies bound to the apical epithelial membrane (110). Furthermore, exogenous antibodies directed against antigens expressed on the apical surface of intestinal epithelial cells prevent neutrophil detachment from the epithelium after transmigration in experimental models (111). Whether prolonged retention of neutrophils via binding to the Fc domain of autoantibodies against lung epitopes contributes autoimmune pulmonary disease in such disorders as systemic lupus erythematosis is not known.

It is recently appreciated that mechanisms are necessary to clear neutrophils from the epithelial surface after successful transmigration. As neutrophils traverse the epithelium and arrive at the luminal (apical) aspect of the tissue, we presume that it is disadvantageous for prolonged exposure to activated neutrophils. A monoclonal antibody screen of apical neutrophil ligands revealed the existence of a mechanism to actively promote the clearance of neutrophils from the luminal surface of mucosal epithelia (112). This antibody (clone OE-1) was found to recognize decay accelerating factor (DAF, also known as CD55). DAF is classically known as a complement regulatory protein, which inhibits complement-mediated cell lysis and is highly expressed on the apical aspect of mucosal (e.g., lung and intestine) epithelial cells. While not fully understood at present, mapping of the binding site for the OE-1 antibody revealed that DAF may compete with ICAM-1 to dispel neutrophils from the epithelial surface after transmigration. CD97 expressed on neutrophils has been shown to bind and interact with DAF (113), although molecular details of this response are not well understood.

THE EFFECT OF NEUTROPHIL TRANSMIGRATION ON THE EPITHELIUM

One of the principal functions of epithelia is to form a selective barrier between various body compartments of different composition or between the body and the environment, a function that is achieved by inter-epithelial junctions termed tight and adherens junctions (114). During leukocyte transmigration, the epithelial barrier must open (at least transiently) to allow the passage of leukocytes. During immune surveillance or even during a normal immune response to an invading pathogen, leukocytes migrate across epithelia without damaging the epithelia (6, 94, 115, 116). To achieve paracellular transmigration without damage to the epithelium, there are close cell–cell contacts and highly regulated mechanisms responsible for signaling the opening and closing of the tight junctions without compromising barrier function (24). Some evidence exists that neutrophils may actively "reseal" epithelial junctions after transmigration. For example, upon arrival at the apical membrane surface, neutrophils release adenine nucleotides (ATP and AMP), which are subsequently metabolized to adenosine (117). Adenosine liberated in this manner is then available to bind apically expressed adenosine receptors, wherein one of the functional endpoints is the reestablishment of epithelial tight junction complexes (118).

In pathologic states, the passage of large numbers of activated neutrophils can result in damage to the epithelium (119, 120), both due to the release of toxic substances and by the mechanical force exerted by the neutrophil pseudopod resulting in microscopic wounds in the epithelium (94, 114). This neutrophil-mediated damage to the epithelial barrier results in the paracellular permeability, which in turn leads to the leakage of fluids that characterize acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) and diarrheal illnesses and facilitates the entry of bacterial toxins and microorganism into the tissues. We propose that there are three distinct mechanisms involved in opening the epithelium: (*1*) highly regulated disassembly and resassembly of TJ, (*2*) mechanical force resulting in epithelial wounds, and (*3*) degradative effects of neutrophil-derived mediators. We will discuss each of these mechanisms below. Furthermore, we propose that the distinct mechanisms by which neutrophils can migrate across the epithelial barrier may unify apparently conflicting reports of the effects of neutrophil transmigration on epithelial permeability (6).

Regulated Disassembly of TJ by Intracellular Signaling Pathways Triggered by Neutrophil Interactions

Although neutrophils transmigrate across the epithelium via the paracellular space (50, 92–94), the transmigration does not inevitably result in increased epithelial permeability, as demonstrated both *in vitro* (94, 115) and *in vivo* (6, 121). Small numbers of neutrophils can transmigrate without any change in permeability, as measured by electrical resistance and macromolecular flux (6, 15, 94, 120), and although larger numbers of transmigrating neutrophils (especially if activated) induce increased epithelial permeability, this can be transient and reversible (24, 93, 120, 122). This suggests that there are mechanisms by which tight junctions are opened and closed to allow for paracellular neutrophil transmigration without marked and permanent increased permeability or damage to the epithelium. There is a developing literature that begins to

define the mechanisms by which neutrophil adhesion to the epithelium triggers intracellular signaling pathways that culminate in a rapid, organized opening and closing of tight junctions to allow for the passage of neutrophils with only a minimal transient or no increase in permeability.

Early ultrastructural studies revealed that transmigrating neutrophils make close contacts with other neutrophils and with adjacent epithelial cells, such that an barrier impermeable to macromolecules is maintained (24, 93, 94). Notably, the ultrastructural appearance of these intimate contacts is similar to those seen during neutrophil transendothelial migration (24), and it has been demonstrated that these points of intimate contacts are comprised by specific endothelial cell surface proteins, notably JAM A, which forms a transient ring around the transmigrating neutrophil (123). In addition, there are functional data to suggest that adhesion of neutrophils to the basolateral epithelial surface may be the critical event that triggers the opening and resealing of tight junctions sufficiently to allow for transmigration (115, 124). Adhesion of neutrophils to the basolateral epithelial surface results in epithelial permeability that is independent of neutrophil transmigration or soluble factors released by neutrophils (19, 24, 115, 124, 125). Probably because of this adhesion-mediated increased permeability, neutrophil transmigration is more efficient in the physiologic basolateral-to-apical direction (19). The increased permeability induced by early adhesive events suggests that neutrophil adhesion to the surface of epithelial cells triggers intracellular signaling events within the epithelial cell leading to opening of the tight junctions. Neutrophil adhesion to the epithelial surface triggers phosphorylation of myosin light chain (MLC) (124), which in turn regulates contraction of the actomyosin ring, leading to opening of the TJ (126). In addition, neutrophil adhesion stimulates tyrosine phosphorylation of TJ proteins (124), which is known to regulate permeability (124, 127). The specific kinases responsible for phosphorylation of MLC or TJ proteins are beginning to be identified (128, 129). Interestingly, immunofluorescence studies have revealed that adhesion-dependent epithelial paracellular permeability is not due to loss or redistribution of junctional proteins away from the junction, which occurs only during neutrophil transmigration (124). The regulated opening of the junctions is quickly followed by rapid closure of the junction, in which JAM-A plays a critical role (84). Consistent with this putative role, JAM-A–deficient mice display increased permeability in a model of intestinal inflammation (130). The closure of the junctions leads to a rapid resealing of the epithelium and only transient alterations in permeability (84), which is critical to maintain barrier function during immune surveillance under physiologic conditions.

While the mere adhesion of neutrophils to the epithelial cell surface can trigger rapid opening and closing of the tight junctions, resulting in only transiently increased permeability, neutrophil transmigration itself may result in a higher degree of permeability that may or may not be reversible. These effects are attributable to both the mechanical forces of transmigration with the creation of microscopic defects and to soluble factors released by activated neutrophils as they migrate. The magnitude of these effects and the rapidity of their repair determine whether gross changes in macromolecular permeability and disease progression result from neutrophil transmigration. We will discuss each of these in turn.

EPITHELIAL WOUNDS DUE TO NEUTROPHIL TRANSMIGRATION

During transepithelial neutrophil migration, "scout" neutrophils are the initial cells to interact with and cross the epithelium individually. As transmigration progresses, neutrophils tend to migrate in clusters following in the "tracks" of the leading leukocytes (19, 96, 120). The migration of large numbers of activated neutrophils ("high-density migration") results in the formation of large epithelial wounds due to the mechanical separation of epithelial cells at the site of the interepithelial junction (94, 96, 120). These "wounds" are thought to represent precursors of the macroscopic areas of denuded epithelium (ulcerated lesions) that characterize inflammatory mucosal diseases such as IBD and ALI/ARDS (15). Although there are conflicting reports regarding the contribution of neutrophil-derived soluble mediators to the creation of these wounds (24, 96), the preponderance of evidence suggests that these wounds are at least in part created by mechanical forces (24, 116, 120). The repair of these wounds, which is achieved initially by epithelial cell flattening, extension of lamellipodia, and contraction of actin/myosin rings (120), may determine the extent to which permeability persists, resulting in leakage of fluid in disease states such as diarrheal illnesses and ALI.

EFFECT OF NEUTROPHIL-SOLUBLE MEDIATORS ON EPITHELIAL PERMEABILITY

In addition to the effect of neutrophil adhesion on opening of TJ and the mechanical effects of neutrophil transmigration on epithelial permeability, soluble mediators released by transmigrating neutrophils may influence epithelial permeability and function. These soluble mediators include proteases, cationic peptides, and reactive oxygen species (ROS). The importance of soluble mediators in neutrophil–epithelial interactions is underscored by the fact that neutrophils do not release toxic substances while in circulation, but only once they are adherent to the endothelium, interstitium, or epithelium (131). These soluble mediators and their effects on the epithelium will be discussed below.

Neutrophil Elastase

Of the neutrophil proteinases, elastase, a serine proteinase stored in azurophilic granules and released upon neutrophil activation, is the most completely characterized. The role of elastase in the pathogenesis of neutrophil-mediated inflammatory diseases, including ALI/ARDS and inflammatory bowel disease, is strongly supported in the literature. The BAL fluid (132–135) and plasma (136) of patients with ALI/ARDS are characterized by high levels of elastase, and these levels correlate with the severity of lung injury (134, 136). In animal models, elastase administration causes lung injury (137–139) and elastase inhibition has a protective effect on lung injury (140–144), as measured by several variables including permeability of the alveolocapillary membrane. There is also strong evidence to support the pathogenic role of elastase in the pathogenesis of inflammatory bowel disease (145–148) as well as cystic fibrosis (138, 149, 150) and chronic obstructive pulmonary disease (151, 152). Although the use of proteinase (elastase) inhibitors has had variable effects in ALI (153, 154), this may be because of limited study design (151), because patients are administered the inhibitor after the disease process has initiated (155), or because concentrated elastase activity may exist in a "protected space" between neutrophils and epithelia from which antiproteinases are excluded (156).

Yet, while elastase likely plays a pathogenic role in tissue destruction and permeability in some inflammatory diseases, it remains unclear whether this is due to the destructive effects of elastase on the epithelium *per se* in addition to its well-established degradative effects on the basement membrane

matrix (151, 157) and endothelium (158, 159). The effects of elastase on the epithelium are controversial. We have used an *in vitro* model to demonstrate that elastase released by neutrophils traversing an epithelial monolayer induces increased epithelial permeability via reorganization of the actin cytoskeleton and the intercellular junctions of epithelial cells adjacent to transmigrating neutrophils (96). Importantly, these effects of elastase on epithelial permeability facilitate further neutrophil transmigration, resulting ultimately in the creation of circular defects (wounds) in the monolayer, as has also been observed by others (120, 160). Although there may be several distinct mechanisms by which elastase disrupts the apical junctions and increases epithelial permeability, we have described proteolytic cleavage of E-cadherin by elastase (96), analogous to the effect of elastase on endothelial VE-cadherin (158), as one potential mechanism. Thus, degradation or down-regulation of junctional proteins may enable migrating neutrophils to "loosen" the interepithelial junctions, thereby inducing an increase in epithelial permeability and promoting transmigration of trailing neutrophils (161, 162).

