Neutrophils in development of multiple organ failure in sepsis

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Multiple organ failure is a major threat to the survival of patients with sepsis and systemic inflammation. In the UK and in the USA, mortality rates are currently comparable with and projected to exceed those from myocardial infarction. The immune system combats microbial infections but, in severe sepsis, its untoward activity seems to contribute to organ dysfunction. In this Review we propose that an inappropriate activation and positioning of neutrophils within the microvasculature contributes to the pathological manifestations of multiple organ failure. We further suggest that targeting neutrophils and their interactions with blood vessel walls could be a worthwhile therapeutic strategy for sepsis.

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Sepsis is defined as the systemic inflammatory response to infection, with its severe form associated with evidence of organ dysfunction—ie, tissue hypoperfusion and hypoxia, lactic acidosis, oliguria, or altered cerebral function.[1,2] Despite prompt treatment with antibiotics, provision of adequate fluid resuscitation, and technological support of organ function, the mortality rate is approximately 35%.[3] Most infections are due to bacteria[4] and the majority of patients have lung dysfunction associated with cardiovascular instability and deteriorating renal function.[5] Several factors are implicated in the pathogenesis of organ failure, such as the endocrine[6] and immune systems;[7] disseminated intravascular coagulation;[8] genetic susceptibility;[9] and a derangement of energy metabolism, possibly in mitochondria.[10]

In sepsis, the initiating stimuli of systemic inflammation are often bacterial components, which induce the secretion of pro-inflammatory cytokines such as interleukin 1β, interleukin 6, and tumour necrosis factor α (TNFα) predominantly from cells of the immune system. High circulating concentrations of these cytokines sometimes indicate an increased risk of mortality[11] but treatments antagonising their activities have not improved patients' survival.[12] The initial cytokinaemia is accompanied by a compensatory response of raised concentrations of circulating anti-inflammatory cytokines (eg, interleukin 10), which are associated with poor patients' outcome and a downregulated immune response (immunoparalysis).[13] This latter proposal stems from findings of impaired lymphocyte responsiveness and of decreased numbers of lymphocytes in the circulation and tissue of patients with sepsis.[14]

Neutrophils have a pivotal role in the defence against bacterial infections, as shown by neutropenia (eg, after chemotherapy), which increases susceptibility to infection and to sepsis. However, overwhelming activation of neutrophils is known to elicit tissue damage. Here, we review the evidence, especially from investigative studies of patients rather than large-scale clinical intervention, which implicates aberrant neutrophil activity with the organ failure of severe sepsis. We postulate that organ dysfunction arises from neutrophils activated by an excessive and inappropriate response to an infectious stimulus and to the inflammatory milieu generated. We also address whether modification of neutrophil function could lead to therapeutic benefit in patients with sepsis.

Neutrophils and organ failure

Neutrophils are ideally suited to the elimination of pathogenic bacteria because of their large stores of proteolytic enzymes and rapid production of reactive oxygen species to degrade internalised pathogens.[15] If these lytic factors[16] or pro-inflammatory cytokines[17] are released extracellularly from tissue-infiltrating neutrophils, local damage will ensue.[18–21] Indeed, neutrophil-induced tissue injury occurs at sites of localised bacterial infection, which, in its extreme form, leads to abscess formation, although any generalised tissue infiltration or organ damage in this situation is rare. By contrast, in severe sepsis, local infection is accompanied by systemic neutrophil activation.

Examination of autopsy specimens from patients with multiple organ failure reveals localisation of neutrophils that varies from sequestration and aggregation in renal blood vessels[22–24] to large-scale tissue infiltration of the lung.[24] In the acute respiratory distress syndrome (ARDS), a more severe form of acute lung injury that could accompany sepsis,[25] the intensity of neutrophil infiltrates correlates with impaired lung function and with high concentrations of neutrophil-derived proteolytic enzymes in the bronchoalveolar lavage.[26]

Organ failure is not always associated with gross morphological changes. One study showed little concordance between cell death in an organ and its dysfunction,[27] an

Search strategy and selection criteria

We searched the library of the Royal Society of Medicine, UK (1960–2005) and MEDLINE (1960–2005). We used the search terms "neutrophils", "sepsis", and "organ failure". We largely selected publications in the past 10 years, but did not exclude commonly referenced and highly regarded older publications. We also searched the reference lists of articles identified by this search strategy and selected those we judged relevant. Review articles and book chapters are cited to provide more details and references.

