Review

Science review: Cell membrane expression (connectivity) regulates neutrophil delivery, function and clearance

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Abstract

As the principal cellular component of the inflammatory host defense and contributor to host injury after severe physiologic insult, the neutrophil is inherently coupled to patient outcome in both health and disease. Extensive research has focused on the mechanisms that regulate neutrophil delivery, function, and clearance from the inflammatory microenvironment. The neutrophil cell membrane mediates the interaction of the neutrophil with the extracellular environment; it expresses a complex array of adhesion molecules and receptors for various ligands, including mediators, cytokines, immunoglobulins, and membrane molecules on other cells. This article presents a review and analysis of the evidence that the neutrophil membrane plays a central role in regulating neutrophil delivery (production, rolling, adhesion, diapedesis, and chemotaxis), function (priming and activation, microbicidal activity, and neutrophil-mediated host injury), and clearance (apoptosis and necrosis). In addition, we review how change in neutrophil membrane expression is synonymous with change in neutrophil function *in vivo*. Employing a complementary analysis of the neutrophil as a complex system, neutrophil membrane expression may be regarded as a measure of neutrophil connectivity, with altered patterns of connectivity representing functionally distinct neutrophil states. Thus, not only does the neutrophil membrane mediate the processes that characterize the neutrophil lifecycle, but characterization of neutrophil membrane expression represents a technology with which to evaluate neutrophil function.

Keywords apoptosis, chemotaxis, connectivity, delivery, neutrophil, receptors

Tissue inflammation, manifesting clinically as rubor, calor, tumor, and dolor, has been a focus of investigation since the beginning of medical science. Inflammation may be defined as a condition or state that tissues enter as a response to injury or insult. The neutrophil is the most important and the most extensively studied cell involved in the inflammatory response. As the principal circulating phagocyte, the neutrophil is the first and most abundant leukocyte to be delivered to a site of infection or inflammation, and is thus an integral component of the innate immune system. In addition to its role in host defense, the neutrophil is implicated in the pathogenesis of tissue injury and of persistent inflammatory diseases. The paradoxic roles of the neutrophil in host defense and host injury have fueled intense scientific inquiry into the processes of neutrophil delivery to a site of inflammation, neutrophil function within the inflammatory environment, and neutrophil clearance from that milieu.

The aim of the present review is to highlight the importance of neutrophil cell membrane expression in the participation and regulation of neutrophil delivery, function, and clearance from its environment. The relationship between altered receptor expression and altered neutrophil function in humans and *in vivo* are emphasized. The review concludes with a brief dis-

FADD = Fas-associated death domain; FasL = Fas ligand; FMLP = f-Met-Leu-Phe; G-CSF = granulocyte colony-stimulating factor; GM-CSF = granulocyte/macrophage colony-stimulating factor; GRO = growth-related oncogene; H_2O_2 = hydrogen peroxide; ICAM = intercellular adhesion molecule; IL = interleukin; MIP = macrophage inflammatory protein; NADPH = reduced nicotinamide adenine dinucleotide phosphate; NF-κB = nuclear factor-κB; $O_2^{-\bullet}$ = superoxide anion; PECAM = platelet–endothelial cell adhesion molecule; TNF = tumor necrosis factor.

cussion and interpretation of the importance of membrane receptor expression as a measure of cellular 'connectivity', and provides suggestions for future research into the role of neutrophils in the inflammatory response.

Neutrophil delivery to the inflammatory microenvironment

Neutrophil production and storage

The neutrophil lifecycle begins with a bone marrow phase, followed by a circulating phase; it ends with a tissue phase. Within the bone marrow, neutrophils originate from self-renewing myeloid stem cells; the myeloblast differentiates into the promyloblast, and then into the myelocyte. These cells differentiate into metamyelocytes as well as segmented band neutrophils, which are occasionally seen in circulation during a stress response. The metamyelocyte is the precursor to polymorphonuclear leukocytes, which are commonly referred to as granulocytes, including eosinophils, basophils, and neutrophils. The process of neutrophil maturation and differentiation within the marrow takes approximately 14 days, and has undergone considerable investigation [1]. Neutrophil production is estimated to vary from 10^8 to 10^{11} cells/day, depending on the measurement technique used [1,2]. This is mediated by a variety of hematopoietic growth factors, most notably granulocyte colony-stimulating factor (G-CSF) and granulocyte/macrophage colony-stimulating factor (GM-CSF) [3].

Growth factors exert their effect through interaction with membrane receptors, with subsequent induction of intracellular tyrosine phosphorylation and activation of multiple signaling cascades [4]. Variation in receptor expression and modulation by soluble mediators occurs during cell maturation [5]. In addition to other factors, GM-CSF and G-CSF mediate proliferation and differentiation of neutrophil bone marrow stem cells, allowing for substantial variation in neutrophil production, which increases as much as 10-fold during a stress response [2]. Pathologic function of growth factor receptors leads to hematologic illness [6,7], and a reduction in marrow G-CSF receptor expression is associated with myeloid maturation arrest and neutropenia following severe burn injury [8]. Thus, neutrophil production, differentiation, and maturation depend upon physiologic interaction of growth factors with receptors on neutrophil myeloid precursors.

After release from the bone marrow, neutrophils enter the circulating compartment (i.e. the second phase of their lifecycle). In circulation, neutrophils have a half-life of 6–9 hours. Neutrophils comprise more than 50% of circulating leukocytes and more than 90% of circulating phagocytes, and reversibly move from circulating to marginating pools. Marginated neutrophils are those that are 'stored' in the capillaries of certain tissues, most notably in the lung, and are much greater in number than are those that are free in circulation at any given time [9]. The lung harbours large numbers of marginating neutrophils because of the tremendous number of small capillaries (with diameter less than that of the neutrophil), forcing neutrophils to deform in order to pass through these capillaries [10]. The marginating pool of neutrophils allows for rapid mobilization in response to infection or other stresses. Despite the rapid turnover, human neutrophil counts are relatively stable, averaging 3000–4000 neutrophils/mm^3. Neutrophil delivery occurs in the postcapillary venule as a sequential series of well studied processes (Fig. 1).

Margination

Neutrophil transmigration from the intravascular to the extravascular (exudate) milieu predominantly occurs in the postcapillary venule within the systemic circulation and in the capillary in the pulmonary circulation [11]. Neutrophil exudation is facilitated and mediated by a combination of mechanical, chemical, and molecular processes; these are distinct events that are linked in a temporal sequence. The first step is 'margination', or movement of the neutrophil from the central stream to the periphery of a vessel. In postcapillary venules, when the vessel diameter is 50% larger than the diameter of the leukocyte, erythrocytes move faster than the larger leukocytes, especially in the center of the vessel, pushing leukocytes to the vessel periphery [12]. Physical forces involved in the erythrocyte–leukocyte interactions govern this radial movement of leukocytes. The importance of erythrocytes has been demonstrated in a rat mesenteric perfusion model, in which no leukocyte margination was observed in the absence of red cells [13]. Neutrophil margination allows for a molecular interaction between the cell surfaces of the neutrophil and endothelial cell to occur, resulting in neutrophil rolling on the vessel wall.