There are conflicting reports in the literature regarding the role of elastase in mediating the permeability induced by neutrophil transmigration. While elastase clearly induces epithelial permeability in several studies (96, 163), in other studies protease inhibitors failed to prevent the fall in resistance induced by neutrophil transmigration and therefore had no effect on the rate of transmigration (24, 115). The conflicting results of the various studies may be attributable to the specificity of the various in inhibitors used, the type of purified elastase used, the directionality of the transmigration experiments, the epithelial cell line used, the culture conditions (such as coating of inserts), the density of neutrophil transmigration, or the methods for measuring permeability (125) or transmigration. Another explanation for these discrepant results is that proteinases are released into a "protected space" between the epithelium and the transmigrating neutrophil, in which they can achieve high concentrations and from which larger molecules such as endogenous antiproteinases are excluded (40, 164). The extent to which experimental conditions compensated for this phenomenon may explain variable results.

In addition, increased epithelial permeability mediated by the opening of interepithelial junctions, diseases in which the pathogenesis involves transepithelial neutrophil migration, such as ALI/ARDS and inflammatory bowel disease (165), are often characterized by loss of epithelial cells resulting in not only permeability (166) but also denuded epithelium that can appear microscopically as ulcerations. In both ALI (167–173) and inflammatory bowel disease (174, 175), the loss of epithelial cells can be due to apoptosis, which is in part triggered by neutrophil transmigration (176). More specifically, recent studies from our laboratory have demonstrated that elastase secreted by transmigrating neutrophil is responsible not only for degradative effects on junctional proteins, leading to epithelial permeability (96), but also induces epithelial cell apoptosis. Importantly, elastase induces epithelial cell apoptosis via nondegradative mechanisms, by cleaving proteinase activated receptors (PARs), leading to the activation of specific intracellular signaling pathways that culminate in apoptosis (177, 178). In addition, PAR activation has also been shown to result in apoptosis and increased permeability in the intestinal epithelium (179). Further support for the role of elastase in epithelial cell apoptosis derives from *in vivo* models in which intratracheal elastase administration induces lung epithelial cell apoptosis (180). Interestingly, elastase has similar pro-apoptotic effects on the endothelium (181). In addition, elastase may also have direct cytotoxic effects on the epithelium (182, 183), although this is controversial (24, 160), and overall the epithelium seems to be less vulnerable than the endothelium to the cytotoxic effects of elastase (184). Finally, unopposed elastase activity may prevent epithelial healing (185).

Whether or not these effects of elastase facilitate or are required for neutrophil transmigration across the basement membrane (138, 186, 187), endothelium (155, 188–190), or epithelium (24, 96, 115) is controversial—although elastase is necessary for neutrophil migration into the lung in some circumstances (142, 151, 158, 191) but not in others (192, 193). Finally, in addition to its effects on epithelial permeability, elastase may also facilitate neutrophil transmigration by modulating de-adhesion from the epithelial surface (194, 195). Ongoing studies in our laboratory aim to elucidate the effects of elastase on epithelial intercellular junctions and apoptosis, the intracellular signaling mechanisms by which these effects occur, and the extent to which these effects facilitate neutrophil transmigration.

Matrix Metalloproteinases

In addition to serine proteases, neutrophils produce matrix metalloproteinases (MMPs), which are also implicated in tissue injury in the acute inflammatory process (196, 197). The BAL fluid (198–200) and plasma (201, 202) of patients with ALI/ARDS have elevated concentrations of MMPs, which correlate with clinical severity (203), and MMP inhibition is protective in experimental models of lung injury (191, 196, 204, 205), although there is some conflicting data in the literature (206, 207). Importantly, the implicated MMPs are derived from neutrophils (197) as well as alveolar macrophages (208). Similarly, several MMPs, including neutrophil-derived MMPs, are up-regulated in both patients with inflammatory bowel disease (209, 210) and animal models of inflammatory bowel disease (211), and MMP expression correlates with disease severity (209, 210). Moreover, MMP deficiency or inhibition attenuates tissue injury in animal models of inflammatory bowel disease (211–214), although it is unclear whether MMPs derived from neutrophils (197, 209) or other cell types (211) are the most critical for disease pathogenesis.

While MMPs clearly degrade the extracellular matrix (ECM), with nearly every ECM component a potential substrate of MMPs (215), the effects of MMPs on the endothelia and epithelia are less clear. Certain MMPs do appear to play a role in maintaining epithelial integrity (211), in part via proteolytic cleavage of E-cadherin and occludin, leading to tight and adherens junction disassembly (215–217). By analogy, endothelial permeability is regulated by MMP degradation of occludin (218) and E-cadherin (219). The identity of the specific MMPs that target specific junctional proteins, resulting in increased permeability of various types of epithelium, remains to be determined.

In addition to mediating tissue injury, MMPs may also pave the way for neutrophil influx, as with elastase. MMPs have the potential to influence neutrophil migration to the inflammatory site through multiple mechanisms, including assembly of the actin cytokeleton, modulation of cell surface adhesion molecules, and proteolysis of the ECM (220). In some experimental models of ALI (204, 221, 222) but not in others (223, 224), MMP inhibition decreases neutrophil influx. This suggests that tissue injury induced by MMPs may indeed facilitate neutrophil transepithelial migration, as is the case with the transepithelial migration of other leukocytes (225) but not with transendothelial neutrophil migration (189). The diverse effects of the various MMPs on tissue injury and neutrophil migration have only begun to be elucidated (187, 211, 223, 226).

Defensins

In addition to proteases, cationic peptides called defensins are a major component of azurophilic granules. Defensins are released principally into the phagolysosome but also into the extracellular space upon neutrophil stimulation (227). Defensins are antimicrobial against both gram-positive and gram-negative bacteria as well as fungi and enveloped viruses via permeabilization of their cell membranes (227). As with other antimicrobial mediators, defensins induce permeability in cultured epithelia via both cytotoxic (228–231) and noncytotoxic (232) mechanisms, and have been shown to cause endothelial injury as well (233). Moreover, when administered intratracheally to mice, defensins induce lung injury, as assessed by lung permeability, oxygen saturation, and mitochondrial damage, in a dose-dependent manner (234). Defensin concentrations have been found to be greatly elevated in the BAL fluid of patients with inflammatory lung diseases, including ARDS (235) and cystic fibrosis (230), and plasma defensin concentrations correlate with severity of lung injury (236). Taken together, this suggests that defensins are likely to play a pathogenic role in diseases characterized by neutrophil-mediated epithelial injury.

Oxidants

In addition to proteinases and defensins, neutrophil-derived oxidants are thought to play a major role in epithelial injury in neutrophil-mediated diseases, including ALI/ARDS and inflammatory bowel disease. Tissue injury due to oxidants is thought to be a key factor in the pathogenesis of ALI/ARDS (237), as levels of plasma (238) and lung (239–241) oxidants, likely of neutrophil origin (242), are increased in patients with ALI/ARDS and correlate with mortality (238). There are also data from clinical trials to suggest that antioxidant therapy may attenuate lung injury (243), although this data is inconclusive (244). Moreover, reactive oxygen and nitrogen species have been shown to induce lung injury, as assessed by permeability and histologic examination, in animal models of ALI (245–250), and the injurious oxidants have been shown to be neutrophil derived (251, 252). In one study, mice whose neutrophils are deficient in NADPH oxidase, the key enzyme necessary to generate a respiratory burst, were protected from increased lung permeability in a sepsis model (253). In inflammatory bowel disease, oxidant levels are elevated in inflamed intestinal mucosa (254, 255), and oxidant-induced injury likely contributes to mucosal injury and clinical exacerbations (256).

In addition to their indirect proinflammatory effects, including the activation of redox-sensitive transcription factors that upregulate expression of various cytokines and chemokines (244, 257), oxidants have a direct effect on epithelial injury. Oxidants have been shown to induce epithelial cell death, either apoptotic (258–265) or necrotic (258–260), depending on the dose and duration of exposure (266), in both animal models of lung injury and in *in vitro* studies. Oxidants also increase epithelial permeability via disruption of tight junctions and redistribution of junctional proteins (267–270). The effects of oxidants on cell death, tight junction integrity, and permeability have been shown in various types of epithelial cells (115, 262, 267, 271, 272). Still, although there is extensive evidence that oxidants induce epithelial permeability, experiments with neutrophils from patients with chronic granulomatous disease suggest that oxidants are not the only factor inducing epithelial permeability during neutrophil transmigration (24) (*see* other mechanisms above), and neutrophil transepithelial migration may proceed independently of oxidant-induced junctional disruption (115). By comparison, although oxidants induce endothelial permeability via cell death (273–275) and disruption of tight junctions (276), neutrophil transmigration can induce endothelial permeability via mechanisms independent of oxidants (184, 277).

Lipid Mediators

Much recent attention has been paid to understanding the innate mechanisms involved in the resolution of inflammation at mucosal sites. Of particular interest are series of lipid mediators termed the lipoxins and resolvins (278). Lipoxins are bioactive eicosanoids derived from membrane arachidonic acid by the combined action of 5-lipoxygenase (LO) and 12-LO or 15-LO (i.e., transcellualr biosynthesis). A number of *in vitro* and *in vivo* studies have revealed that lipoxins, and specifically lipoxin A4 (LXA4), serve as an innate "stop signal," functioning to control local inflammatory processes (278). LXA4 has been demonstrated to inhibit neutrophil transmigration across both endothelia and epithelia both *in vitro* and *in vivo*. Synthetic lipoxin analogs exhibit greater potency for these actions than the native compound, likely due to a longer half-life consequent to decreased metabolism to inactive compounds.

An additional aspect of lipid metabolism was recently defined: that of inflammatory resolution via a switch from proinflammatory (e.g., leukotrienes and prostaglandins) to antiinflammatory (e.g., lipoxins) lipid mediators. Such a switch occurred through temporal induction of 15-LO pathways via cyclic AMP responsive elements in the 15-LO gene and revealed that these functionally distinct lipid profiles drive neutrophils toward a program of inflammatory resolution (278). Extensions of these findings identified the "resolvins" as key control points for initiation of inflammatory resolution. The discovery of resolvins was based on a plethora of previous reports indicating that omega-3 polyunsaturated fatty acids (omega-3 PUFA) are beneficial to a number of cardiovascular and immunoregulatory responses. Ensuing studies revealed the existence of novel series of lipid mediators, derived from either eicosapentanoic acid (C20:5, 18-series resolvins) or docosahexaenoic acid (C22:6, 17-series resolvins), which potently initiate the resolution phase of acute inflammation. One such resolvin, termed RvE1, was recently shown to potently attenuate allergic airway inflammation in a murine model (279).