***Panel*: Diagnostic criteria for sepsis in adult patients**[1]

Infection (recorded or suspected), and some of the following variables:

General
Fever (core temperature >38·3°C)
Hypothermia (core temperature <36°C)
Heart rate >90 beats per min or >2 SD above typical value for age
Tachypnoea
Altered mental status
Clinically significant oedema or positive fluid balance (>20 mL/kg per day)
Hyperglycaemia (plasma glucose 7·7 mmol/L) in the absence of diabetes

Inflammatory
Leucocytosis (>12×10⁹ white blood cells per L)
Leucopenia (<4×10⁹ white blood cells per L)
Normal white-blood-cell count with >10% immature forms
Plasma C-reactive protein >2 SD above typical value for age
Plasma procalcitonin >2 SD above typical value for age

Haemodynamic
Arterial hypotension (SBP <90 mm Hg, MAP <70, or SBP reduction >40 mm Hg in adults or <2 SD below typical value for age)
SvO_2 >70%
Cardiac index >3·5 L/min per mol

Organ dysfunction
Arterial hypoxaemia (PaO_2/F_iO_2<300)
Acute oliguria (urine output <0·5 mL/kg per h or 45 mmol/L for at least 2 h)
Creatinine increase >442 μmol/L
Coagulation abnormalities (INR >1·5 or aPTT >60 s)
Ileus (absent bowel sounds)
Thrombocytopenia (platelet count <100×10⁹ per L)
Hyperbilirubinaemia (plasma total bilirubin ≥70 mmol/L)

Tissue perfusion
Hyperlactataemia (>1 mmol/L)
Decreased capillary refill or mottling

SBP=systolic blood pressure. MAP=mean arterial blood pressure. SVO_2=mixed venous oxygen saturation. INR=international normalised ratio. aPPT=activated partial thromboplastin time. Information reproduced with permission from Levy, et al. SSCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* 2003; **31:** 1250–56 (table 1). Copyright 2003, Society of Critical Care Medicine.

observation that led to the proposal that cell hibernation was a protective mechanism against an overwhelming systemic inflammation.[6] This hypothesis does not preclude the possibility that neutrophil-mediated tissue injury occurs in the microvasculature. Direct visualisation of the vascular bed is provided by orthogonal polarisation spectral imaging, which monitors in-vivo interactions between neutrophils and endothelial cells. However, most studies so far have been undertaken with animals, and the technique's application to patients with sepsis is currently limited by its insufficient resolution and confinement to measurements of the sublingual and cutaneous microvasculature rather than of organ failure in sepsis.[28,29]

During systemic inflammation, homoeostatic mechanisms are compromised in the microcirculation including: endothelial hyperactivity, fibrin deposition, vascular occlusion, and sometimes development of tissue exudates that further impede adequate oxygenation. Neutrophils are purported to participate in these rheological changes through their augmented binding to blood vessel walls and through the formation of leucocytic aggregates.[10,30] Further evidence showing that neutrophil activity might be causal comes from our finding that the removal of blood neutrophils from patients with systemic inflammation by leucodepletion filters enhances respiratory and renal function.[31] Additionally, neutrophils retained by the filters are predisposed to endothelial binding,[32] implying an association between neutrophil interaction with blood vessel walls and organ dysfunction. Findings in animal models of sepsis concur with these clinical observations. Large numbers of neutrophils accumulate in organs undergoing failure, and insult of one organ could trigger the widespread recruitment and sequestration of neutrophils in others with subsequent multiple organ dysfunction.[33,34] Experimental interventions that deplete or antagonise the activity of neutrophils ameliorate organ dysfunction.[35,36] Suppression of neutrophil function in sepsis could alleviate organ failure, but conventional wisdom suggests that maintenance or even enhancement of neutrophil activity is integral to the elimination of initiating pathogens. This dilemma is heightened by conflicting reports concerning the functional status of neutrophils in sepsis and by variations in their circulating numbers.

Abnormal numbers of blood neutrophils

Neutrophils have a 14-day development in the bone marrow and stay temporarily in a storage pool before release into the blood, where they spend 12–14 h in transit from a circulating pool (axial stream) into a marginating pool (contact with blood vessel walls). Thereafter, in the absence of any bacterial infections, neutrophils enter reticuloendothelial organs, such as the liver,[37] or even return to the bone marrow[38] to undergo apoptosis (programmed cell death).[39] The ageing neutrophils shrink into apoptotic bodies, which culminates in their phagocytosis by local macrophages, thereby preventing the onset of tissue damage by lytic factors released from these senescent cells.