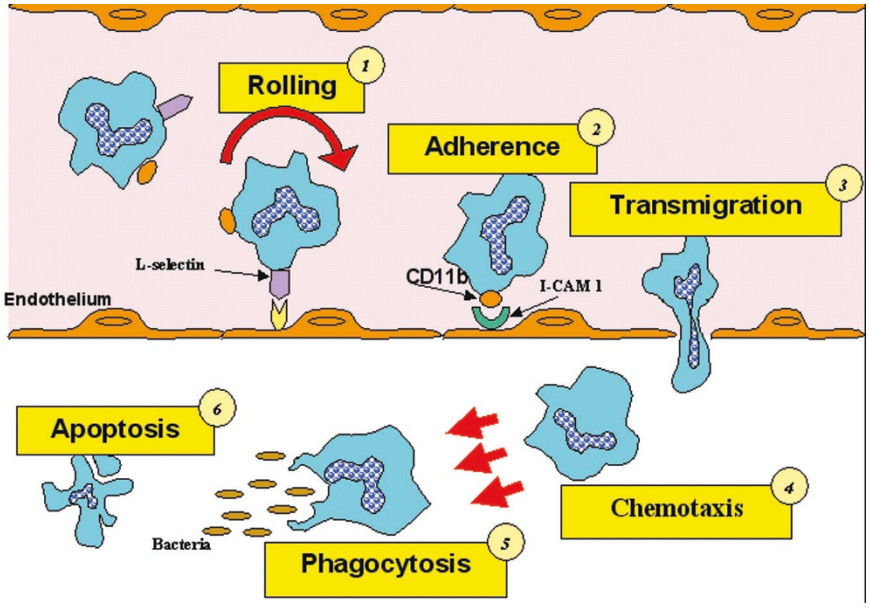
Rolling

A state of weak adhesive interaction between the neutrophil and endothelial cell allows the neutrophil to roll along the surface of the postcapillary venule. 'Rolling' is dependent upon both physical and molecular forces. The neutrophil's ability to roll and adhere to endothelial cells is inversely proportional to the vessel shear rate (i.e. faster moving blood decreases the ability of leukocytes to adhere) [14]. Neutrophil rolling velocity is also directly proportional to luminal red blood cell velocity [15]. Once in proximity to the endothelial cell, a low-affinity adherence occurs and, in conjunction with the shear stress of passing erythrocytes, the neutrophil begins to roll along the endothelial lining of the vessel.

Selectins

Interactions between the surface of the neutrophil and the endothelial cell allow for rolling, and subsequently adherence and diapedesis. The low-affinity interaction involved in rolling is largely governed by selectins and their ligands (Table 1). Selectins are a family of glycoprotein surface adhesion molecules, and include L-selectin (expressed exclusively on leukocytes), E-selectin (expressed exclusively on endothelial cells), and P-selectin (expressed on platelets and endothelial cells). Constitutive expression of L-selectin is maintained on all circulating quiescent leukocytes (except for certain subpopulations of memory T cells) [16].

Figure 1



Neutrophil delivery in the postcapillary venule. ICAM, intercellular adhesion molecule.

Animal intravital microscopy has demonstrated that blocking L-selectin and/or P-selectin with high-dose selectin-binding carbohydrate (fucoidin) decreased both neutrophil rolling and adherence following ischemia/reperfusion [17]. L-selectin and P-selectin gene-deficient mice exhibit diminished rolling [18]. The ligands for neutrophil L-selectin are multiple sialylated carbohydrate determinants, which are linked to mucin-like molecules [16,19]. These selectin ligands on endothelial cells are inducible with lipopolysaccharide or a variety of inflammatory cytokines [20]. In addition to L-selectin mediated rolling, endothelial cell expression of E-selectin is necessary for normal leukocyte recruitment and may initiate leukocyte rolling in certain models [21,22]. The rolling governed by a weak molecular interaction is a prerequisite for a stronger molecular interaction, namely adherence. This has been demonstrated using intravital microscopy in the rat mesenteric microcirculation [23], in human neutrophils in rabbit mesenteric venules [24], and in a cat mesenteric perfusion model [15]. However, other investigators have demonstrated that antibodies to P-selectin will attenuate rolling but not impact on adherence [25]. Blocking L-selectin in animal models reduced neutrophil-mediated tissue injury, which was believed to be dependent upon neutrophil adherence [26]. In addition, soluble L-selectin shed from neutrophils may attenuate TNF-α stimulated neutrophil adherence and subsequent vascular permeability [27]. Thus, those studies suggest that selectins not only mediate rolling, but also impact upon ensuing leukocyte adherence.

Adherence

As with rolling, the cell surface of the neutrophil determines its ability to undergo 'adherence'. In contrast to rolling, which is a dynamic low-affinity adhesive interaction, adherence is a stationary high-affinity (strong) adhesive interaction between the neutrophil and endothelial cell. This interaction is largely mediated by a separate set of adhesion molecules, namely the integrins and their ligands. The importance of integrin-mediated adhesion to neutrophil delivery and host defense was first demonstrated in patients with leukocyte adhesion deficiency type 1 [28]. These patients develop life-threatening bacterial infections; this is because neutrophils are unable to undergo transmigration to sites of inflammation as a result of a genetic mutation in CD18, the β-subunit of the integrin family of adhesion molecules. Neutrophils from healthy control individuals incubated with monoclonal antibodies to integrins, or neutrophils from patients with leukocyte adhesion deficiency-1 both demonstrate deficient adhesion and transmigration through activated endothelial monolayers [29].

Integrins and intercellular adhesion molecules

Integrins are a family of heterodimeric proteins (made up of two different subunits, namely α-subunits and β-subunits) that are expressed on the cell surface, and are integral to the process of cell adhesion. Of this family, the β_2-integrins have attracted the most investigation; they are restricted to leukocytes and are essential to normal leukocyte trafficking. They consist of three distinct α-subunits (CD11a, CD11b, and CD11c) that are bound to a common β-subunit (CD18). Although the distribution of β_2-integrins subclasses differs among leukocyte populations, neutrophils express all three classes. The relative contribution of each α-subunit to leukocyte adherence may vary and depend upon the stimulus leading to adherence and transmigration [30]. Neutrophil

Table 1

Neutrophil and endothelial cell adhesion receptors

Receptor	Cell	Ligand	Cell type	Purpose
L-selectin	Neutrophil	sLe^a, sLe^x	Endothelium	Rolling and weak adhesion of PMNs on EC
CD11a/CD18	Neutrophil	ICAM-1, ICAM-2, ICAM-3	Endothelium	Adhesion of PMNs on EC
CD11b/CD18	Neutrophil	ICAM-1	Endothelium	Adhesion of PMNs on EC
		iC3b	Complement	Phagocytosis?
		Fibrinogen	–	–
		Factor X	–	–
CD11c/CD18	Neutrophil	iC3b	Complement	Phagocytosis?
		Fibrinogen	–	–
E-selectin	Endothelium	sLe^x	Neutrophil	Firm PMN/EC adhesion
P-selectin	Endothelium, platelets	sLe^x	Endothelium	Firm PMN/EC adhesion
		PSGL-1	Neutrophil	Firm PMN/EC adhesion
PECAM-1	Endothelium	CD31/α_v	Leukocytes	Diapedesis of PMN through EC
	Neutrophil		–	–
ICAM-3	Neutrophil	CD11a/CD18	Leukocytes	Antigen presentation

ICAM, intercellular adhesion molecule; PECAM, platelet–endothelial cell adhesion molecule; PMN, polymorphonuclear leukocyte; PSGL, P-selectin glycoprotein ligand.

integrins interact with complementary surface molecule ligands on endothelial cells in order to generate the high-affinity bond that characterizes adherence (Table 1). Particularly important to neutrophils, intercellular adhesion molecule (ICAM)-1 on endothelial cells serves as the ligand for both CD11a/CD18 and CD11b/CD18, whereas ICAM-2 is capable of binding CD11a only [31].