CONCLUSIONS

In summary, the pathogenesis of inflammatory diseases affecting mucosal surfaces in the lung and GI tract involves the disruption of endothelial and epithelial barriers. There is considerable evidence that a balance must exist between pro- and anti-inflammatory mechanisms during active states of disease. A tipping of this balance in either direction results in either establishment of chronic inflammation or the active resolution of acute inflammation. Herein, we have delineated the mechanisms by which neutrophils migrate from the intravascular space across the epithelium, including the roles of adhesion molecules such as β_2 integrins, intercellular adhesion molecules, junctional adhesion molecules, carbohydrate ligands, and others. We have included evidence from models of both alveolar and bronchial epithelium as well as intestinal and other types of epithelium, and address the apparent similarities and differences between the interactions of neutrophils with the different types of epithelial cells as well as endothelial cells. We have discussed the mechanisms by which neutrophils injure the epithelium, including regulated disassembly of tight junctions, mechanical force, and degradative effects of soluble mediators including elastase, MMPs, defensins, and oxidants. This injury leads to epithelial cell apoptosis and sloughing, resulting in enhanced permeability, which in the lung allows for extravasa-

tion of edema into the alveolar spaces, manifesting clinically as bilateral infiltrates, compromised gas exchange, and diminished lung compliance. We hope that an improved understanding of the mechanisms by which neutrophil migrate across and injure epithelia will ultimately lead to the development of therapeutic endeavors with the goal to prevent or mitigate lung injury and hasten repair of the damaged lung.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

1. Nathan C. Points of control in inflammation. *Nature* 2002;420:846–852.
2. Malech HL, Gallin JI. Current concepts: immunology. Neutrophils in human diseases. *N Engl J Med* 1987;317:687–694.
3. Nauseef WM. How human neutrophils kill and degrade microbes: an integrated view. *Immunol Rev* 2007;219:88–102.
4. Babior BM, Lambeth JD, Nauseef W. The neutrophil NADPH oxidase. *Arch Biochem Biophys* 2002;397:342–344.
5. Martin TR. Neutrophils and lung injury: getting it right. *J Clin Invest* 2002;110:1603–1605.
6. Martin TR, Pistorese BP, Chi EY, Goodman RB, Matthay MA. Effects of leukotriene B4 in the human lung: recruitment of neutrophils into the alveolar spaces without a change in protein permeability. *J Clin Invest* 1989;84:1609–1619.
7. Wiener-Kronish JP, Albertine KH, Matthay MA. Differential responses of the endothelial and epithelial barriers of the lung in sheep to *Escherichia coli* endotoxin. *J Clin Invest* 1991;88:864–875.
8. Worthen GS, Haslett C, Rees AJ, Gumbay RS, Henson JE, Henson PM. Neutrophil-mediated pulmonary vascular injury: synergistic effect of trace amounts of lipopolysaccharide and neutrophil stimuli on vascular permeability and neutrophil sequestration in the lung. *Am Rev Respir Dis* 1987;136:19–28.
9. Shasby DM, Vanbenthuysen KM, Tate RM, Shasby SS, McMurtry I, Repine JE. Granulocytes mediate acute edematous lung injury in rabbits and in isolated rabbit lungs perfused with phorbol myristate acetate: role of oxygen radicals. *Am Rev Respir Dis* 1982;125:443–447.
10. Abraham E, Carmody A, Shenkar R, Arcaroli J. Neutrophils as early immunologic effectors in hemorrhage- or endotoxemia-induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L1137–L1145.
11. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol* 2007;7:678–689.
12. Zarbock A, Ley K. Mechanisms and consequences of neutrophil interaction with the endothelium. *Am J Pathol* 2008;172:1–7.
13. Yago T, Zarnitsyna VI, Klopocki AG, McEver RP, Zhu C. Transport governs flow-enhanced cell tethering through L-selectin at threshold shear. *Biophys J* 2007;92:330–342.
14. Zen K, Parkos CA. Leukocyte-epithelial interactions. *Curr Opin Cell Biol* 2003;15:557–564.
15. Parkos CA. Cell adhesion and migration: I. Neutrophil adhesive interactions with intestinal epithelium. *Am J Physiol* 1997;273:G763–G768.
16. Walker DC, Behzad AR, Chu F. Neutrophil migration through preexisting holes in the basal laminae of alveolar capillaries and epithelium during streptococcal pneumonia. *Microvasc Res* 1995;50:397–416.
17. Lin Y, Xia L, Turner JD, Zhao X. Morphologic observation of neutrophil diapedesis across bovine mammary gland epithelium in vitro. *Am J Vet Res* 1995;56:203–207.
18. Smart SJ, Casale TB. Pulmonary epithelial cells facilitate TNF-alpha-induced neutrophil chemotaxis: a role for cytokine networking. *J Immunol* 1994;152:4087–4094.
19. Parkos CA, Delp C, Arnaout MA, Madara JL. Neutrophil migration across a cultured intestinal epithelium: dependence on a CD11b/CD18-mediated event and enhanced efficiency in physiological direction. *J Clin Invest* 1991;88:1605–1612.
20. Liu L, Mul FP, Lutter R, Roos D, Knol EF. Transmigration of human neutrophils across airway epithelial cell monolayers is preferentially in the physiologic basolateral-to-apical direction. *Am J Respir Cell Mol Biol* 1996;15:771–780.
21. Kidney JC, Proud D. Neutrophil transmigration across human airway epithelial monolayers: mechanisms and dependence on electrical resistance. *Am J Respir Cell Mol Biol* 2000;23:389–395.
22. Hu M, Du Q, Vancurova I, Lin X, Miller EJ, Simms HH, Wang P. Proapoptotic effect of curcumin on human neutrophils: activation of the p38 mitogen-activated protein kinase pathway. *Crit Care Med* 2005;33:2571–2578.
23. Mul FP, Zuurbier AE, Janssen H, Calafat J, van Wetering S, Hiemstra PS, Roos D, Hordijk PL. Sequential migration of neutrophils across monolayers of endothelial and epithelial cells. *J Leukoc Biol* 2000;68:529–537.
24. Nash S, Stafford J, Madara JL. The selective and superoxide-independent disruption of intestinal epithelial tight junctions during leukocyte transmigration. *Lab Invest* 1988;59:531–537.
25. Parkos CA, Colgan SP, Diamond MS, Nusrat A, Liang TW, Springer TA, Madara JL. Expression and polarization of intercellular adhesion molecule-1 on human intestinal epithelia: consequences for CD11b/CD18-mediated interactions with neutrophils. *Mol Med* 1996;2:489–505.
26. Luscinskas FW, Brock AF, Arnaout MA, Gimbrone Jr MA. Endothelial-leukocyte adhesion molecule-1-dependent and leukocyte (CD11/CD18)-dependent mechanisms contribute to polymorphonuclear leukocyte adhesion to cytokine-activated human vascular endothelium. *J Immunol* 1989;142:2257–2263.
27. Lo SK, Van Seventer GA, Levin SM, Wright SD. Two leukocyte receptors (CD11a/CD18 and CD11b/CD18) mediate transient adhesion to endothelium by binding to different ligands. *J Immunol* 1989;143:3325–3329.
28. Agace WW, Patarroyo M, Svensson M, Carlemalm E, Svanborg C. *Escherichia coli* induces transuroepithelial neutrophil migration by an intercellular adhesion molecule-1-dependent mechanism. *Infect Immun* 1995;63:4054–4062.
29. McDonald RJ, St George JA, Pan LC, Hyde DM. Neutrophil adherence to airway epithelium is reduced by antibodies to the leukocyte CD11/CD18 complex. *Inflammation* 1993;17:145–151.
30. Celi A, Cianchetti S, Petruzzelli S, Carnevali S, Baliva F, Giuntini C. ICAM-1-independent adhesion of neutrophils to phorbol ester-stimulated human airway epithelial cells. *Am J Physiol* 1999;277:L465–L471.
31. Jagels MA, Daffern PJ, Zuraw BL, Hugli TE. Mechanisms and regulation of polymorphonuclear leukocyte and eosinophil adherence to human airway epithelial cells. *Am J Respir Cell Mol Biol* 1999;21:418–427.
32. Tosi MF, Hamedani A, Brosovich J, Alpert SE. ICAM-1-independent, CD18-dependent adhesion between neutrophils and human airway epithelial cells exposed in vitro to ozone. *J Immunol* 1994;152:1935–1942.
33. Tosi MF, Stark JM, Smith CW, Hamedani A, Gruenert DC, Infeld MD. Induction of ICAM-1 expression on human airway epithelial cells by inflammatory cytokines: effects on neutrophil-epithelial cell adhesion. *Am J Respir Cell Mol Biol* 1992;7:214–221.
34. Miyata R, Iwabuchi K, Watanabe S, Sato N, Nagaoka I. Short exposure of intestinal epithelial cells to TNF-alpha and histamine induces Mac-1-mediated neutrophil adhesion independent of protein synthesis. *J Leukoc Biol* 1999;66:437–446.
35. Colgan SP, Parkos CA, Delp C, Arnaout MA, Madara JL. Neutrophil migration across cultured intestinal epithelial monolayers is modulated by epithelial exposure to IFN-gamma in a highly polarized fashion. *J Cell Biol* 1993;120:785–798.
36. Hawkins HK, Heffelfinger SC, Anderson DC. Leukocyte adhesion deficiency: clinical and postmortem observations. *Pediatr Pathol* 1992;12:119–130.
37. Blake KM, Carrigan SO, Issekutz AC, Stadnyk AW. Neutrophils migrate across intestinal epithelium using beta2 integrin (CD11b/CD18)-independent mechanisms. *Clin Exp Immunol* 2004;136:262–268.
38. Carrigan SO, Weppler AL, Issekutz AC, Stadnyk AW. Neutrophil differentiated HL-60 cells model Mac-1 (CD11b/CD18)-independent neutrophil transepithelial migration. *Immunology* 2005;115:108–117.
39. Ridger VC, Wagner BE, Wallace WA, Hellewell PG. Differential effects of CD18, CD29, and CD49 integrin subunit inhibition on neutrophil migration in pulmonary inflammation. *J Immunol* 2001;166:3484–3490.
40. Winn RK, Mileski WJ, Kovach NL, Doerschuk CM, Rice CL, Harlan JM. Role of protein synthesis and CD11/CD18 adhesion complex in neutrophil emigration into the lung. *Exp Lung Res* 1993;19:221–235.
41. Doerschuk CM, Winn RK, Coxson HO, Harlan JM. CD18-dependent and -independent mechanisms of neutrophil emigration in the pulmonary and systemic microcirculation of rabbits. *J Immunol* 1990;144:2327–2333.
42. Hellewell PG, Young SK, Henson PM, Worthen GS. Disparate role of the beta 2-integrin CD18 in the local accumulation of neutrophils in