The criteria for sepsis include a neutrophil count that is high, low, or contains more than 10% of immature cells (panel).[1] High numbers of blood neutrophils could be due to excessive recruitment from the bone marrow, the return of marginated cells into the circulatory pool, or both.[40,41] At present, no therapeutic strategy has aimed at reducing the raised numbers of neutrophils in sepsis, presumably because the presence of too many circulating neutrophils during bacterial infection is assumed to be better than too few.

Colony stimulating factors

Cytokines that possess the potential to release neutrophils from the bone marrow include granulocyte-colony

stimulating factor (G-CSF) and granulocyte-macrophage (GM)-CSF. Both augment the number of circulating neutrophils, promote their maturation and activation,[42] and extend neutrophil lifespan.[43] In healthy individuals, G-CSF blood concentrations are very low, unlike during the acute stage of infection, when a several-fold increase and consequent rise in neutrophil numbers takes place.[44] During sepsis, circulating concentrations of G-CSF are often increased, and peak concentrations precede a rise in neutrophil numbers[45] and an augmentation of the respiratory burst activity of neutrophils.[46]

An association between neutropenia and an especially poor prognosis in sepsis has led to attempts to increase the number and maturity of neutrophils by the use of G-CSF or GM-CSF.[47] Restoration of typical neutrophil numbers by recombinant G-CSF is particularly effective in patients with sepsis who have low percentages of immature neutrophils and low concentrations of endogenous G-CSF.[48] However, interest in the therapeutic application of G-CSF in sepsis is tempered by contradictory findings from its use in animal models. Initial reports showing that G-CSF treatment boosted the number of neutrophils[49] and enhanced survival[50] were questioned by studies in which G-CSF treatment reduced the number of blood neutrophils, increased tissue injury and mortality,[51] and exacerbated the augmented lung injury associated with neutropenia recovery.[52] Two multicentre trials showed that recombinant G-CSF did not reduce mortality in patients with sepsis.[53,54]

Leucopenia and organ failure

The finding that neutrophil-mediated lung injury occurs in patients with neutropenia shows that organ dysfunction might be initiated by only a few neutrophils sequestered in the microvasculature.[55,56] Accordingly, perhaps of more pathogenetic importance to severe sepsis is not the total number of neutrophils in the circulation, but the presence of a cellular subset whose phenotype and level of activation favours induction of tissue damage.[57] In animal models of sepsis, immature neutrophils preferentially accumulate in pulmonary microvessels in which their activation induces substantial tissue damage through the release of defensins, which are proteolytic enzymes.[58,59]

Thus, despite abnormal numbers of blood neutrophils being used to diagnose systemic inflammation, the contribution of neutrophils to the pathology of sepsis could emerge from defining populations of neutrophils, such as those that are predisposed to endothelial interaction. A relevant suggestion is that neutrophils are not functionally homogeneous, but consist of subpopulations with distinct phenotypic and secretory profiles.[60]

Tissue extravasation

Bacterial elimination is dependent on the rapid recruitment of blood neutrophils into sites of infection. The neutrophils must first adhere to blood vessel walls before actively migrating into the surrounding tissue in response to chemical stimuli (chemotaxis). Here, we discuss all these stages with respect to the behaviour of neutrophils in sepsis.

Adherence to blood vessel walls

Binding of neutrophils to the vascular endothelium is controlled by the sequential activities of two families of adhesion molecules; the selectins and integrins. The selectins promote the initial rolling or tethering of the neutrophils to the endothelium under the shear force of blood flow, whereas the integrins induce firm adhesion.[61] There are two subfamilies of integrins (β1 and β2). The integrins are heterodimers with an α chain and a common β chain, which defines the respective family. Many adhesion molecules have interchangeable terms (table 1).

On the neutrophil surface, L-selectin (CD62L) interacts with specific oligosaccharide moieties on endothelial-cell surface glycoproteins, whereas on the endothelium, E-selectin (CD62E) and P-selectin (CD62P) similarly recognise specific neutrophil carbohydrate motifs. Transient rolling helps neutrophils to make contact with inflammatory factors, such as interleukin 8 and platelet-activating factor that are expressed on the endothelial surface (figure 1). This interaction stimulates the neutrophils to rapidly upregulate and increase the avidity of the β2 integrins (CD11a and CD11b) for the endothelial ligand, ICAM-1 (intercellular adhesion molecule 1, CD54). The endothelial expression of CD54 itself is enhanced by factors (eg, TNFα, interleukin 1,