Animal intravital microscopy has demonstrated the importance of the integrin β-subunit CD18 to adhesion but not to rolling [32,33]. Multiple studies have demonstrated that anti-CD11/CD18 antibodies are associated with reduced inflammation and injury in models of allograft rejection, endotoxin challenge, hemorrhagic shock, aspiration pneumonia, bacterial pneumonia, and ischemia/reperfusion, among others [34]. Although CD18-dependent neutrophil transmigration is essential for physiologic neutrophil delivery, CD18-independent neutrophil transmigration has been demonstrated in rabbit models of respiratory and peritoneal infection, and respiratory and hepatic ischemia/reperfusion [35–38]; this may depend on the type of bacteria at the site of infection [39]. In addition to β_2-integrin mediated adhesion, Kubes and coworkers [40] demonstrated that expression of β_1-integrins (specifically $\alpha_4\beta_1$) may be induced by activation or by transmigration in order to mediate adhesion on human neutrophils. Notwithstanding the complexity of adhesion molecule interaction, the membrane of the neutrophil and of the endothelial cell must undergo firm adhesion in order for the process of neutrophil transmigration to progress.

Receptor adherence in receptor molecular biology is evaluated by receptor affinity, which relates to the strength of interaction between a single antigen-binding site and a single antigenic determinant, as well as by receptor avidity, which represents the strength of binding of a molecule with multiple binding sites, such as the binding of a complex antigen with multiple antibodies. Affinity depends upon noncovalent bonds between binding sites and is measured using an affinity constant. Avidity represents the overall binding of antibodies to antigen, and may be greater than the sum of the affinities if cooperative effects exist (i.e. binding at one site promotes binding at another). Both receptor affinity and avidity may be differentially regulated in leukocyte–endothelial cell interactions involving the β_2-integrin (CD11a/CD18) [41,42].

Both integrins on neutrophils, as well as ICAMs on endothelial cells, demonstrate marked variability in expression and adhesiveness. Augmented neutrophil expression of CD11b/CD18 is induced from intracellular pools by various cytokines, including f-Met-Leu-Phe (FMLP), GM-CSF, C5a, tumor necrosis factor (TNF)-α, and others; however, increased neutrophil adhesiveness may be more significantly related to conformational changes in the CD11b/CD18 protein complex [43]. Chemoattractants such as the chemokine IL-8 will activate integrin adhesiveness as well as help to direct leukocyte migration [44,45]. In addition to constitutive expression of ICAM-1 and ICAM-2 on endothelial cells, ICAM-1 expression may be augmented by numerous inflammatory mediators [46–48]. Thus, under the influence of

inflammatory mediators, changes in number and conformation of neutrophil integrins and upregulation of endothelial cell ICAM expression will induce a transition from selectin-dependent rolling to integrin/ICAM-dependent adherence [49], subsequently leading to diapedesis, which is the next step in neutrophil delivery.

Diapedesis

Following adherence, the neutrophil must pass through the endothelial monolayer and basement membrane to enter the extravascular inflammatory (exudate) environment. *In vitro* adherence of neutrophils on activated endothelial cells will cause a disruption in endothelial cell–cell interaction and augment endothelial cell permeability – an effect that may be blocked with anti-integrin monoclonal antibodies [50]. Transmission electron microscopy in a human umbilical vein neutrophil transmigration model suggested that diapedesis of neutrophils occurs at endothelial cell tricellular corners (the intersection of three endothelial cells) [51]. Endothelial adhesion molecules are necessary for diapedesis and transmigration. Leukocyte adherence and emigration observed after ischemia/reperfusion and in response to leukotriene-B_4 or platelet-activating factor is decreased with monoclonal antibodies to various adhesion glycoproteins, including CD18, CD11b, ICAM-1, and L-selectin [52,53]. Thus, membrane-mediated adherence is a prerequisite for diapedesis – a process that is also mediated by neutrophil–endothelial cell membrane interaction.

Platelet–endothelial cell adhesion molecule-1

Other adhesion molecules, such as platelet–endothelial cell adhesion molecule (PECAM)-1, are specifically involved in the process of diapedesis. PECAM-1 is constitutively expressed and concentrated on the lateral borders of endothelial cells where diapedesis is observed to take place, as well as on the surface of neutrophils, some T cells, monocytes, and platelets. Blocking PECAM-1 with monoclonal antibodies will increase neutrophil adhesion to endothelial cells mediated by CD11b/CD18 [54,55], thus inhibiting the ability of the neutrophil to undergo diapedesis. Monoclonal antibodies to PECAM-1 will arrest leukocyte transmigration by 70–90% without interfering with normal leukocyte adhesion to endothelial monolayers; leukocytes remain tightly bound to the apical surface of the endothelial cell, precisely over the intercellular junction [56]. The importance of endothelial and neutrophil expression of PECAM-1 was confirmed using *in vivo* murine intravital microscopy [57]. Thus, PECAM-1 appears to allow the neutrophil to evade adhesion at intercellular junctions so that diapedesis leading to neutrophil transmigration may take place.

In summary, the process of neutrophil transmigration is regulated by a multistep process that involves sequential events, each of which are necessary for progression to the next. These cellular processes are governed by molecular interactions between receptors and their ligands expressed on neutrophils and endothelial cells. The cell membrane of the neutrophil allows it to interact with endothelial cells. Leukocyte delivery may be regulated by altering the expression and efficacy of the various adhesion receptors dynamically *in vivo*, leading to site-specific leukocyte accumulation. In addition to adhesion receptors and ligands mediating neutrophil–endothelial cell interactions, leukocyte delivery requires further neutrophil cell membrane participation, specifically responding to soluble mediators in the extracellular inflammatory environment.

Chemotaxis

In addition to intercellular adhesion, leukocytes require a chemoattractant gradient in order to complete the process of transmigration. Chemoattractants are soluble molecules that confer directionality on cell movement; cells migrate in the direction of increasing concentration of a chemoattractant in a process termed 'chemotaxis'. Neutrophils have long been known to undergo chemotaxis toward damaged or inflamed tissue [58].

The production of chemoattractants in the inflammatory environment is from a combination of sources, including bacterial byproducts and cell wall constituents, complement factors, and chemokines produced by inflammatory and noninflammatory cells. For example, in addition to neutrophils themselves [59], monocytes, smooth muscle cells, epithelial cells, endothelial cells, and fibroblasts are capable of generating IL-8 (a potent neutrophil chemoattractant) when they are stimulated with an proinflammatory agonist such as IL-1 or TNF-α [60].