pulmonary and cutaneous inflammation in the rabbit. *Am J Respir Cell Mol Biol* 1994;10:391–398.
43. Mizgerd JP, Kubo H, Kutkoski GJ, Bhagwan SD, Scharffetter-Kochanek K, Beaudet AL, Doerschuk CM. Neutrophil emigration in the skin, lungs, and peritoneum: different requirements for CD11/CD18 revealed by CD18-deficient mice. *J Exp Med* 1997;186:1357–1364.
44. Downey GP, Worthen GS, Henson PM, Hyde DM. Neutrophil sequestration and migration in localized pulmonary inflammation. Capillary localization and migration across the interalveolar septum. *Am Rev Respir Dis* 1993;147:168–176.
45. Khan AI, Kerfoot SM, Heit B, Liu L, Andonegui G, Ruffell B, Johnson P, Kubes P. Role of CD44 and hyaluronan in neutrophil recruitment. *J Immunol* 2004;173:7594–7601.
46. Si-Tahar M, Sitaraman S, Shibahara T, Madara JL. Negative regulation of epithelium-neutrophil interactions via activation of CD44. *Am J Physiol Cell Physiol* 2001;280:C423–C432.
47. Wang Q, Teder P, Judd NP, Noble PW, Doerschuk CM. CD44 deficiency leads to enhanced neutrophil migration and lung injury in *Escherichia coli* pneumonia in mice. *Am J Pathol* 2002;161:2219–2228.
48. Colgan SP, Parkos CA, McGuirk D, Brady HR, Papayianni AA, Frendl G, Madara JL. Receptors involved in carbohydrate binding modulate intestinal epithelial-neutrophil interactions. *J Biol Chem* 1995;270:10531–10539.
49. Jaye DL, Parkos CA. Neutrophil migration across intestinal epithelium. *Ann N Y Acad Sci* 2000;915:151–161.
50. Parkos CA, Colgan SP, Madara JL. Interactions of neutrophils with epithelial cells: lessons from the intestine. *J Am Soc Nephrol* 1994;5:138–152.
51. Parkos CA, Colgan SP, Bacarra AE, Nusrat A, Delp-Archer C, Carlson S, Su DH, Madara JL. Intestinal epithelia (T84) possess basolateral ligands for CD11b/CD18-mediated neutrophil adherence. *Am J Physiol* 1995;268:C472–C479.
52. Diamond MS, Staunton DE, de Fougerolles AR, Stacker SA, Garcia-Aguilar J, Hibbs ML, Springer TA. ICAM-1 (CD54): a counter-receptor for Mac-1 (CD11b/CD18). *J Cell Biol* 1990;111:3129–3139.
53. Sakamoto S, Okanoue T, Itoh Y, Sakamoto K, Nishioji K, Nakagawa Y, Yoshida N, Yoshikawa T, Kashima K. Intercellular adhesion molecule-1 and CD18 are involved in neutrophil adhesion and its cytotoxicity to cultured sinusoidal endothelial cells in rats. *Hepatology* 1997;26:658–663.
54. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J Clin Invest* 1989;83:2008–2017.
55. Taguchi M, Sampath D, Koga T, Castro M, Look DC, Nakajima S, Holtzman MJ. Patterns for RANTES secretion and intercellular adhesion molecule 1 expression mediate transepithelial T cell traffic based on analyses in vitro and in vivo. *J Exp Med* 1998;187:1927–1940.
56. Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990;247:456–459.
57. Paolieri F, Battifora M, Riccio AM, Pesce G, Canonica GW, Bagnasco M. Intercellular adhesion molecule-1 on cultured human epithelial cell lines: influence of proinflammatory cytokines. *Allergy* 1997;52:521–531.
58. Huang GT, Eckmann L, Savidge TC, Kagnoff MF. Infection of human intestinal epithelial cells with invasive bacteria upregulates apical intercellular adhesion molecule-1 (ICAM)-1) expression and neutrophil adhesion. *J Clin Invest* 1996;98:572–583.
59. Wuthrich RP. Tumor necrosis factor-alpha- and interleukin-1-stimulated intercellular adhesion molecule-1 expression by murine renal tubular epithelial cells is transcriptionally regulated and involves protein kinase C. *Ren Physiol Biochem* 1992;15:302–306.
60. Ishikura H, Takahashi C, Kanagawa K, Hirata H, Imai K, Yoshiki T. Cytokine regulation of ICAM-1 expression on human renal tubular epithelial cells in vitro. *Transplantation* 1991;51:1272–1275.
61. Lhotta K, Neumayer HP, Joannidis M, Geissler D, Konig P. Renal expression of intercellular adhesion molecule-1 in different forms of glomerulonephritis. *Clin Sci (Lond)* 1991;81:477–481.
62. Tosi MF, Stark JM, Hamedani A, Smith CW, Gruenert DC, Huang YT. Intercellular adhesion molecule-1 (ICAM-1)-dependent and ICAM-1-independent adhesive interactions between polymorphonuclear leukocytes and human airway epithelial cells infected with parainfluenza virus type 2. *J Immunol* 1992;149:3345–3349.
63. Look DC, Rapp SR, Keller BT, Holtzman MJ. Selective induction of intercellular adhesion molecule-1 by interferon-gamma in human airway epithelial cells. *Am J Physiol* 1992;263:L79–L87.
64. Subauste MC, Choi DC, Proud D. Transient exposure of human bronchial epithelial cells to cytokines leads to persistent increased expression of ICAM-1. *Inflammation* 2001;25:373–380.
65. Atsuta J, Sterbinsky SA, Plitt J, Schwiebert LM, Bochner BS, Schleimer RP. Phenotyping and cytokine regulation of the BEAS-2B human bronchial epithelial cell: demonstration of inducible expression of the adhesion molecules VCAM-1 and ICAM-1. *Am J Respir Cell Mol Biol* 1997;17:571–582.
66. Kang BH, Crapo JD, Wegner CD, Letts LG, Chang LY. Intercellular adhesion molecule-1 expression on the alveolar epithelium and its modification by hyperoxia. *Am J Respir Cell Mol Biol* 1993;9:350–355.
67. Burns AR, Takei F, Doerschuk CM. Quantitation of ICAM-1 expression in mouse lung during pneumonia. *J Immunol* 1994;153:3189–3198.
68. Bloemen PG, Van den Tweel MC, Henricks PA, Engels F, Van de Velde MJ, Blomjous FJ, Nijkamp FP. Stimulation of both human bronchial epithelium and neutrophils is needed for maximal interactive adhesion. *Am J Physiol* 1996;270:L80–L87.
69. Wegner CD, Wolyniec WW, LaPlante AM, Marschman K, Lubbe K, Haynes N, Rothlein R, Letts LG. Intercellular adhesion molecule-1 contributes to pulmonary oxygen toxicity in mice: role of leukocytes revised. *Lung* 1992;170:267–279.
70. Kumasaka T, Quinlan WM, Doyle NA, Condon TP, Sligh J, Takei F, Beaudet A, Bennett CF, Doerschuk CM. Role of the intercellular adhesion molecule-1(ICAM-1) in endotoxin-induced pneumonia evaluated using ICAM-1 antisense oligonucleotides, anti-ICAM-1 monoclonal antibodies, and ICAM-1 mutant mice. *J Clin Invest* 1996;97:2362–2369.
71. Xu H, Gonzalo JA, St Pierre Y, Williams IR, Kupper TS, Cotran RS, Springer TA, Gutierrez-Ramos JC. Leukocytosis and resistance to septic shock in intercellular adhesion molecule 1-deficient mice. *J Exp Med* 1994;180:95–109.
72. Lo SK, Everitt J, Gu J, Malik AB. Tumor necrosis factor mediates experimental pulmonary edema by ICAM-1 and CD18-dependent mechanisms. *J Clin Invest* 1992;89:981–988.
73. Jahn HU, Krull M, Wuppermann FN, Klucken AC, Rosseau S, Seybold J, Hegemann JH, Jantos CA, Suttorp N. Infection and activation of airway epithelial cells by *Chlamydia pneumoniae*. *J Infect Dis* 2000;182:1678–1687.
74. Tonetti MS, Imboden MA, Lang NP. Neutrophil migration into the gingival sulcus is associated with transepithelial gradients of interleukin-8 and ICAM-1. *J Periodontol* 1998;69:1139–1147.
75. Tabary O, Corvol H, Boncoeur E, Chadelat K, Fitting C, Cavaillon JM, Clement A, Jacquot J. Adherence of airway neutrophils and inflammatory response are increased in CF airway epithelial cell-neutrophil interactions. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L588–L596.
76. Ostermann G, Weber KS, Zernecke A, Schroder A, Weber C. JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes. *Nat Immunol* 2002;3:151–158.
77. Santoso S, Sachs UJ, Kroll H, Linder M, Ruf A, Preissner KT, Chavakis T. The junctional adhesion molecule 3 (JAM-3) on human platelets is a counterreceptor for the leukocyte integrin Mac-1. *J Exp Med* 2002;196:679–691.
78. Chavakis T, Keiper T, Matz-Westphal R, Hersemeyer K, Sachs UJ, Nawroth PP, Preissner KT, Santoso S. The junctional adhesion molecule-C promotes neutrophil transendothelial migration in vitro and in vivo. *J Biol Chem* 2004;279:55602–55608.
79. Del Maschio A, De Luigi A, Martin-Padura I, Brockhaus M, Bartfai T, Fruscella P, Adorini L, Martino G, Furlan R, De Simoni MG, *et al.* Leukocyte recruitment in the cerebrospinal fluid of mice with experimental meningitis is inhibited by an antibody to junctional adhesion molecule (JAM). *J Exp Med* 1999;190:1351–1356.
80. Shaw SK, Perkins BN, Lim YC, Liu Y, Nusrat A, Schnell FJ, Parkos CA, Luscinskas FW. Reduced expression of junctional adhesion molecule and platelet/endothelial cell adhesion molecule-1 (CD31) at human vascular endothelial junctions by cytokines tumor necrosis factor-alpha plus interferon-gamma Does not reduce leukocyte transmigration under flow. *Am J Pathol* 2001;159:2281–2291.
81. Sircar M, Bradfield PF, Aurrand-Lions M, Fish RJ, Alcaide P, Yang L, Newton G, Lamont D, Sehrawat S, Mayadas T, *et al.* Neutrophil transmigration under shear flow conditions in vitro is junctional adhesion molecule-C independent. *J Immunol* 2007;178:5879–5887.