	Alternative name	Type of cell expressed on
Selectins		
CD62E	E-selectin	Endothelium
CD62L	L-selectin	Neutrophils
CD62P	P-selectin	Endothelium
β1 integrins		
α chain		
CD49d	VLA-4 (very late antigen 4)	Small proportion of neutrophils in sepsis
Common β chain		
CD29	..	..
β2 integrins		
α chain		
CD11a	LFA-1 (leucocyte function antigen-1)	Neutrophils
CD11b	Mac-1 (macrophage-1)	Neutrophils
Common β chain		
CD18	..	..
Ligand for β1 integrins		
CD106	VCAM-1 (vascular cell adhesion molecule-1)	Endothelium; induced by pro-inflammatory cytokines
Ligand for β2 integrins		
CD54	ICAM-1 (intercellular adhesion molecule-1)	Endothelium; upregulated by pro-inflammatory cytokines

CD number=cluster of differentiation. Every CD number describes a cell surface protein that might have a distinct function or acts as a cell marker (or both).

Table 1: **Adhesion molecules relevant to neutrophil-endothelial cell interaction**

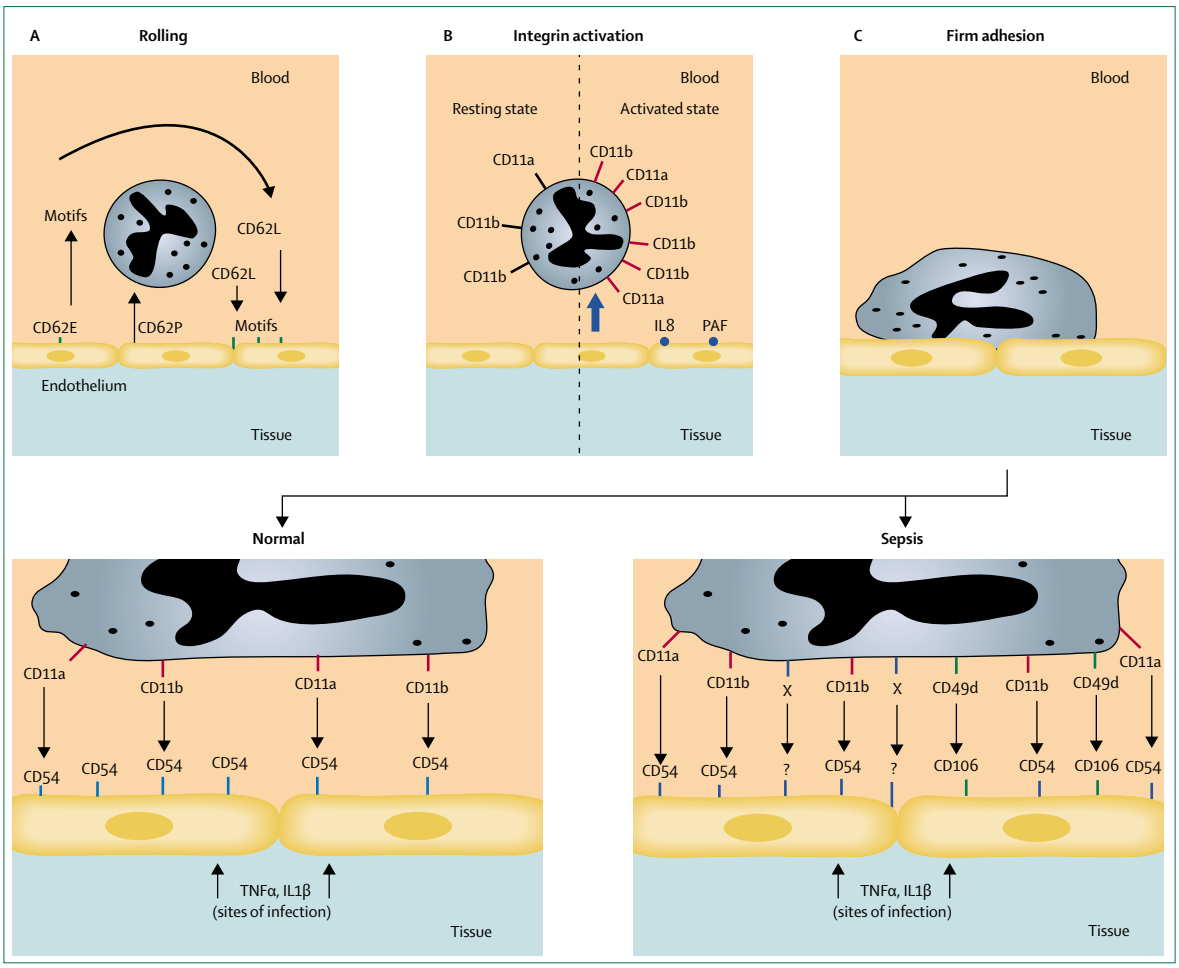

Figure 1: **Attachment of neutrophils to endothelium at sites of infection**
The binding of circulating neutrophils to postcapillary venules that are adjacent to sites of infection depends on three consecutively related events: rolling of the neutrophils on the endothelium, activation of the neutrophils by inflammatory stimuli expressed on the endothelial surface, and firm adhesion. (A) Selectins on the neutrophils (L-selectin) and on the endothelial cells (E-selectin and P-selectin) recognise complementary carbohydrate motifs, which induce the rolling or tethering of neutrophils. (B) Tethering allows interaction with inflammatory molecules (platelet-activating factor and interleukin 8), whose expression on the endothelium arises from the underlying infection. Activation of the neutrophils by these inflammatory molecules increases the surface expression and avidity of the β2 integrins, CD11a and CD11b, which promotes firm adhesion to endothelium. (C) For healthy neutrophils, firm adhesion is promoted by CD11a and CD11b interacting with CD54, which is upregulated by locally generated pro-inflammatory cytokines (eg, TNFα and interleukin 1). For neutrophils from patients with sepsis, similar molecular interactions, but of an increased intensity, also take place. Binding is further augmented by CD49d recognising CD106, whose expression is also induced by TNFα and interleukin 1, and probably by the expression of additional determinants (X), which await identification. PAF=platelet-activating factor. IL=interleukin.