Chemoattractants serve not only to direct leukocytes to specific areas of inflammation but also to recruit specific subpopulations of leukocytes to inflamed tissue, such as neutrophils in response to acute bacterial infection, eosinophils at sites of chronic allergic inflammation or parasitic infection, and monocytes in chronic inflammatory diseases. Chemoattractant mediators may thus be classified on the basis of their spectrum of leukocyte activity (Table 2). Classical chemoattractants include N-formylated peptides produced by bacteria, such as FMLP, polypeptides (e.g. C5a), and lipids (e.g. leukotriene-B_4), which act as chemoattractants for various nonspecific leukocyte populations [61–63]. Chemoattractant cytokines, or chemokines, are a novel family of chemoattractants that confer specificity to leukocyte subset responsiveness, and are well reviewed elsewhere [64,65]. Extensive *in vitro* and *in vivo* investigation has identified IL-8 as a principal factor in neutrophil delivery [66–69]. Other chemokines that are specific for neutrophils include epithelial cell derived neutrophil activating peptide; neutrophil activating peptide-2; growth-related oncogene (GRO)-α, GRO-β and GRO-δ; and macrophage inflammatory protein (MIP)-2α and MIP-2β. These chemokines are structurally similar, and consist of the first two cysteine (C) amino acid residues separated by a separate amino acid (X), and are referred to as CXC

Table 2

Neutrophil chemoattractants

Neutrophil specific	Leukocyte nonspecific
IL-8	C5a
Granulocyte chemotactic protein (GCP)-2	Tumor necrosis factor (TNF)
Epithelial cell-derived neutrophil attractant (ENA)-78	Monocyte chemoattractant protein (MCP)-1, MCP-2, MCP-3, MCP-4
Neutrophil-activating peptide (NAP)-2	f-Met-Leu-Phe (FMLP)
Growth-related oncogene (GRO)-α, GRO-β, GRO-γ	Macrophage chemotactic and activating factor (MCAF)
Macrophage inflammatory protein (MIP)-1, MIP-2	Platelet-activating factor (PAF)
	Regulated upon activation, normal T cell expressed and secreted (RANTES)
Platelet factor (PF)-4	I-309
Mast cell-derived chemotactic factor	Casein
5-Hydroxyeicosatetraenoic acid	Leukotriene-B_4 (LTB_4)

chemokines or α chemokines. A separate family of chemokines are known as CC chemokines, because the first two cysteine residues are in juxtaposition. Monocyte chemoattractant protein-1, -2 and -3; MIP-1α and MIP-1β; and RANTES (regulated upon activation, normal T cell expressed and secreted) are members of the CC family, or β chemokines. The activity of the CC supergene family of chemokines is predominantly oriented toward monocytes [70]. Thus, chemoattractants help to explain how leukocytes localize to specific inflammatory sites, and how specific leukocyte populations are recruited to those sites.

Chemoattractant receptors

Leukocyte delivery is further regulated by chemoattractant receptors that exhibit specificity for both the type of leukocyte on which they are expressed and the ligand to which they bind. The specificity of chemoattractant-induced leukocyte chemotaxis is related to differential expression of chemokine receptors, a superfamily of G-protein-coupled receptors with seven transmembrane regions [71,72]. Although chemokine receptors share similar structures, they differ in their ligand specificity (Table 3). For example, IL-8 receptor A (CXC R1) and IL-8 receptor B (CXC R2) have a 78% identical amino acid sequence, and both bind IL-8; however, although IL-8 receptor A is specific for IL-8, IL-8 receptor B has multiple agonists, including other CXC chemokines such GRO-α, GRO-β, GRO-δ, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil activating peptide-78 [73]. Neutrophil transmigration appears to depend to a greater degree on IL-8 receptor A than on IL-8 receptor B, because antibodies directed against IL-8 receptor A inhibited the majority (78%) of IL-8 induced chemotaxis [74]. In contrast, IL-8 receptor B has been implicated in transendothelial migration of T cells [75]. In addition, chemoattractant receptors are expressed on specific leukocyte subsets (Table 3); whereas receptors to the classical chemoattractants are expressed on monocytes, neutrophils, eosinophils and basophils, CXC chemokine receptors are primarily restricted to neutrophils [16].

Thus, chemokine receptors display both ligand and leukocyte specificity. These complex rules defining the interactions between specific chemoattractants and leukocytes are the mechanisms that allow the host response to deliver specific subsets of leukocytes to localized areas of infection or inflammation. Chemoattractant receptors not only mediate the process of chemotaxis, but changes in receptor expression within the inflammatory environment confer changes on cell function. Before discussing changes in neutrophil cell surface expression, we consider neutrophil function and clearance from the inflammatory microenvironment.

Neutrophil function in the inflammatory microenvironment

Neutrophil priming and activation

Neutrophils can exist in various stable functional states. The different states are associated with different patterns of altered membrane expression (Table 4). Quiescent neutrophils can be 'activated' by various inflammatory mediators in order to produce reactive oxygen metabolites (the respiratory burst) and destructive proteolytic enzymes (see below). In addition to being activated, the neutrophil can be 'primed' to produce an augmented or exaggerated response to an activating stimulus. Priming is defined as an enhancement or amplification of the neutrophil respiratory burst in response to a given activating stimulus following exposure to the priming agent [76]. Altering the neutrophil from a 'resting' state to a 'primed' state does not activate the respiratory burst directly but will potentiate the neutrophil response to a subsequent stimulus [77].

Various mediators have been found to cause neutrophil priming, including adenosine triphosphate [78], platelet-acti-

Table 3

Neutrophil chemoattractant receptors and their ligands

Class	Receptors	Ligands
C-X-C receptors	CXCR1 (IL-8 receptor A)	IL-8
	CXCR2 (IL-8 receptor B)	IL-8, GRO, NAP-2, ENA-78, GCP-2
	CXCR3	Mig, IP-10
	CXCR4	SDF-1
C-C receptors	CCR1	MIP-1α, MIP-1β, MCP-3
	CCR2A, CCR2B	MCP-1, MCP-3
	CCR3	Eotaxin, RANTES, MCP-3
	CCR4	MIP-1α, RANTES, MCP-1
	CCR5	MIP-1α, MIP-1β, RANTES
	CCR6	MIP-3α
	CCR7	ELC
	CCR8	I-309
Non-C-X-C	C5aR	C5a
	FMLPr	FMLP

ELC, Epstein-Barr virus-induced molecule 1 ligand chemokine (CCL19); ENA, epithelial cell derived neutrophil activating peptide; FMLP, f-Met-Leu-Phe; GCP, granulocyte chemotactic protein; GRO, growth-related oncogene; IP, inducible protein; IP-10, interferon-gamma inducible protein; MCP, monocyte chemoattractant protein; Mig, monokine induced by interferon-gamma (CXCL9); MIP, macrophage inflammatory protein; NAP, neutrophil-activating peptide; RANTES, regulated upon activation, normal T cell expressed and secreted; SDF, stromal derived factor.

vating factor [79], IL-8 [80], IL-6 [81], lipopolysaccharide [82], and leukotriene-B_4 [83]. An alteration in cell surface receptor expression has been proposed to mediate the priming phenomenon; for example, GM-CSF and TNF cause an increase in neutrophil FMLP receptor expression when primed [84,85]. However, other investigators have demonstrated diminished or unchanged numbers of receptors with other priming agents, or that the priming effect was temporally unrelated to increase in receptor numbers [86–88]. Other groups found that the priming effect altered the signal transduction cascade distal to the FMLP receptor, involving a direct activation of G-proteins [89]. An immediate and rapid rise in intracellular [Ca^{2+}] is implicated in the ability of a group of agents to cause priming, including IL-8, adenosine triphosphate, leukotriene-B_4, and platelet-activating factor [78,90]. Under certain conditions, however, priming secondary to FMLP occurs without any rise in [Ca^{2+}] [91]. Other priming agents, such as TNF-α, GM-CSF, and lipopolysaccharide, are associated with less rapid rises in intracellular [Ca^{2+}], and require longer incubation periods to achieve the priming effect [92]. Priming effects are further complicated by the fact that priming agents exhibit synergy [93,94]. Neutrophil priming and subsequent activation has been hypothesized to play an important role in endothelial cell and end-organ injury and in the pathogenesis of multiple organ dysfunction [95], which is supported by data from an animal ischemia/reperfusion model [96] and observations in human neutrophils following trauma [97].