82. Martin-Padura I, Lostaglio S, Schneemann M, Williams L, Romano M, Fruscella P, Panzeri C, Stoppacciaro A, Ruco L, Villa A, *et al.* Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *J Cell Biol* 1998;142:117–127.
83. Zen K, Babbin BA, Liu Y, Whelan JB, Nusrat A, Parkos CA. JAM-C is a component of desmosomes and a ligand for CD11b/CD18-mediated neutrophil transepithelial migration. *Mol Biol Cell* 2004;15:3926–3937.
84. Liu Y, Nusrat A, Schnell FJ, Reaves TA, Walsh S, Pochet M, Parkos CA. Human junction adhesion molecule regulates tight junction resealing in epithelia. *J Cell Sci* 2000;113:2363–2374.
85. Burns AR, Smith CW, Walker DC. Unique structural features that influence neutrophil emigration into the lung. *Physiol Rev* 2003;83: 309–336.
86. Corada M, Chimenti S, Cera MR, Vinci M, Salio M, Fiordaliso F, De Angelis N, Villa A, Bossi M, Staszewsky LI, *et al.* Junctional adhesion molecule-A-deficient polymorphonuclear cells show reduced diapedesis in peritonitis and heart ischemia-reperfusion injury. *Proc Natl Acad Sci USA* 2005;102:10634–10639.
87. Reaves TA, Chin AC, Parkos CA. Neutrophil transepithelial migration: role of toll-like receptors in mucosal inflammation. *Mem Inst Oswaldo Cruz* 2005;100:191–198.
88. Aurrand-Lions M, Lamagna C, Dangerfield JP, Wang S, Herrera P, Nourshargh S, Imhof BA. Junctional adhesion molecule-C regulates the early influx of leukocytes into tissues during inflammation. *J Immunol* 2005;174:6406–6415.
89. Diamond MS, Alon R, Parkos CA, Quinn MT, Springer TA. Heparin is an adhesive ligand for the leukocyte integrin Mac-1 (CD11b/CD1). *J Cell Biol* 1995;130:1473–1482.
90. Zen K, Liu Y, Cairo D, Parkos CA. CD11b/CD18-dependent interactions of neutrophils with intestinal epithelium are mediated by fucosylated proteoglycans. *J Immunol* 2002;169:5270–5278.
91. Dorscheid DR, Conforti AE, Hamann KJ, Rabe KF, White SR. Characterization of cell surface lectin-binding patterns of human airway epithelium. *Histochem J* 1999;31:145–151.
92. Evans CW, Taylor JE, Walker JD, Simmons NL. Transepithelial chemotaxis of rat peritoneal exudate cells. *Br J Exp Pathol* 1983;64: 644–654.
93. Milks LC, Brontoli MJ, Cramer EB. Epithelial permeability and the transepithelial migration of human neutrophils. *J Cell Biol* 1983;96: 1241–1247.
94. Nash S, Stafford J, Madara JL. Effects of polymorphonuclear leukocyte transmigration on the barrier function of cultured intestinal epithelial monolayers. *J Clin Invest* 1987;80:1104–1113.
95. Feng D, Nagy JA, Pyne K, Dvorak HF, Dvorak AM. Neutrophils emigrate from venules by a transendothelial cell pathway in response to FMLP. *J Exp Med* 1998;187:903–915.
96. Ginzberg HH, Cherapanov V, Dong Q, Cantin A, McCulloch CA, Shannon PT, Downey GP. Neutrophil-mediated epithelial injury during transmigration: role of elastase. *Am J Physiol Gastrointest Liver Physiol* 2001;281:G705–G717.
97. Damiano VV, Cohen A, Tsang AL, Batra G, Petersen R. A morphologic study of the influx of neutrophils into dog lung alveoli after lavage with sterile saline. *Am J Pathol* 1980;100:349–364.
98. Behzad AR, Chu F, Walker DC. Fibroblasts are in a position to provide directional information to migrating neutrophils during pneumonia in rabbit lungs. *Microvasc Res* 1996;51:303–316.
99. Cooper D, Lindberg FP, Gamble JR, Brown EJ, Vadas MA. Transendothelial migration of neutrophils involves integrin-associated protein (CD47). *Proc Natl Acad Sci USA* 1995;92:3978–3982.
100. Parkos CA, Colgan SP, Liang TW, Nusrat A, Bacarra AE, Carnes DK, Madara JL. CD47 mediates post-adhesive events required for neutrophil migration across polarized intestinal epithelia. *J Cell Biol* 1996;132:437–450.
101. Liu Y, Merlin D, Burst SL, Pochet M, Madara JL, Parkos CA. The role of CD47 in neutrophil transmigration. Increased rate of migration correlates with increased cell surface expression of CD47. *J Biol Chem* 2001;276:40156–40166.
102. Chin AC, Parkos CA. Neutrophil transepithelial migration and epithelial barrier function in IBD: potential targets for inhibiting neutrophil trafficking. *Ann N Y Acad Sci* 2006;1072:276–287.
103. Lindberg FP, Bullard DC, Caver TE, Gresham HD, Beaudet AL, Brown EJ. Decreased resistance to bacterial infection and granulocyte defects in IAP-deficient mice. *Science* 1996;274:795–798.
104. Rosseau S, Selhorst J, Wiechmann K, Leissner K, Maus U, Mayer K, Grimminger F, Seeger W, Lohmeyer J. Monocyte migration through the alveolar epithelial barrier: adhesion molecule mechanisms and impact of chemokines. *J Immunol* 2000;164:427–435.
105. Liu Y, Buhring HJ, Zen K, Burst SL, Schnell FJ, Williams IR, Parkos CA. Signal regulatory protein (SIRPalpha), a cellular ligand for CD47, regulates neutrophil transmigration. *J Biol Chem* 2002;277: 10028–10036.
106. Liu Y, O'Connor MB, Mandell KJ, Zen K, Ullrich A, Buhring HJ, Parkos CA. Peptide-mediated inhibition of neutrophil transmigration by blocking CD47 interactions with signal regulatory protein alpha. *J Immunol* 2004;172:2578–2585.
107. Liu Y, Soto I, Tong Q, Chin A, Buhring HJ, Wu T, Zen K, Parkos CA. SIRPbeta1 is expressed as a disulfide-linked homodimer in leukocytes and positively regulates neutrophil transepithelial migration. *J Biol Chem* 2005;280:36132–36140.
108. Moog-Lutz C, Cave-Riant F, Guibal FC, Breau MA, Di Gioia Y, Couraud PO, Cayre YE, Bourdoulous S, Lutz PG. JAML, a novel protein with characteristics of a junctional adhesion molecule, is induced during differentiation of myeloid leukemia cells. *Blood* 2003; 102:3371–3378.
109. Zen K, Liu Y, McCall IC, Wu T, Lee W, Babbin BA, Nusrat A, Parkos CA. Neutrophil migration across tight junctions is mediated by adhesive interactions between epithelial coxsackie and adenovirus receptor and a junctional adhesion molecule-like protein on neutrophils. *Mol Biol Cell* 2005;16:2694–2703.
110. Halstensen TS, Mollnes TE, Garred P, Fausa O, Brandtzaeg P. Epithelial deposition of immunoglobulin G1 and activated complement (C3b and terminal complement complex) in ulcerative colitis. *Gastroenterology* 1990;98:1264–1271.
111. Reaves TA, Colgan SP, Selvaraj P, Pochet MM, Walsh A, Nusrat A, Liang TW, Madara JL, Parkos CA. Neutrophil transepithelial migration: regulation at the apical epithelial surface by Fc-mediated events. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G746–G754.
112. Lawrence DW, Bruyninckx WJ, Louis NA, Lublin DM, Stahl GL, Parkos CA, Colgan SP. Antiadhesive role of apical decay-accelerating factor (CD55) in human neutrophil transmigration across mucosal epithelia. *J Exp Med* 2003;198:999–1010.
113. Qian YM, Haino M, Kelly K, Song WC. Structural characterization of mouse CD97 and study of its specific interaction with the murine decay-accelerating factor (DAF, CD55). *Immunology* 1999;98:303–311.
114. Madara JL. Loosening tight junctions: lessons from the intestine. *J Clin Invest* 1989;83:1089–1094.
115. Parsons PE, Sugahara K, Cott GR, Mason RJ, Henson PM. The effect of neutrophil migration and prolonged neutrophil contact on epithelial permeability. *Am J Pathol* 1987;129:302–312.
116. Edens HA, Parkos CA. Modulation of epithelial and endothelial paracellular permeability by leukocytes. *Adv Drug Deliv Rev* 2000; 41:315–328.
117. Colgan SP, Eltzschig HK, Eckle T, Thompson LF. Physiological roles for ecto-5′-nucleotidase (CD73). *Purinergic Signal* 2006;2:351–360.
118. Lawrence DW, Comerford KM, Colgan SP. Role of VASP in reestablishment of epithelial tight junction assembly after Ca2+ switch. *Am J Physiol Cell Physiol* 2002;282:C1235–C1245.
119. Liu Y, Shaw SK, Ma S, Yang L, Luscinskas FW, Parkos CA. Regulation of leukocyte transmigration: cell surface interactions and signaling events. *J Immunol* 2004;172:7–13.
120. Nusrat A, Parkos CA, Liang TW, Carnes DK, Madara JL. Neutrophil migration across model intestinal epithelia: monolayer disruption and subsequent events in epithelial repair. *Gastroenterology* 1997;113: 1489–1500.
121. Webster RO, Larsen GL, Mitchell BC, Goins AJ, Henson PM. Absence of inflammatory lung injury in rabbits challenged intravascularly with complement-derived chemotactic factors. *Am Rev Respir Dis* 1982;125:335–340.
122. Milks LC, Conyers GP, Cramer EB. The effect of neutrophil migration on epithelial permeability. *J Cell Biol* 1986;103:2729–2738.
123. Ma S, Shaw SK, Yang L, Jones T, Liu Y, Nusrat A, Parkos CA, Luscinskas FW. Dynamics of junctional adhesion moleule 1 (JAM1) during leukocyte transendothelial migration under flow in vitro. *FASEB J* 2003;1189:1758.
124. Edens HA, Levi BP, Jaye DL, Walsh S, Reaves TA, Turner JR, Nusrat A, Parkos CA. Neutrophil transepithelial migration: evidence for sequential, contact-dependent signaling events and enhanced paracellular permeability independent of transjunctional migration. *J Immunol* 2002;169:476–486.
125. Parkos CA, Colgan SP, Delp C, Arnaout MA, Madara JL. Neutrophil migration across a cultured epithelial monolayer elicits a biphasic