lipopolysaccharide) released from sites of bacterial infection. Both L-selectin and CD11b have shown abnormal distributions on neutrophils from patients with sepsis. A reduced expression of L-selectin could be due to autoproteolysis induced by circulating inflammatory stimuli, or following transient contact with blood vessel walls.[62,63] The expression of CD11b on neutrophils in sepsis has been reported to be increased,[63–69] decreased[70,71] and indicative of a poor prognosis,[67] and normal (table 2).[62,72] Since a downregulation of L-selectin and upregulation of CD11b is the phenotype of activated neutrophils, in sepsis, these cells might already be committed to undergo firm contact with endothelium (figure 1). However, no reports so far have shown that the blocking of the expression of L-selectin or β2 integrins on neutrophils from patients with sepsis alters their binding to endothelial cells. From work in our laboratory, which finds that anti-CD11b antibodies are not very effective at inhibiting the interaction of neutrophils from patients with sepsis to endothelial monolayers, it seems that other surface determinants could also contribute to the supranormal adhesiveness of neutrophils in sepsis.[32]

The β1 integrins are mainly associated with lymphocytes and monocytes,[61] but one member, VLA-4 (CD49d), was recently identified on approximately 30% of neutrophils from patients with sepsis.[73] The VLA-4 molecule binds fibronectin and CD106 and, therefore, neutrophils with both β1 and β2 integrins have the potential to adhere to several vascular ligands (figure 1). The endothelial expression of VCAM-1 is induced by the activity of pro-

inflammatory cytokines (eg, TNFα, interleukin 1). In the acute lung injury model of sepsis, the sequestration and infiltration of neutrophils involves β1 and β2 integrin-dependent pathways: the choice of integrin indicates the time-dependent expression of their respective vascular ligands and the nature of the bacterial stimulus.[88,89] By expressing β1 and β2 integrins on their surface, neutrophils from patients with sepsis seem ideally suited to undergo tissue extravasation. Contrary to expectations, these events do not always seem to be the case.