In summary, neutrophil priming occurs through different, interconnected pathways marked by redundancy and synergy, is mediated by intracellular pathways, and is characterized by alteration in surface receptor expression.

Strongly related to priming, neutrophil activation is an integral component of the systemic host response. Neutrophils are the most abundant inflammatory cells, and their activation is essential for host defense against bacterial or fungal infection, as well as being principally involved in host injury in states of persistent inflammation. Our patients live to survive the balance between the paradoxic roles of the neutrophil. Although this subject has been comprehensive reviewed [98,99], the physiologic and pathologic roles of the neutrophil are presented, highlighting the role of the neutrophil cell membrane. Both neutrophil microbicidal activity and neutrophil-induced tissue injury are representative of the function of the activated neutrophil within the exudate inflammatory microenvironment.

Neutrophil microbicidal activity and neutrophil-induced tissue injury

The neutrophil is the principal phagocyte delivered to inflammatory sites; its role is to destroy and ingest pathogens in the circulating and exudate milieu, which is an important component of nonspecific immunity. Deficiencies in neutrophil function are well studied and are clearly linked to increased frequency and severity of bacterial and fungal infections

Table 4

Human neutrophil states: adhesion, chemotaxis, apoptosis and function

PMN state	PMN receptors	PMN functions
Circulating PMN (resting bloodstream PMN, collected by venipuncture)	Adhesion receptors: constitutive expression of L-selectin, PECAM-1 Chemoattractant receptors: constitutive expression of IL-8 receptor A, IL-8 receptor B, C5aR Apoptosis receptors: constitutive expression of TNF-α receptor I, Fas, FasL	PMN–EC interactions: baseline PMN rolling, adhesion on activated endothelium and transmigration Chemotaxis: will undergo chemotaxis to PMN-specific and leukocyte nonspecific chemoattractants Function: minimal PMN respiratory burst (ROI•) and microbicidal activity (proteolytic enzymes) Apoptosis: constitutive apoptosis (PMN half-life ~6 h)
Primed PMN (PMN stimulated with priming agent *in vitro*)	Adhesion receptors: increased expression of CD11b, L-selectin, PECAM-1, ↔FMLPr Chemoattractant receptors: ?IL-8 receptor A, ?IL-8 receptor B, ↔C5aR Apoptosis receptors: ?TNF-α receptor I, ?Fas, ?FasL Other: CD14, ↑LTB_4r, ↑PAFr	PMN–EC interactions: unclear impact on rolling, adhesion, diapedesis Chemotaxis: no change in chemotaxis Function: when activated, display increased respiratory burst and microbicidal activity after activation Apoptosis: delayed constitutive apoptosis
Activated PMN (PMN stimulated with activating agent *in vitro*)	Adhesion receptors: ↑CD11b, ↑FMLPr, ?L-selectin, PECAM-1 Chemoattractant receptors: ↓IL-8 receptor A, ↓IL-8 receptor B, ↔C5aR Apoptosis receptors: unknown Other: ↓C3br, ↓1C3b	PMN–EC interactions: ↑PMN rolling and adhesion, ?transmigration Chemotaxis: ↔chemotaxis to C5a, LTB_4/ZAS; ↑?chemotaxis to FMLP Function: ↑respiratory burst (ROI•) and microbicidal activity (proteolytic enzymes); ↑phagocytosis Apoptosis: delayed apoptosis
Exudate PMN (PMN collected from dermal exudate milieu *in vivo*)	Adhesion receptors: ↑CD11b, ↑Mac-1, ↓L-selectin, ↓PECAM-1 Chemoattractant receptors: ↓IL-8 receptor A, ↓IL-8 receptor B, ↑C5ar Function: ↑FMLPr Apoptosis receptors: ↓binding to TNF-α, ?↓TNF receptor I, ↔Fas, FasL	PMN–EC interactions: unknown Chemotaxis: ↑baseline chemotaxis, ↓chemotaxis to IL-8, ↑chemotaxis to C5a Function: ↑respiratory burst (ROI•), ↑microbicidal activity and phagocytosis Apoptosis: ↓constitutive apoptosis; ↓TNF-α-induced, but not Fas-induced apoptosis
Septic PMN (PMN collected from circulation in septic patients *in vivo*)	Adhesion receptors: ↓L-selectin, ?CD11b, ?FMLPr, ?PECAM-1 Chemoattractant receptors: ↓IL-8 receptor A, ↓IL-8 receptor B, ↓C5aR Apoptosis receptors: ↓TNF-α receptor I, ?Fas, ?FasL	PMN–EC interactions: unknown Chemotaxis: ↓chemotaxis to IL-8 and C5a Function: ? ↑respiratory burst (ROI•), ?↑microbicidal activity and phagocytosis Apoptosis: ↓constitutive apoptosis; ↓TNF-α-induced, but not Fas induced, apoptosis
Unresponsive or apoptotic PMN	Adhesion receptors: ↓L-selectin, ?CD11b, ?PECAM-1 Chemoattractant receptors: unknown Apoptosis receptors: ? ↓TNF receptor I, ?Fas, ?FasL Other: ↓PAFr	PMN–EC interactions: no interaction Chemotaxis: ↓chemotaxis Function: ↓respiratory burst (ROI•), ↓phagocytosis Apoptosis: unresponsive PMN undergo apoptosis. and apoptotic PMN are unresponsive

?, unknown/controversial; EC, endothelial cell; FasL, Fas ligand; FMLP, f-Met-Leu-Phe; LT, leukotriene; PAF, platelet-activating factor; PECAM, platelet–endothelial cell adhesion molecule; PMN, polymorphonuclear leukocyte; ROI, reactive oxygen intermediates; TNF, tumor necrosis factor; ZAS, zymosan activated serum.

[100]. Simultaneously, the neutrophil's destructive capacity leads to host injury in numerous disease states [101]. This paradox is at the heart of the difficulty in creating effective immunomodulation for critically ill patients.

Cell surface receptors on the neutrophil are essential to the process of phagocytosis and simultaneous activation of microbicidal mechanisms. Using mechanisms similar to those used in chemotactic movement, the membrane of the neu-

trophil is capable of extending pseudopodia and engulfing micro-organisms. Opsonins will bind to neutrophil receptors and trigger phagocytosis. Opsonins principally include complement fragments and antibodies. IgG, which comprises 85% of circulating immunoglobulin, will bind to IgG receptors. These membrane-bound glycoprotein complexes are expressed on hematopoietic and endothelial cells, consist of three classes (FcγI, FcγII, FcγIII, and FcRB), and when bound to IgG they cause tyrosine kinase mediated alteration in cell function [102].