resistance response representing sequential effects on transcellular and paracellular pathways. *J Cell Biol* 1992;117:757–764.
126. Turner JR. 'Putting the squeeze' on the tight junction: understanding cytoskeletal regulation. *Semin Cell Dev Biol* 2000;11:301–308.
127. Huber D, Balda MS, Matter K. Transepithelial migration of neutrophils. *Invasion Metastasis* 1998;18:70–80.
128. Serikov VB, Choi H, Chmiel KJ, Wu R, Widdicombe JH. Activation of extracellular regulated kinases is required for the increase in airway epithelial permeability during leukocyte transmigration. *Am J Respir Cell Mol Biol* 2004;30:261–270.
129. Kale G, Naren AP, Sheth P, Rao RK. Tyrosine phosphorylation of occludin attenuates its interactions with ZO-1, ZO-2, and ZO-3. *Biochem Biophys Res Commun* 2003;302:324–329.
130. Laukoetter MG, Nava P, Lee WY, Severson EA, Capaldo CT, Babbin BA, Williams IR, Koval M, Peatman E, Campbell JA, *et al.* JAM-A regulates permeability and inflammation in the intestine in vivo. *J Exp Med* 2007;204:3067–3076.
131. Downey GP, Dong Q, Kruger J, Dedhar S, Cherapanov V. Regulation of neutrophil activation in acute lung injury. *Chest* 1999;116:46S–54S.
132. Lee CT, Fein AM, Lippmann M, Holtzman H, Kimbel P, Weinbaum G. Elastolytic activity in pulmonary lavage fluid from patients with adult respiratory-distress syndrome. *N Engl J Med* 1981;304:192–196.
133. Suter PM, Suter S, Girardin E, Roux-Lombard P, Grau GE, Dayer JM. High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, and elastase, in patients with adult respiratory distress syndrome after trauma, shock, or sepsis. *Am Rev Respir Dis* 1992;145:1016–1022.
134. Idell S, Kucich U, Fein A, Kueppers F, James HL, Walsh PN, Weinbaum G, Colman RW, Cohen AB. Neutrophil elastase-releasing factors in bronchoalveolar lavage from patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 1985;132:1098–1105.
135. Cochrane CG, Spragg RG, Revak SD, Cohen AB, McGuire WW. The presence of neutrophil elastase and evidence of oxidation activity in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 1983;127:S25–S27.
136. Donnelly SC, MacGregor I, Zamani A, Gordon MW, Robertson CE, Steedman DJ, Little K, Haslett C. Plasma elastase levels and the development of the adult respiratory distress syndrome. *Am J Respir Crit Care Med* 1995;151:1428–1433.
137. Tremblay GM, Vachon E, Larouche C, Bourbonnais Y. Inhibition of human neutrophil elastase-induced acute lung injury in hamsters by recombinant human pre-elafin (trappin-2). *Chest* 2002;121:582–588.
138. Delacourt C, Herigault S, Delclaux C, Poncin A, Levame M, Harf A, Saudubray F, Lafuma C. Protection against acute lung injury by intravenous or intratracheal pretreatment with EPI-HNE-4, a new potent neutrophil elastase inhibitor. *Am J Respir Cell Mol Biol* 2002; 26:290–297.
139. Janusz MJ, Hare M. Inhibition of human neutrophil elastase and cathepsin G by a biphenyl disulfonic acid copolymer. *Int J Immunopharmacol* 1994;16:623–632.
140. Baird BR, Cheronis JC, Sandhaus RA, Berger EM, White CW, Repine JE. O2 metabolites and neutrophil elastase synergistically cause edematous injury in isolated rat lungs. *J Appl Physiol* 1986;61:2224–2229.
141. Gossage JR, Kuratomi Y, Davidson JM, Lefferts PL, Snapper JR. Neutrophil elastase inhibitors, SC-37698 and SC-39026, reduce endotoxin-induced lung dysfunction in awake sheep. *Am Rev Respir Dis* 1993;147:1371–1379.
142. Sakamaki F, Ishizaka A, Urano T, Sayama K, Nakamura H, Terashima T, Waki Y, Tasaka S, Hasegawa N, Sato K, *et al.* Effect of a specific neutrophil elastase inhibitor, ONO-5046, on endotoxin-induced acute lung injury. *Am J Respir Crit Care Med* 1996;153:391–397.
143. Kawabata K, Hagio T, Matsumoto S, Nakao S, Orita S, Aze Y, Ohno H. Delayed neutrophil elastase inhibition prevents subsequent progression of acute lung injury induced by endotoxin inhalation in hamsters. *Am J Respir Crit Care Med* 2000;161:2013–2018.
144. Takayama M, Ishibashi M, Ishii H, Kuraki T, Nishida T, Yoshida M. Effects of neutrophil elastase inhibitor (ONO-5046) on lung injury after intestinal ischemia-reperfusion. *J Appl Physiol* 2001;91:1800–1807.
145. Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol* 2008;103:162–169.
146. Tarlton JF, Whiting CV, Tunmore D, Bregenholt S, Reimann J, Claesson MH, Bland PW. The role of up-regulated serine proteases and matrix metalloproteinases in the pathogenesis of a murine model of colitis. *Am J Pathol* 2000;157:1927–1935.
147. Gouni-Berthold I, Baumeister B, Wegel E, Berthold HK, Vetter H, Schmidt C. Neutrophil-elastase in chronic inflammatory bowel disease: a marker of disease activity? *Hepatogastroenterology* 1999;46: 2315–2320.
148. Adeyemi EO, Hodgson HJ. Faecal elastase reflects disease activity in active ulcerative colitis. *Scand J Gastroenterol* 1992;27:139–142.
149. Rees DD, Brain JD. Effects of cystic fibrosis airway secretions on rat lung: role of neutrophil elastase. *Am J Physiol* 1995;269:L195–L202.
150. Cantin AM, Woods DE, Cloutier D, Heroux J, Dufour EK, Leduc R. Leukocyte elastase inhibition therapy in cystic fibrosis: role of glycosylation on the distribution of alpha-1-proteinase inhibitor in blood versus lung. *J Aerosol Med* 2002;15:141–148.
151. Shapiro SD. Neutrophil elastase: path clearer, pathogen killer, or just pathologic? *Am J Respir Cell Mol Biol* 2002;26:266–268.
152. Kuraki T, Ishibashi M, Takayama M, Shiraishi M, Yoshida M. A novel oral neutrophil elastase inhibitor (ONO-6818) inhibits human neutrophil elastase-induced emphysema in rats. *Am J Respir Crit Care Med* 2002;166:496–500.
153. Zeiher BG, Artigas A, Vincent JL, Dmitrienko A, Jackson K, Thompson BT, Bernard G. Neutrophil elastase inhibition in acute lung injury: results of the STRIVE study. *Crit Care Med* 2004;32: 1695–1702.
154. Ryugo M, Sawa Y, Takano H, Matsumiya G, Iwai S, Ono M, Hata H, Yamauchi T, Nishimura M, Fujino Y, *et al.* Effect of a polymorphonuclear elastase inhibitor (sivelestat sodium) on acute lung injury after cardiopulmonary bypass: findings of a double-blind randomized study. *Surg Today* 2006;36:321–326.
155. Lee WL, Downey GP. Leukocyte elastase: physiological functions and role in acute lung injury. *Am J Respir Crit Care Med* 2001;164:896–904.
156. Moraes TJ, Plumb J, Martin R, Vachon E, Cherepanov V, Koh A, Loeve C, Jongstra-Bilen J, Zurawska JH, Kus JV, *et al.* Abnormalities in the pulmonary innate immune system in cystic fibrosis. *Am J Respir Cell Mol Biol* 2006;34:364–374.
157. Havemann K, Gramse M. Physiology and pathophysiology of neutral proteinases of human granulocytes. *Adv Exp Med Biol* 1984;167:1–20.
158. Carden D, Xiao F, Moak C, Willis BH, Robinson-Jackson S, Alexander S. Neutrophil elastase promotes lung microvascular injury and proteolysis of endothelial cadherins. *Am J Physiol* 1998;275:H385–H392.
159. Harlan JM, Killen PD, Harker LA, Striker GE, Wright DG. Neutrophil-mediated endothelial injury in vitro mechanisms of cell detachment. *J Clin Invest* 1981;68:1394–1403.
160. Chung Y, Kercsmar CM, Davis PB. Ferret tracheal epithelial cells grown in vitro are resistant to lethal injury by activated neutrophils. *Am J Respir Cell Mol Biol* 1991;5:125–132.
161. Dogan A, Wang ZD, Spencer J. E-cadherin expression in intestinal epithelium. *J Clin Pathol* 1995;48:143–146.
162. Hanby AM, Chinery R, Poulsom R, Playford RJ, Pignatelli M. Down-regulation of E-cadherin in the reparative epithelium of the human gastrointestinal tract. *Am J Pathol* 1996;148:723–729.
163. Peterson MW, Walter ME, Nygaard SD. Effect of neutrophil mediators on epithelial permeability. *Am J Respir Cell Mol Biol* 1995;13:719–727.
164. Campbell EJ, Senior RM, McDonald JA, Cox DL. Proteolysis by neutrophils: relative importance of cell-substrate contact and oxidative inactivation of proteinase inhibitors in vitro. *J Clin Invest* 1982;70: 845–852.
165. Lencer WI. Patching a leaky intestine. *N Engl J Med* 2008;359:526–528.
166. Bojarski C, Gitter AH, Bendfeldt K, Mankertz J, Schmitz H, Wagner S, Fromm M, Schulzke JD. Permeability of human HT-29/B6 colonic epithelium as a function of apoptosis. *J Physiol* 2001;535:541–552.
167. Bardales RH, Xie SS, Schaefer RF, Hsu SM. Apoptosis is a major pathway responsible for the resolution of type II pneumocytes in acute lung injury. *Am J Pathol* 1996;149:845–852.
168. Martin TR, Hagimoto N, Nakamura M, Matute-Bello G. Apoptosis and epithelial injury in the lungs. *Proc Am Thorac Soc* 2005;2:214–220.
169. Matute-Bello G, Liles WC, Steinberg KP, Kiener PA, Mongovin S, Chi EY, Jonas M, Martin TR. Soluble Fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS). *J Immunol* 1999; 163:2217–2225.
170. Matute-Bello G, Martin TR. Science review: apoptosis in acute lung injury. *Crit Care* 2003;7:355–358.
171. Albertine KH, Soulier MF, Wang Z, Ishizaka A, Hashimoto S, Zimmerman GA, Matthay MA, Ware LB. Fas and fas ligand are