Chemotaxis

Blood neutrophils respond to chemotactic factors released at the source of infection, such as the complement peptide C5a, leukotriene B_4, platelet-activating factor, the bacterial peptide formyl-methionyl-leucyl-phenylalanine (fMLP), and interleukin 8. The cells migrate from an area of low concentration (ie, blood vessel walls) to an area of high concentration (site of infection or inflammation), whereupon the chemotactic factors become potent activators of neutrophils (figure 2).[90]

In patients with sepsis, movement of blood neutrophils into experimentally induced skin blisters is defective (figure 2).[62,80,81] The migration of isolated neutrophils in response to leukotriene B_4 is impeded, although whether the reported migration to fMLP is impaired is contested by other researchers (table 2).[78,79]

Interleukin 8 binds to the high-affinity receptors CXCR1 and CXCR2. CXCR2 is also a receptor for other chemokines. Neutrophils from patients with sepsis have a reduced expression of CXCR2 but not CXCR1[69,79] and a chemotactic responsiveness to interleukin 8 that might be normal[79] or impaired.[69] The pathological importance of these molecules has been shown in a mouse model of sepsis in which blockade of CXCR1 and CXCR2 signalling inhibited multiple organ failure and disseminated intravascular coagulation.[91]

In patients with acute respiratory distress syndrome, neutrophils undergo a large-scale migration into the lungs, and the concentration of interleukin 8 in bronchoalveolar lavage correlates with mortality.[92] In the systemic circulation, neutrophils enter tissue via postcapillary venules, but in the pulmonary circulation, emigration occurs via the capillaries.[93] The lumen of the pulmonary capillary is so narrow that the enforced neutrophil contact with the vessel walls extends neutrophil transit time and prevents rolling along the endothelium.[94,95] Lung sequestration of neutrophils is therefore more likely to be dependent on the contribution of the integrins rather than of the selectins, which is consistent with the finding that neutrophils from patients with sepsis have low expression of L-selectin,[62,63] and increased expression of CD11b[63–69] and CD49d.[73] Also, neutrophils might readily enter the lung as opposed to other organs because of the distinctive phenotype of the capillary endothelium and, as shown in animal models, because of the responsiveness of a subset of neutrophils to chemotactic factors or to microorganisms associated with pulmonary infection.[96]

	Effect relative to normal neutrophils	Reference
Adherence to cultured endothelium	Enhanced	32
Adhesion molecules		
Selectin—CD62L	Downregulated	62,63
β2 integrin—CD11b	Upregulated	64–69
	Downregulated	70,71
	Similar	62,72
β1 integrin—VLA-4/CD49d	Upregulated	73
Apoptosis	Extended	48,74,75–77
Chemotaxis	Decreased relative to fMLP, leukotriene B_4, and interleukin 8	69,78
	Similar to fMLP and interleukin 8	79
Chemotactic receptors		
CXCR1	Similar	
CXCR2	Downregulated	69,79
Cytotoxicity	Increased priming	77
Migration into experimentally induced lesions	Impaired	62,80,81
Phagocytosis	Enhanced	74,82,83
Phagocytosis receptors (CD64)	Increased	84
Priming of respiratory burst	Increased	74,85–87

Table 2: **Function of neutrophils and expression of related molecules in patients with sepsis and those at risk of organ failure**

Neutrophil priming, survival, and circulating factors

Neutrophils exist in three states: resting (unstimulated), primed (encounter with an inflammatory agonist or microbial-derived product that has lowered the threshold stimulus needed for activation), and activated (undertaking a defined function). The transition of neutrophils from a resting state in the circulation to an activated state at a site of infection is triggered by an ordered sequence of signals from priming stimuli—eg, C5a, lipopolysaccharide, and cytokines.[97–99] This effect benefits neutrophils at extravascular sites of infection, but vascular damage could occur if primed neutrophils already bound to endothelial cells were to encounter a second priming stimulus.[100] Circulating neutrophils from patients with sepsis are already primed, as shown by their increased oxidative activity and enhanced expression of the intracellular transcription gene, nuclear factor kB (NFκB).[74,85–87] Reduced activation of NFκB in neutrophils from patients with sepsis is associated with improved survival.[101]

Removal of neutrophils by apoptosis is a homoeostatic mechanism that prevents damage to healthy tissues that would otherwise occur after necrotic cell lysis. This process is central to the prevention and resolution of inflammation. Neutrophil apoptosis is inhibited in patients with systemic inflammation, systemic infections, severe sepsis, and those at risk of multiple organ failure.[45,74–77] The effect is generally agreed to be due to the activity of circulating factors that include lipopolysaccharide, lipoteichoic acid,