Human neutrophils constitutively express two distinct Fcγ receptors, namely FcγRIIa (CD32) and FcγRIIIb (CD16), both of which cause cell activation through the same intracellular pathways [103]. Changes in receptor expression alter the ability of neutrophils to respond to opsonins. For example, although FcγRIIIb and FcγRIIa are low-affinity, constitutively expressed receptors on circulating neutrophils in healthy control individuals, FcγRI (CD64) is a high-affinity IgG receptor, which is induced by inflammatory cytokines [104] and is expressed in circulating neutrophils in patients with bacterial infections [105] and septic shock [106].

When opsonized particulate matter is encountered by the neutrophil, the plasma membrane flows around the offending agent, engulfing it completely with minimal extracellular fluid. Phagocytosis is immediately followed by release of cytosolic granules into the phagocytic vacuoles, converting the phagosome into a phagolysosome. A synergistic combination of potent oxidants and enzymes serve to destroy the targets ingested by the neutrophil within the phagosome [107]. In addition, neutrophils may be activated by soluble stimuli, an interaction that is again mediated by the neutrophil membrane, through cytokine and chemokine receptors, immunoglobulin (Fc) receptors, and adhesion molecules, among others. In contrast to ingestion of particulate stimuli, activation of a neutrophil by soluble stimuli will yield release of its toxic components into the extracellular space; this process is of clinical significance in inflammatory disease states.

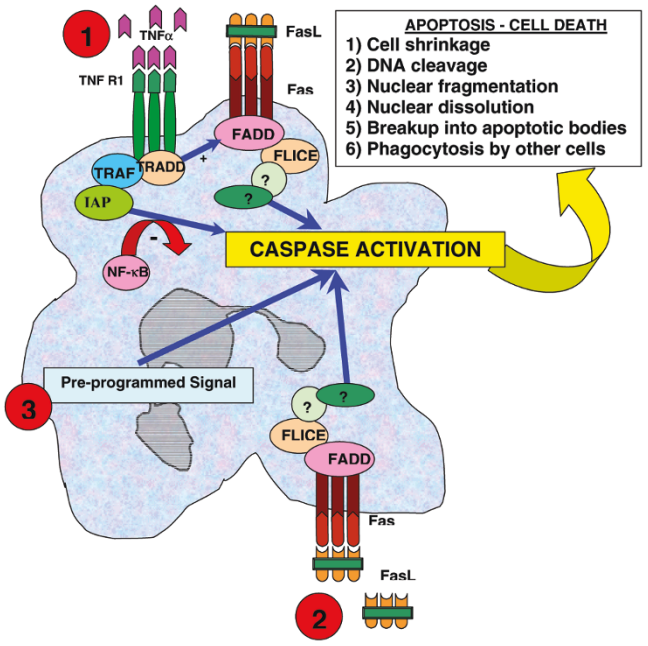
Neutrophil toxins are divided into two groups based on their localization within the cell: intracellular granules and plasma membrane [101]. At least four distinct classes of intracellular granules have been characterized within neutrophils, containing microbicidal peptides, proteins, and enzymes such as elastase, proteinases and myeloperoxidase [108]. These enzymes are released into phagocytic vacuoles or into the extracellular environment, depending upon the stimulus. Concurrently, neutrophil membrane reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is activated. The activated NADPH oxidase converts oxygen to the superoxide anion ($O_2^{-\bullet}$), a process known as the respiratory burst. The majority of $O_2^{-\bullet}$ then dismutates to hydrogen peroxide (H_2O_2). In addition to residing on the surface of the neutrophil, NADPH oxidase is assembled intracellularly in stimulated neutrophils [109]. Hypochlorous acid is formed when myeloperoxidase oxidases chlorine in the presence of H_2O_2. In addition to the direct toxic effects of $O_2^{-\bullet}$, proteolytic enzymes and hypochlorous acid, neutrophil endothelial cell injury may also occur through combination of H_2O_2 with reduced iron within the endothelial cell, forming the highly reactive and toxic hydroxyl radical [110]. Reactive nitrogen species, including nitric oxide, act independently and synergistically with reactive oxygen species to augment neutrophil delivery, and form secondary cytotoxic species [98]. Thus, neutrophil microbicidal activity is mediated by a synergistic combination of membrane respiratory burst and intracellular granules.

Neutrophil-mediated tissue injury is dependent upon a balance of competing protective and destructive pathways. To protect the host against the damaging products generated by neutrophils, there exist antioxidants and powerful protease inhibitors within the extracellular matrix, such as α_1-protease inhibitor, α_2-macroglobulin, and secretory leukoproteinase inhibitor [111]. To counteract the neutralizing effect of the protease inhibitors, hypochlorous acid will inactivate the antiproteases in the immediate vicinity of the neutrophil [101]. Neutrophils also contain an endogenous supply of antioxidants, protecting themselves and the surrounding tissue. Also contributing to the balance of inflammation, the rate of clearance of neutrophils through apoptosis correlates with degree and resolution of inflammation, and is discussed below in greater depth. The balance of inflammatory and anti-inflammatory mediators is coupled with the neutrophil's paradoxic roles. An inflammatory response associated with severe sepsis may be harmful, whereas the inflammatory response is necessary to clear infection, as demonstrated in an elegant murine cecal ligation and puncture model utilizing variable caliber of puncture. Inflammatory responses may be localized or systemic, and interventions that yield a reduction in neutrophil-mediated inflammatory injury in one organ may predispose to infection at other sites. Genetic factors are clearly involved in determining host response to physiologic insult, and have only recently been subjected to active investigation. Improved understanding of these factors are essential if we are to understand better how to intervene effectively in patients with overwhelming persistent inflammation.

Neutrophil clearance from the inflammatory microenvironment: apoptosis and necrosis

Apoptosis is the principal means by which physiologic cell death occurs (Fig. 2), although abnormal apoptosis is associated with various pathologic illness states. It is a highly orchestrated, much studied form of cell death in which cells commit suicide by cleaving their DNA into relatively uniform short segments, dividing the cell into membrane-packaged parcels of intracellular contents (including intact organelles) that are then phagocytosed by surrounding cells. Physiologic cell death is crucial to the varied functions of multicellular organisms, including normal tissue development, homeostasis, and neural and immune system development [112]. Because illness may reflect an altered balance between cell

Figure 2



Neutrophil apoptosis pathways. Note that Fas, FADD, and FLICE are also known as APO-1, MORT-1, and MACH, respectively. FADD, Fas-associated death domain; FasL, Fas ligand; FLICE, FADD-like IL-1β converting enzyme (ICE); IAP, inhibitor of apoptosis protein; NF-κB, nuclear factor-κB; TNFR, tumor necrosis factor receptor; TRADD, TNFR-associated protein death domain; TRAF, TNFR-associated factor.

proliferation and cell death, too little or too much apoptosis has been implicated in human diseases such as Alzheimer's disease and cancer [113].

Apoptosis, a term introduced by Kerr in 1972 [114], denotes a form of cell death under genetic control that results in removal of a cell with no inflammatory reaction. A cell undergoing apoptosis will shrink. Its nucleus will undergo karyorrhexis (fragmentation) and karyolysis (dissolution), its DNA undergoes specific internucleosomal cleavage (resulting in DNA segments of approximately 185 base pairs in length), and the cell will ultimately break up into apoptotic bodies containing pyknotic nuclear debris [115]. Surrounding cells, even those that are not 'professional phagocytes' such as epithelial cells, will phagocytose the apoptotic bodies. The phagocytosis of apoptotic bodies containing intact cellular organelles allows for efficient recycling of valuable intracellular contents, without causing an inflammatory response.