up-regulated in pulmonary edema fluid and lung tissue of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Pathol* 2002;161:1783–1796.
172. Kawasaki M, Kuwano K, Hagimoto N, Matsuba T, Kunitake R, Tanaka T, Maeyama T, Hara N. Protection from lethal apoptosis in lipopolysaccharide-induced acute lung injury in mice by a caspase inhibitor. *Am J Pathol* 2000;157:597–603.
173. Li X, Shu R, Filippatos G, Uhal BD. Apoptosis in lung injury and remodeling. *J Appl Physiol* 2004;97:1535–1542.
174. Gitter AH, Wullstein F, Fromm M, Schulzke JD. Epithelial barrier defects in ulcerative colitis: characterization and quantification by electrophysiological imaging. *Gastroenterology* 2001;121:1320–1328.
175. Iwamoto M, Koji T, Makiyama K, Kobayashi N, Nakane PK. Apoptosis of crypt epithelial cells in ulcerative colitis. *J Pathol* 1996;180:152–159.
176. Le'Negrate G, Selva E, Auberger P, Rossi B, Hofman P. Sustained polymorphonuclear leukocyte transmigration induces apoptosis in T84 intestinal epithelial cells. *J Cell Biol* 2000;150:1479–1488.
177. Ginzberg HH, Shannon PT, Suzuki T, Hong O, Vachon E, Moraes T, Abreu MT, Cherepanov V, Wang X, Chow CW, *et al.* Leukocyte elastase induces epithelial apoptosis: role of mitochondial permeability changes and Akt. *Am J Physiol Gastrointest Liver Physiol* 2004; 287:G286–G298.
178. Suzuki T, Moraes TJ, Vachon E, Ginzberg HH, Huang TT, Matthay MA, Hollenberg MD, Marshall J, McCulloch CA, Abreu MT, *et al.* Proteinase-activated receptor-1 mediates elastase-induced apoptosis of human lung epithelial cells. *Am J Respir Cell Mol Biol* 2005;33: 231–247.
179. Chin AC, Vergnolle N, MacNaughton WK, Wallace JL, Hollenberg MD, Buret AG. Proteinase-activated receptor 1 activation induces epithelial apoptosis and increases intestinal permeability. *Proc Natl Acad Sci USA* 2003;100:11104–11109.
180. Lucey EC, Keane J, Kuang PP, Snider GL, Goldstein RH. Severity of elastase-induced emphysema is decreased in tumor necrosis factor-alpha and interleukin-1beta receptor-deficient mice. *Lab Invest* 2002; 82:79–85.
181. Yang JJ, Kettritz R, Falk RJ, Jennette JC, Gaido ML. Apoptosis of endothelial cells induced by the neutrophil serine proteases proteinase 3 and elastase. *Am J Pathol* 1996;149:1617–1626.
182. Barnett CC Jr, Moore EE, Mierau GW, Partrick DA, Biffl WL, Elzi DJ, Silliman CC. ICAM-1–CD18 interaction mediates neutrophil cytotoxicity through protease release. *Am J Physiol* 1998;274:C1634–C1644.
183. Kercsmar CM, Davis PB. Resistance of human tracheal epithelial cells to killing by neutrophils, neutrophil elastase, and *Pseudomonas* elastase. *Am J Respir Cell Mol Biol* 1993;8:56–62.
184. Smedly LA, Tonnesen MG, Sandhaus RA, Haslett C, Guthrie LA, Johnston Jr RB, Henson PM, Worthen GS. Neutrophil-mediated injury to endothelial cells: enhancement by endotoxin and essential role of neutrophil elastase. *J Clin Invest* 1986;77:1233–1243.
185. Ashcroft GS, Lei K, Jin W, Longenecker G, Kulkarni AB, Greenwell-Wild T, Hale-Donze H, McGrady G, Song XY, Wahl SM. Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. *Nat Med* 2000;6:1147–1153.
186. Huber AR, Weiss SJ. Disruption of the subendothelial basement membrane during neutrophil diapedesis in an in vitro construct of a blood vessel wall. *J Clin Invest* 1989;83:1122–1136.
187. Delclaux C, Delacourt C, D'Ortho MP, Boyer V, Lafuma C, Harf A. Role of gelatinase B and elastase in human polymorphonuclear neutrophil migration across basement membrane. *Am J Respir Cell Mol Biol* 1996;14:288–295.
188. Mackarel AJ, Russell KJ, Brady CS, FitzGerald MX, O'Connor CM. Interleukin-8 and leukotriene-B(4), but not formylmethionyl leucyl-phenylalanine, stimulate CD18-independent migration of neutrophils across human pulmonary endothelial cells *in vitro. Am J Respir Cell Mol Biol* 2000;23:154–161.
189. Mackarel AJ, Cottell DC, Russell KJ, FitzGerald MX, O'Connor CM. Migration of neutrophils across human pulmonary endothelial cells is not blocked by matrix metalloproteinase or serine protease inhibitors. *Am J Respir Cell Mol Biol* 1999;20:1209–1219.
190. Cepinskas G, Noseworthy R, Kvietys PR. Transendothelial neutrophil migration: role of neutrophil-derived proteases and relationship to transendothelial protein movement. *Circ Res* 1997;81:618–626.
191. Carney DE, Lutz CJ, Picone AL, Gatto LA, Ramamurthy NS, Golub LM, Simon SR, Searles B, Paskanik A, Snyder K, *et al.* Matrix metalloproteinase inhibitor prevents acute lung injury after cardiopulmonary bypass. *Circulation* 1999;100:400–406.
192. Hirche TO, Atkinson JJ, Bahr S, Belaaouaj A. Deficiency in neutrophil elastase does not impair neutrophil recruitment to inflamed sites. *Am J Respir Cell Mol Biol* 2004;30:576–584.
193. Yamazaki T, Ooshima H, Usui A, Watanabe T, Yasuura K. Protective effects of ONO-5046*Na, a specific neutrophil elastase inhibitor, on postperfusion lung injury. *Ann Thorac Surg* 1999;68:2141–2146.
194. Champagne B, Tremblay P, Cantin A, St Pierre Y. Proteolytic cleavage of ICAM-1 by human neutrophil elastase. *J Immunol* 1998;161:6398–6405.
195. Cai TQ, Wright SD. Human leukocyte elastase is an endogenous ligand for the integrin CR3 (CD11b/CD18, Mac-1, alpha M beta 2) and modulates polymorphonuclear leukocyte adhesion. *J Exp Med* 1996; 184:1213–1223.
196. Steinberg J, Halter J, Schiller HJ, Dasilva M, Landas S, Gatto LA, Maisi P, Sorsa T, Rajamaki M, Lee HM, *et al.* Metalloproteinase inhibition reduces lung injury and improves survival after cecal ligation and puncture in rats. *J Surg Res* 2003;111:185–195.
197. Plitas G, Gagne PJ, Muhs BE, Ianus IA, Shaw JP, Beudjekian M, Delgado Y, Jacobowitz G, Rockman C, Shamamian P. Experimental hindlimb ischemia increases neutrophil-mediated matrix metalloproteinase activity: a potential mechanism for lung injury after limb ischemia. *J Am Coll Surg* 2003;196:761–767.
198. Delclaux C, d'Ortho MP, Delacourt C, Lebargy F, Brun-Buisson C, Brochard L, Lemaire F, Lafuma C, Harf A. Gelatinases in epithelial lining fluid of patients with adult respiratory distress syndrome. *Am J Physiol* 1997;272:L442–L451.
199. Torii K, Iida K, Miyazaki Y, Saga S, Kondoh Y, Taniguchi H, Taki F, Takagi K, Matsuyama M, Suzuki R. Higher concentrations of matrix metalloproteinases in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. *Am J Respir Crit Care Med* 1997; 155:43–46.
200. Ricou B, Nicod L, Lacraz S, Welgus HG, Suter PM, Dayer JM. Matrix metalloproteinases and TIMP in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1996;154:346–352.
201. Steinberg J, Fink G, Picone A, Searles B, Schiller H, Lee HM, Nieman G. Evidence of increased matrix metalloproteinase-9 concentration in patients following cardiopulmonary bypass. *J Extra Corpor Technol* 2001;33:218–222.
202. Pugin J, Verghese G, Widmer MC, Matthay MA. The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of acute respiratory distress syndrome. *Crit Care Med* 1999;27: 304–312.
203. Fligiel SE, Standiford T, Fligiel HM, Tashkin D, Strieter RM, Warner RL, Johnson KJ, Varani J. Matrix metalloproteinases and matrix metalloproteinase inhibitors in acute lung injury. *Hum Pathol* 2006; 37:422–430.
204. Kim JH, Suk MH, Yoon DW, Lee SH, Hur GY, Jung KH, Jeong HC, Lee SY, Lee SY, Suh IB, *et al.* Inhibition of matrix metalloproteinase-9 prevents neutrophilic inflammation in ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol* 2006;291:L580–L587.
205. Kim KH, Burkhart K, Chen P, Frevert CW, Randolph-Habecker J, Hackman RC, Soloway PD, Madtes DK. Tissue inhibitor of metalloproteinase-1 deficiency amplifies acute lung injury in bleomycin-exposed mice. *Am J Respir Cell Mol Biol* 2005;33:271–279.
206. Van de Louw A, Jean D, Frisdal E, Cerf C, d'Ortho MP, Baker AH, Lafuma C, Duvaldestin P, Harf A, Delclaux C. Neutrophil proteinases in hydrochloric acid- and endotoxin-induced acute lung injury: evaluation of interstitial protease activity by in situ zymography. *Lab Invest* 2002;82:133–145.
207. Betsuyaku T, Shipley JM, Liu Z, Senior RM. Gelatinase B deficiency does not protect against lipopolysaccharide-induced acute lung injury. *Chest* 1999;116:17S–18S.
208. D'Ortho MP, Jarreau PH, Delacourt C, Macquin-Mavier I, Levame M, Pezet S, Harf A, Lafuma C. Matrix metalloproteinase and elastase activities in LPS-induced acute lung injury in guinea pigs. *Am J Physiol* 1994;266:L209–L216.
209. Baugh MD, Perry MJ, Hollander AP, Davies DR, Cross SS, Lobo AJ, Taylor CJ, Evans GS. Matrix metalloproteinase levels are elevated in inflammatory bowel disease. *Gastroenterology* 1999;117:814–822.
210. Bailey CJ, Hembry RM, Alexander A, Irving MH, Grant ME, Shuttleworth CA. Distribution of the matrix metalloproteinases stromelysin, gelatinases A and B, and collagenase in Crohn's disease and normal intestine. *J Clin Pathol* 1994;47:113–116.
211. Castaneda FE, Walia B, Vijay-Kumar M, Patel NR, Roser S, Kolachala VL, Rojas M, Wang L, Oprea G, Garg P, *et al.* Targeted deletion of metalloproteinase 9 attenuates experimental colitis in mice: central