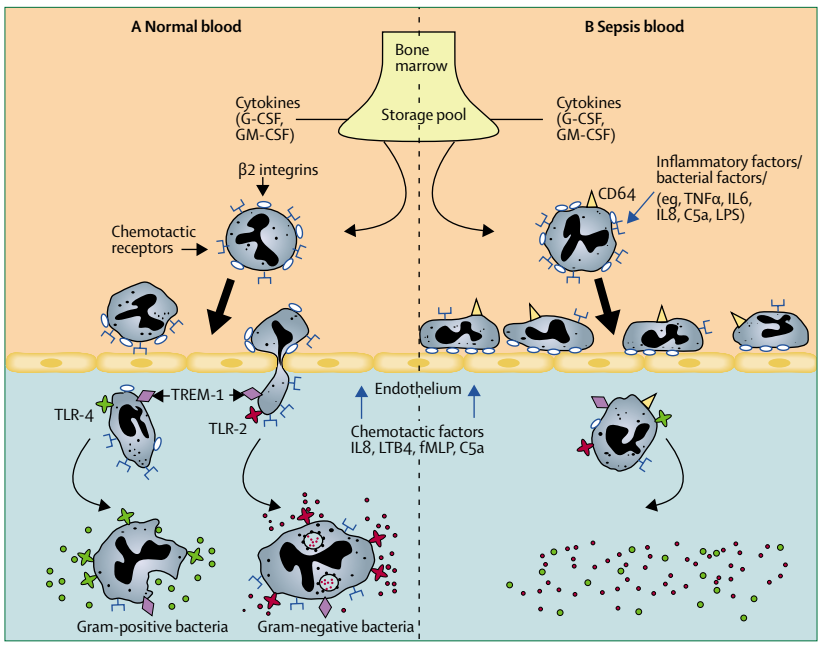

Figure 2: **Recruitment of neutrophils to bacterial infection in non-pulmonary tissue in (A) healthy individuals and (B) sepsis patients**

In response to bacterial infection, cytokines (eg, G-CSF/GM-CSF) are generated, which induce the release of neutrophils from the bone marrow. (A) In the normal state, large numbers of blood neutrophils enter sites of bacterial infection by first adhering to the activated endothelium of local postcapillary venules (PCV), before migrating along a concentration gradient of chemotactic factors (eg, C5a, fMLP, leukotriene B_4, interleukin 8) produced at the source of infection. Elimination of gram-positive bacteria is favoured by neutrophils expressing Toll-like receptor 2 (TLR2), whereas gram-negative bacteria are associated with neutrophils bearing TLR4. The expression of TREM-1 (triggering receptor expressed on myeloid cells) on neutrophils is common in all bacterial infections. Thereafter, bacteria are destroyed by phagocytosis. (B) In patients with sepsis, after stimulation of blood neutrophils by high concentrations of circulating inflammatory factors (eg, interleukin 1, TNFα, G-CSF, C5a, nitric oxide) or bacterial products (eg, lipopolysaccharide or lipoteichoic acid), surface integrins and CD64 (high-affinity Fc receptor that binds monomeric IgG) are upregulated to promote firm endothelial adhesion to postcapillary venules. However, some of these factors also downregulate the expression of chemotactic receptors. Consequently, many of the neutrophils bind very strongly to endothelium and are less responsive to underlying chemotactic factors than are healthy neutrophils. LPS=lipopolysaccharide. IL=interleukin. LTB4=leukotriene B_4. Red areas=gram-positive bacteria. Green=gram-negative bacteria.

and pro-inflammatory cytokines[45,102–105] although binding to endothelium that is activated by pro-inflammatory cytokines extends the lifespan of neutrophils by contrast with unstimulated endothelium, which exacerbates cell death.[106] The extended lifespan of tissue-infiltrating neutrophils increases the probability of these cells inducing extracellular damage through their uncontrolled release of oxygen radicals and proteolytic enzymes.

In sepsis, the survival of neutrophils in tissue could be further augmented by locally derived anti-apoptotic factors, as illustrated in the acute respiratory distress syndrome, in which the low level of apoptosis in lung alveolar neutrophils is related to the concentration of interleukin 2 in bronchoalveolar lavage fluids.[107] This inhibition of apoptosis takes place through the dysregulation of a complex network of intracellular signalling and of organelle function[108] that include an increase in tyrosine phosphorylation and a sustained mitochondrial transmembrane potential.[109,110] Extended neutrophil survival in patients with sepsis contrasts with the increased apoptosis of lymphocytes in the lymphoid tissue and subsequent immunoparalysis.[14]