The lack of inflammation associated with apoptosis is crucial to the distinction between apoptosis and other forms of cell death. For example, ischemic cell death (termed oncosis) is characterized by cellular swelling, organelle swelling, blebbing and increased membrane permeability, and nonspecific DNA breakup, which will evolve to cell membrane dissolution, or necrosis [115]. Particularly important to the neutrophil, oncosis and necrosis involve the spillage of intracellular contents into the extracellular environment, with resultant inflammation. The lack of inflammation associated with neutrophil clearance through apoptosis has led to intensive investigation regarding the regulation of neutrophil apoptosis. Here we focus on the role of neutrophil membrane expression in the process of apoptosis. First, alteration in receptor expression occurs during the process of apoptosis, providing a means to detect apoptosis; second, the neutrophil membrane mediates the activation of apoptosis through death receptors.

Alterations in cell membrane expression in apoptotic cells may be used to detect apoptosis in the laboratory. It was noted that phagocytosis is inhibited by phosphatidylserine, regardless of species (human or murine) or type of apoptotic cell (lymphocyte or neutrophil) [116]. Phosphatidylserine normally resides on the inner membrane leaflet, but is expressed on the outer membrane as an early feature of apoptosis [117] and is implicated in macrophage recognition of apoptotic cells [118]. Flow cytometry analysis using a fluorescent-labeled molecule (annexin V) that specifically binds to phosphatidylserine facilitates the quantification of cells that express phosphatidylserine and thus are undergoing apoptosis [119,120]. The phosphatidylserine-binding technique detects early apoptosis, and provides clear differentiation between necrotic and apoptotic cells.

Death receptors

In addition to genetically controlled, pre-programmed apoptosis, cells may be instructed to undergo apoptosis by the binding of neutrophil membrane death receptors, which transmit signals initiated by the binding of a death ligand [121]. Death receptors are part of the TNF receptor gene superfamily, and contain a cytoplasmic sequence that has been named the 'death domain' – a sequence of approximately 80 base pairs near the carboxyl-terminus that is located within the intracellular region of the receptor and mediates its cytotoxicity [122,123]. The best characterized and presumably most important death receptors are Fas (CD95) and TNF receptor I (the p55 or 55 kDa TNF receptor) [123,124]. Neutrophils express both of these receptors, which may be activated by their ligands to induce rapid cell death. Other more recently discovered death receptors include death receptor-3, -4, and -5; these receptors are not expressed on neutrophils, have not yet been investigated with respect to neutrophil apoptosis, or are not recognized as significant to neutrophil homeostasis [121]. Following activation of a death receptor, a receptor-specific complex cascade of intracellular events results in apoptosis.

Fas

When Fas ligand (FasL) interacts with Fas (a death receptor), the cell expressing the Fas will undergo rapid apoptosis [125,126]. The Fas–FasL apoptotic pathway has been demonstrated to play important roles in immune system

development and function, including the regulation of T-cell development and apoptosis, and killing of inflammatory cells at 'immune-privileged' sites [127–130]. Fas and FasL are of crucial importance to initiation of apoptosis in human neutrophils. Anti-Fas antibodies accelerate neutrophil apoptosis to a greater degree than do lymphocytes and monocytes [131]. FasL exists either in soluble form or as a cell surface molecule, forming part of the TNF family. FasL can bind three Fas molecules simultaneously, causing clustering of the death domains and leading to binding of specific intracellular proteins. Fas-associated death domain (FADD) and FADD-like IL-1β converting enzyme bind to the death domain, and activate a family of specific cysteine proteases called 'caspases' [121]. Caspases represent the machinery of cell death: they inactivate proteins that protect against apoptosis; they disable and deregulate proteins in general; and they participate in direct disassembly of cell structures, including the reorganization of the cytoskeleton and disruption of the nucleus [132].

Tumor necrosis factor receptor I

TNF-α dramatically increases apoptosis rates in circulating neutrophils of healthy human controls [133,134]. Similar to FasL, three TNF-α molecules can trimerize on TNF receptor I, leading to clustering of death domains and to binding by TNF receptor associated death domain. Two distinct and independent signaling pathways then proceed [135]: activation of nuclear factor-κB (NF-κB); and activation of the caspase pathway, leading to apoptosis (mediated through FADD, similar to the Fas pathway) [135]. NF-κB regulates a wide variety of genes that are involved in the synthesis of hematopoietic growth factors, chemokines, and leukocyte adhesion molecules [136–138]. Recent evidence also implicates NF-κB activation as an important survival mechanism in granulocytes. It has been shown to downregulate TNF-mediated apoptosis in a negative feedback mechanism [139–141]. The survival mechanism mediated by NF-κB explains why TNF-α may not trigger apoptosis unless protein synthesis is blocked. Given that activation of the death receptor TNF receptor I leads to competing pathways, TNF-α will have differential effects on neutrophil apoptosis, depending on the activation state of the neutrophil [142].

The signaling pathways initiated by both TNF and FasL may be 'modulated' by a variety of mediators in the inflammatory environment. Specifically, delayed apoptosis in states of persistent inflammation has been extensively investigated. Many inflammatory mediators cause a delay in constitutive neutrophil apoptosis, and include IL-2 [143], IL-6 [144], IL-8 [145], G-CSF [146], GM-CSF [147], C5a, and lipopolysaccharide [134,148]. In addition to constitutive apoptosis, inducible apoptosis mediated by the Fas pathway is suppressed by a variety of inflammatory mediators, including IL-8 [149], G-CSF, GM-CSF, interferon-γ, and TNF-α [150]. This delay in Fas-mediated apoptosis secondary to inflammatory cytokines may be diminished in elderly persons [151]. In addition, inflammatory mediators may alter intracellular factors within neutrophils in order to delay apoptosis; these factors include mitochondrial stability and caspases activity [152], in addition to NF-κB activation. Other agents in the inflammatory microenvironment that have been demonstrated to modulate neutrophil apoptosis include immune complexes [153], reactive oxygen intermediates [154], and red blood cells (possibly secondary to scavenging oxidants) [155]. In addition, engagement of neutrophil adhesion receptors will delay apoptosis [156]. Thus, through alterations that occur during and after neutrophil delivery to the exudate environment, numerous agents modulate the rate of constitutive and inducible neutrophil apoptosis.

Neutrophil cell surface expression in the exudate environment

Neutrophils display altered membrane expression and cell function following transmigration. Using monoclonal antibodies directed toward surface molecules, characterization of the neutrophil cell surface reveals significant and consistent alteration in exudate neutrophil membrane expression. Our laboratory has previously demonstrated that exudate polymorphonuclear neutrophils have enhanced microbicidal activity, superoxide production, and augmented expression of CD16 and the FMLP receptor, and are refractory to further stimulation with TNF [157]. Multiple studies have confirmed that human exudate neutrophils collected in skin windows are primed for enhanced metabolic activation and phagocytic activity [158–161]. In addition to altered function within the inflammatory environment, exudate neutrophils demonstrate altered membrane expression, including receptors that mediate adhesion, chemotaxis, and function.