role of epithelial-derived MMP. *Gastroenterology* 2005;129:1991–2008.
212. Sykes AP, Bhogal R, Brampton C, Chander C, Whelan C, Parsons ME, Bird J. The effect of an inhibitor of matrix metalloproteinases on colonic inflammation in a trinitrobenzenesulphonic acid rat model of inflammatory bowel disease. *Aliment Pharmacol Ther* 1999;13:1535–1542.
213. Di Sebastiano P, di Mola FF, Artese L, Rossi C, Mascetta G, Pernthaler H, Innocenti P. Beneficial effects of Batimastat (BB-94), a matrix metalloproteinase inhibitor, in rat experimental colitis. *Digestion* 2001;63:234–239.
214. Medina C, Videla S, Radomski A, Radomski MW, Antolin M, Guarner F, Vilaseca J, Salas A, Malagelada JR. Increased activity and expression of matrix metalloproteinase-9 in a rat model of distal colitis. *Am J Physiol Gastrointest Liver Physiol* 2003;284:G116–G122.
215. Ravi A, Garg P, Sitaraman SV. Matrix metalloproteinases in inflammatory bowel disease: boon or a bane? *Inflamm Bowel Dis* 2007;13:97–107.
216. Gorodeski GI. Estrogen decrease in tight junctional resistance involves matrix-metalloproteinase-7-mediated remodeling of occludin. *Endocrinology* 2007;148:218–231.
217. Pflugfelder SC, Farley W, Luo L, Chen LZ, de Paiva CS, Olmos LC, Li DQ, Fini ME. Matrix metalloproteinase-9 knockout confers resistance to corneal epithelial barrier disruption in experimental dry eye. *Am J Pathol* 2005;166:61–71.
218. Alexander JS, Elrod JW. Extracellular matrix, junctional integrity and matrix metalloproteinase interactions in endothelial permeability regulation. *J Anat* 2002;200:561–574.
219. Navaratna D, McGuire PG, Menicucci G, Das A. Proteolytic degradation of VE-cadherin alters the blood-retinal barrier in diabetes. *Diabetes* 2007;56:2380–2387.
220. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. *Genes Dev* 2000;14:2123–2133.
221. McCann UG II, Gatto LA, Searles B, Carney DE, Lutz CJ, Picone AL, Schiller HJ, Nieman GF. Matrix metalloproteinase inhibitor: differential effects on pulmonary neutrophil and monocyte sequestration following cardiopulmonary bypass. *J Extra Corpor Technol* 1999;31:67–75.
222. Warner RL, Lewis CS, Beltran L, Younkin EM, Varani J, Johnson KJ. The role of metalloelastase in immune complex-induced acute lung injury. *Am J Pathol* 2001;158:2139–2144.
223. Betsuyaku T, Shipley JM, Liu Z, Senior RM. Neutrophil emigration in the lungs, peritoneum, and skin does not require gelatinase B. *Am J Respir Cell Mol Biol* 1999;20:1303–1309.
224. Owen CA, Hu Z, Lopez-Otin C, Shapiro SD. Membrane-bound matrix metalloproteinase-8 on activated polymorphonuclear cells is a potent, tissue inhibitor of metalloproteinase-resistant collagenase and serpinase. *J Immunol* 2004;172:7791–7803.
225. Ichiyasu H, McCormack JM, McCarthy KM, Dombkowski D, Preffer FI, Schneeberger EE. Matrix metalloproteinase-9-deficient dendritic cells have impaired migration through tracheal epithelial tight junctions. *Am J Respir Cell Mol Biol* 2004;30:761–770.
226. Warner RL, Beltran L, Younkin EM, Lewis CS, Weiss SJ, Varani J, Johnson KJ. Role of stromelysin 1 and gelatinase B in experimental acute lung injury. *Am J Respir Cell Mol Biol* 2001;24:537–544.
227. Aarbiou J, Rabe KF, Hiemstra PS. Role of defensins in inflammatory lung disease. *Ann Med* 2002;34:96–101.
228. Aarbiou J, Tjabringa GS, Verhoosel RM, Ninaber DK, White SR, Peltenburg LT, Rabe KF, Hiemstra PS. Mechanisms of cell death induced by the neutrophil antimicrobial peptides alpha-defensins and LL-37. *Inflamm Res* 2006;55:119–127.
229. Van Wetering S, Mannesse-Lazeroms SP, Dijkman JH, Hiemstra PS. Effect of neutrophil serine proteinases and defensins on lung epithelial cells: modulation of cytotoxicity and IL-8 production. *J Leukoc Biol* 1997;62:217–226.
230. Soong LB, Ganz T, Ellison A, Caughey GH. Purification and characterization of defensins from cystic fibrosis sputum. *Inflamm Res* 1997;46:98–102.
231. Sakamoto N, Mukae H, Fujii T, Ishii H, Yoshioka S, Kakugawa T, Sugiyama K, Mizuta Y, Kadota J, Nakazato M, Kohno S. Differential effects of alpha- and beta-defensin on cytokine production by cultured human bronchial epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2005;288:L508–L513.
232. Nygaard SD, Ganz T, Peterson MW. Defensins reduce the barrier integrity of a cultured epithelial monolayer without cytotoxicity. *Am J Respir Cell Mol Biol* 1993;8:193–200.
233. Okrent DG, Lichtenstein AK, Ganz T. Direct cytotoxicity of polymorphonuclear leukocyte granule proteins to human lung-derived cells and endothelial cells. *Am Rev Respir Dis* 1990;141:179–185.
234. Zhang H, Porro G, Orzech N, Mullen B, Liu M, Slutsky AS. Neutrophil defensins mediate acute inflammatory response and lung dysfunction in dose-related fashion. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L947–L954.
235. Ashitani J, Mukae H, Ihiboshi H, Taniguchi H, Mashimoto H, Nakazato M, Matsukura S. *Nihon Kyobu Shikkan Gakkai Zasshi* 1996;34:1349–1353. (Defensin in plasma and in bronchoalveolar lavage fluid from patients with acute respiratory distress syndrome).
236. Ashitani J, Mukae H, Arimura Y, Sano A, Tokojima M, Nakazato M. High concentrations of alpha-defensins in plasma and bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome. *Life Sci* 2004;75:1123–1134.
237. Lang JD, McArdle PJ, O'Reilly PJ, Matalon S. Oxidant-antioxidant balance in acute lung injury. *Chest* 2002;122:314S–320S.
238. Quinlan GJ, Lamb NJ, Tilley R, Evans TW, Gutteridge JM. Plasma hypoxanthine levels in ARDS: implications for oxidative stress, morbidity, and mortality. *Am J Respir Crit Care Med* 1997;155:479–484.
239. Sznajder JI, Fraiman A, Hall JB, Sanders W, Schmidt G, Crawford G, Nahum A, Factor P, Wood LD. Increased hydrogen peroxide in the expired breath of patients with acute hypoxemic respiratory failure. *Chest* 1989;96:606–612.
240. Sittipunt C, Steinberg KP, Ruzinski JT, Myles C, Zhu S, Goodman RB, Hudson LD, Matalon S, Martin TR. Nitric oxide and nitrotyrosine in the lungs of patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2001;163:503–510.
241. Zhu S, Ware LB, Geiser T, Matthay MA, Matalon S. Increased levelste and surfactant protein a nitration in the pulmonary edema fluid of patients with acute lung injury. *Am J Respir Crit Care Med* 2001;163:166–172.
242. Winterbourn CC, Buss IH, Chan TP, Plank LD, Clark MA, Windsor JA. Protein carbonyl measurements show evidence of early oxidative stress in critically ill patients. *Crit Care Med* 2000;28:143–149.
243. Gadek JE, DeMichele SJ, Karlstad MD, Pacht ER, Donahoe M, Albertson TE, Van Hoozen C, Wennberg AK, Nelson JL, Noursalehi M. Effect of enteral feeding with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants in patients with acute respiratory distress syndrome. Enteral Nutrition in ARDS Study Group. *Crit Care Med* 1999;27:1409–1420.
244. Guo RF, Ward PA. Role of oxidants in lung injury during sepsis. *Antioxid Redox Signal* 2007;9:1991–2002.
245. Johnson KJ, Fantone 3rd JC, Kaplan J, Ward PA. In vivo damage of rat lungs by oxygen metabolites. *J Clin Invest* 1981;67:983–993.
246. Berisha HI, Pakbaz H, Absood A, Said SI. Nitric oxide as a mediator of oxidant lung injury due to paraquat. *Proc Natl Acad Sci USA* 1994;91:7445–7449.
247. Kristof AS, Goldberg P, Laubach V, Hussain SN. Role of inducible nitric oxide synthase in endotoxin-induced acute lung injury. *Am J Respir Crit Care Med* 1998;158:1883–1889.
248. Olivera WG, Ridge KM, Sznajder JI. Lung liquid clearance and Na,K-ATPase during acute hyperoxia and recovery in rats. *Am J Respir Crit Care Med* 1995;152:1229–1234.
249. Crapo JD. Morphologic changes in pulmonary oxygen toxicity. *Annu Rev Physiol* 1986;48:721–731.
250. Folz RJ, Abushamaa AM, Suliman HB. Extracellular superoxide dismutase in the airways of transgenic mice reduces inflammation and attenuates lung toxicity following hyperoxia. *J Clin Invest* 1999;103:1055–1066.
251. Auten RL Jr, Mason SN, Tanaka DT, Welty-Wolf K, Whorton MH. Anti-neutrophil chemokine preserves alveolar development in hyperoxia-exposed newborn rats. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L336–L344.
252. Auten RL, Whorton MH, Nicholas Mason S. Blocking neutrophil influx reduces DNA damage in hyperoxia-exposed newborn rat lung. *Am J Respir Cell Mol Biol* 2002;26:391–397.
253. Gao XP, Standiford TJ, Rahman A, Newstead M, Holland SM, Dinauer MC, Liu QH, Malik AB. Role of NADPH oxidase in the mechanism of lung neutrophil sequestration and microvessel injury induced by Gram-negative sepsis: studies in p47phox−/− and gp91phox−/− mice. *J Immunol* 2002;168:3974–3982.
254. Keshavarzian A, Banan A, Farhadi A, Komanduri S, Mutlu E, Zhang Y, Fields JZ. Increases in free radicals and cytoskeletal protein oxidation and nitration in the colon of patients with inflammatory bowel disease. *Gut* 2003;52:720–728.

255. McKenzie SJ, Baker MS, Buffinton GD, Doe WF. Evidence of oxidant-induced injury to epithelial cells during inflammatory bowel disease. *J Clin Invest* 1996;98:136–141.
256. Naito Y, Takagi T, Yoshikawa T. Molecular fingerprints of neutrophil-dependent oxidative stress in inflammatory bowel disease. *J Gastroenterol* 2007;42:787–798.
257. Zaher TE, Miller EJ, Morrow DM, Javdan M, Mantell LL. Hyperoxia-induced signal transduction pathways in pulmonary epithelial cells. *Free Radic Biol Med* 2007;42:897–908.
258. Barazzone C, Horowitz S, Donati YR, Rodriguez I, Piguet PF. Oxygen toxicity in mouse lung: pathways to cell death. *Am J Respir Cell Mol Biol* 1998;19:573–581.
259. Kazzaz JA, Xu J, Palaia TA, Mantell L, Fein AM, Horowitz S. Cellular oxygen toxicity: oxidant injury without apoptosis. *J Biol Chem* 1996; 271:15182–15186.
260. Jyonouchi H, Sun S, Abiru T, Chareancholvanich S, Ingbar DH. The effects of hyperoxic injury and antioxidant vitamins on death and proliferation of human small airway epithelial cells. *Am J Respir Cell Mol Biol* 1998;19:426–436.
261. Zhang X, Shan P, Sasidhar M, Chupp GL, Flavell RA, Choi AM, Lee PJ. Reactive oxygen species and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase mediate hyperoxia-induced cell death in lung epithelium. *Am J Respir Cell Mol Biol* 2003;28:305–315.
262. Buckley S, Barsky L, Driscoll B, Weinberg K, Anderson KD, Warburton D. Apoptosis and DNA damage in type 2 alveolar epithelial cells cultured from hyperoxic rats. *Am J Physiol* 1998;274:L714–L720.
263. Zhang XF, Foda HD. *Zhonghua Jie He He Hu Xi Za Zhi* 2004;27:465–468. (Pulmonary apoptosis and necrosis in hyperoxia-induced acute mouse lung injury).
264. De Paepe ME, Mao Q, Chao Y, Powell JL, Rubin LP, Sharma S. Hyperoxia-induced apoptosis and Fas/FasL expression in lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L647–L659.
265. Otterbein LE, Chin BY, Mantell LL, Stansberry L, Horowitz S, Choi AM. Pulmonary apoptosis in aged and oxygen-tolerant rats exposed to hyperoxia. *Am J Physiol* 1998;275:L14–L20.
266. Pagano A, Barazzone-Argiroffo C. Alveolar cell death in hyperoxia-induced lung injury. *Ann N Y Acad Sci* 2003;1010:405–416.
267. Rao RK, Baker RD, Baker SS, Gupta A, Holycross M. Oxidant-induced disruption of intestinal epithelial barrier function: role of protein tyrosine phosphorylation. *Am J Physiol* 1997;273:G812–G823.
268. Rao RK, Basuroy S, Rao VU, Karnaky Jr KJ, Gupta A. Tyrosine phosphorylation and dissociation of occludin-ZO-1 and E-cadherin-beta-catenin complexes from the cytoskeleton by oxidative stress. *Biochem J* 2002;368:471–481.
269. Bailey TA, Kanuga N, Romero IA, Greenwood J, Luthert PJ, Cheetham ME. Oxidative stress affects the junctional integrity of retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 2004;45: 675–684.
270. Musch MW, Walsh-Reitz MM, Chang EB. Roles of ZO-1, occludin, and actin in oxidant-induced barrier disruption. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G222–G231.
271. Jyonouchi H, Sun S, Mizokami M, Ingbar DH. Cell density and antioxidant vitamins determine the effects of hyperoxia on proliferation and death of MDCK epithelial cells. *Nutr Cancer* 1997;28:115–124.
272. Chapman KE, Waters CM, Miller WM. Continuous exposure of airway epithelial cells to hydrogen peroxide: protection by KGF. *J Cell Physiol* 2002;192:71–80.
273. Sacks T, Moldow CF, Craddock PR, Bowers TK, Jacob HS. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. An in vitro model of immune vascular damage. *J Clin Invest* 1978;61:1161–1167.
274. Varani J, Fligiel SE, Till GO, Kunkel RG, Ryan US, Ward PA. Pulmonary endothelial cell killing by human neutrophils. Possible involvement of hydroxyl radical. *Lab Invest* 1985;53:656–663.
275. Weiss SJ, Young J, LoBuglio AF, Slivka A, Nimeh NF. Role of hydrogen peroxide in neutrophil-mediated destruction of cultured endothelial cells. *J Clin Invest* 1981;68:714–721.
276. Kevil CG, Oshima T, Alexander B, Coe LL, Alexander JS. H(2)O(2)-mediated permeability: role of MAPK and occludin. *Am J Physiol Cell Physiol* 2000;279:C21–C30.
277. Harlan JM, Schwartz BR, Reidy MA, Schwartz SM, Ochs HD, Harker LA. Activated neutrophils disrupt endothelial monolayer integrity by an oxygen radical-independent mechanism. *Lab Invest* 1985;52:141–150.
278. Serhan CN, Chiang N. Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. *Br J Pharmacol* 2008;153:S200–S215.
279. Haworth O, Cernadas M, Yang R, Serhan CN, Levy BD. Resolvin E1 regulates interleukin 23, interferon-gamma and lipoxin A4 to promote the resolution of allergic airway inflammation. *Nat Immunol* 2008;9:873–879.
280. Su X, Johansen M, Looney MR, Brown EJ, Matthay MA. CD47 deficiency protects mice from lipopolysaccharide-induced acute lung injury and Escherichia coli pneumonia. *J Immunol* 2008;180:6947–6953.