Adhesion receptors are altered after transmigration. Our laboratory and others have found increased expression of CD11b, decreased L-selectin, and decreased PECAM-1 expression in exudate neutrophils following transmigration [57,157,162,163]. The loss of PECAM-1 is particularly interesting because it mediates adhesion to endothelial cell corners and is necessary for diapedesis (see discussion above) [56,164]. The alteration in adhesion molecule expression may allow the neutrophil to complete the process of diapedesis, and undergoes chemotaxis to a site of inflammation or infection.

Evidence suggests that change in the membrane expression in exudate neutrophils is closely tied to the mobilization of secretory vesicles. Exudate neutrophils collected in skin windows displayed increased surface expression of alkaline phosphatase, complement receptor 1, and CD11b/CD18, but a complete loss of L-selectin following transmigration, and the increase in the content of surface molecules in the plasma membrane correlated with complete mobilization of secretory vesicles [165]. Loss of specific granules also correlated with increased number of FMLP receptors in exudate neutrophils [161]. Thus, the changes to membrane expression are intrinsic to the change in neutrophil function.

Exudate neutrophils exhibit a reduced number of chemoattractant receptors, along with reduced chemotaxis. In animal models, exudate neutrophils demonstrate reduced chemotactic response [166]. In humans, neutrophils isolated from skin windows have diminished chemotactic ability when compared with circulating neutrophils [159]. Exudate neutrophils from pustules in a single patient exhibited markedly reduced chemotaxis to C5a, FMLP, leukotriene-B_4, and IL-8 when compared with circulating neutrophils [167]. Bronchoalveolar lavage neutrophils in patients with chronic respiratory tract infections have reduced IL-8 receptor A and IL-8 receptor B when compared with circulating neutrophils [168]. In addition to demonstrating alterations in chemoattractant receptor expression, dynamic and variable alterations in neutrophil chemoattractant receptors in vivo correlate with changes in cell function (chemotaxis). We have shown that compared to control circulating neutrophils, exudate neutrophils (i.e. neutrophils that have undergone transmigration to the extravascular inflammatory environment from healthy subjects) simultaneously exhibit increased C5a receptors, increased C5a chemotaxis, reduced IL-8 receptors (both IL-8 receptor A and B), and reduced IL-8 chemotaxis. In a separate but related experiment again comparing to control circulating neutrophils, circulating neutrophils isolated from septic patients (APACHE II 23.6 ± 7.8) displayed reduced C5a receptors, reduced C5a chemotaxis, a lesser decrease in IL-8 receptors with no change in IL-8 chemotaxis. These observations of *in vivo* receptor alteration and cell function suggest both specific and generalizable conclusions, including: (1) diminished chemoattractant receptors and chemotaxis in septic neutrophils may account for decreased neutrophil delivery to peripheral sites observed in these patients [169]; (2) exudate neutrophil chemotaxis may depend more on C5a than on IL-8; and (3) change in neutrophil chemoattractant receptor expression appears to regulate neutrophil chemotaxis *in vivo*.

Exudate neutrophils have delayed rates of physiologic cell death, or apoptosis. In humans, exudate neutrophils have decreased surface expression of FcγRIII [157] and FcγRIII is known to be decreased in apoptosis [170,171]. In an animal model, pulmonary exudate neutrophils exhibited delayed constitutive and induced apoptosis [172]. In humans, using a sterile skin blister skin window technique, we found that exudate neutrophils had delayed apoptosis, a reduction in TNF-α membrane binding, and a decreased susceptibility to TNF-α-induced apoptosis [173]. Human salivary neutrophils do not respond to a combination of TNF-α and cycloheximide, unlike circulating neutrophils [174]. Thus, exudate neutrophils have delayed apoptosis, and will be less responsive to certain apoptotic stimuli such as TNF.

In summary, following delivery to the inflammatory environment, neutrophils are primed for enhanced bactericidal activity, have altered expression of chemoattractant receptors and chemotaxis, and are refractory to cell death. These mechanisms have presumably developed in order to facilitate neutrophil effector function in host defense. Participating in and being altered by the multiple sequential steps that are involved in the neutrophil's path from the circulation to the inflammatory environment, the neutrophil membrane is changed into a new configuration, reflecting the fact that the function of the neutrophil (its overall properties) have changed also.

Neutrophil connectivity

The cell membrane of the neutrophil is the principal means by which the neutrophil interacts and communicates with its environment, and it is not surprising that the membrane mediates the processes that are inherent to the neutrophil's lifecycle. As a complementary interpretation of this observation, the neutrophil membrane offers a measure of neutrophil 'connectivity', that is, the degree and nature of its interconnectedness with other elements within the host response. This concept becomes essential when evaluating a complex nonlinear system, such the systemic host response to trauma, shock, or sepsis [175].

A complex system may be thought of as one that is able to exist in stable states, with systemic properties and functions that are wholly distinct from the innumerable, interconnected, interdependent parts of the system, which are continuously engaged in a dynamic web of nonlinear relationships. The systemic host response, with its interdependent metabolic, neural, endocrine, immune, and inflammatory systems, may be regarded as a complex system [175]. In addition, the neutrophil itself may be regarded as a complex system in its own right, with its own systemic or 'emergent' properties. Emergent properties of the neutrophil might include adhesiveness, chemotactic ability, activation state, and rate of cell death, all of which represent a measure of cell function. We previously observed that changes in variability (patterns of change over time) and connectivity (patterns of interconnection over space) of the elements of a complex system may be utilized as a measure of changes in the systemic properties of that complex system, which define whether a patient is healthy or ill [175]. The demonstration that altered connectivity (i.e. neutrophil membrane expression) is associated with altered emergent properties (i.e. function) of the neutrophil represents a demonstration of this hypothesis on a smaller scale. These observations suggest further hypotheses for investigation. For example, utilizing dynamic measurement of neutrophil membrane expression as a technology to analyze neutrophil function, it may be possible to identify which patients might benefit from attempted immunomodulation, and when an intervention should be performed.

Conclusion

As the foremost circulating phagocyte, which is essential to normal effective host defense and is responsible for host tissue injury in states of persistent inflammation, the neutrophil has undergone extensive investigation. The present review arrived

at two principal conclusions: the neutrophil membrane mediates the processes that are integral to neutrophil delivery, function, and clearance; and alterations in membrane expression occur with changes in cell function. The neutrophil membrane mediates neutrophil delivery, including neutrophil–endothelial cell interactions, rolling, adhesion, and diapedesis. During this process, and in the interstitial inflammatory environment, the neutrophil responds to various chemoattractants, based on the presence and binding capacity of the appropriate receptors. In the inflammatory environment, neutrophil membrane receptors participate in phagocytosis, priming, and activation, leading to release of a toxic arsenal of granules and activation of the membrane-bound respiratory burst. Following completion of its function, the neutrophil is cleared via physiologic cell death or apoptosis, a process that is activated by membrane-bound death receptors. In summary, because the neutrophil membrane is the principal means by which the cell interacts with its surroundings, it is the principle mediator of neutrophil development during the neutrophil lifecycle. The second principal conclusion that may be derived from the evaluation of neutrophil membrane expression and function is that alterations in the neutrophil membrane are synonymous with alterations in cell function, and observation whose clinical significance merits exploration. Thus, in addition to the neutrophil membrane mediating cell processes during the neutrophil lifecycle, changes in membrane expression allows for *in vivo* regulation of cellular function.

Competing interests

None declared.

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