Circulating factors might also be responsible for the reported phenotype and functional status of blood neutrophils in sepsis. Pretreatment of neutrophils with interleukin 8, the blood concentration of which is frequently increased in sepsis,[111] inhibits migration across endothelial monolayers[112] whereas the intravenous administration of interleukin 8 to rabbits prevents neutrophil emigration from mesenteric venules.[113] Similarly, patients with sepsis have raised blood concentrations of C5a, at concentrations that deactivate the chemotactic responsiveness of neutrophils in vitro.[97] High concentrations of TNFα in the blood of sepsis patients also impede neutrophil migration,[114] and incubation of normal neutrophils with TNFα inhibits apoptosis,[115] enhances the production of reactive oxygen species,[116] but suppresses CXCR2 expression.[117] These findings suggest that neutrophil dysfunction in severe sepsis is not a primary mechanism but a consequence of systemic activation. By extending the survival of neutrophils and impeding their migration across the vasculature, circulating factors have the potential to extend neutrophil-endothelial cell interactions and enhance vascular damage.

Recognition and phagocytosis of bacteria

Neutrophils from sepsis patients show enhanced internalisation and destruction of micro-organisms (table 2),[74,82,83] although opinion is divided as to whether phagocytosis is augmented or impeded at sites of infection in animals.[118,119] So far, most investigations of the phagocytic potential of neutrophils in patients with sepsis have focused on the distribution and expression of neutrophil receptors implicated in bacterial recognition and internalisation rather than the functional activities of the cells.

Neutrophil binding of bacteria is greatly augmented when the pathogens are coated with IgG. The high-affinity receptor for IgG is CD64, which is absent from resting neutrophils and is considered to be a marker of activated neutrophils. Its expression is induced by interferon γ and by GM-CSF.[120,121] Most neutrophils from patients with sepsis express CD64,[84] and an upregulation of CD64 on neonatal neutrophils is regarded as an indicator of sepsis.[122] An increased expression of CD64 is associated with augmented respiratory burst activity[46] and this molecule is present on most neutrophils that bind to cultured endothelium, an interaction that is impeded by anti-CD64 antibodies.[123] Binding to bacteria also occurs via CD14, the receptor for lipopolysaccharide that is present on all monocytes. This receptor is weakly expressed on neutrophils[124] but becomes upregulated in response to bacterial infections.[125] Other receptors that enhance phagocytosis and bacterial recognition include the C3b receptor, which binds complement peptide C3b; and CD16 and CD32, which like CD64 also bind the Fc sites (tail regions) of IgG. All of these receptors are adequately expressed on neutrophils from patients with sepsis.

The Toll-like receptors (TLRs), so called because they are homologues of the *Drosophila* protein Toll, are pattern recognition receptors that control innate immune responses to various microbial ligands. There are currently 11 forms. TLR4, which is closely associated with CD14, is a signal-transducing receptor for lipopolysaccharide whereas TLR2 preferentially recognises gram-positive bacteria.[126] Unstimulated neutrophils from healthy individuals have few of these two receptor types on their surface, although TLR2 is upregulated by G-CSF and GM-CSF.[127] Agonists of either receptor initiate a respiratory burst, release interleukin 8, shed L-selectin, and increase CD11b expression.[128,129] Both TLR2[130] and TLR4 agonists could directly delay neutrophil apoptosis, but indirect effects mediated via monocytes and macrophages could be more important for extended neutrophil survival.[129] Although activation of TLR2 and TLR4 downregulates neutrophil chemokine receptors, in particular CXCR2,[128,130] the cells still retain a limited migration to interleukin 8.[131] The interplay between signalling through TLRs and chemotaxis is complex, as shown by the finding in mice that TLR4 activation enhances the chemokine responsiveness of neutrophils, by downregulating the expression of G-protein receptor kinases, which participate in CXCR desensitisation.[132] The differential responsiveness of TLR2 or TLR4 might depend on the contribution of other surface receptors such as CD18 in response to gram-negative bacteria, and by a CD18-independent pathway in response to gram-positive bacteria.[133] Pharmacological modification of TLRs is proposed as a future therapeutic strategy in patients with septic shock.[134]

Another group of pattern-recognition receptors is the TREM (triggering receptor expressed on myeloid cells) family. Human tissue infected with bacteria is infiltrated by neutrophils with high amounts of TREM-1, and the blocking of TREM-1 expression in experimentally induced sepsis enhances survival.[135] Signalling through TREM-1 releases interleukin 8 and upregulates surface adhesion molecules.[136] The notion that TREM-1 could have substantial pathogenetic importance in sepsis is lent support by findings showing that increased TREM-1 expression on neutrophils is confined to acute inflammatory reactions, precipitated by infectious agents, and that a fall in the soluble form of TREM-1 in the plasma of patients with sepsis favours outcomes.[137]

Limitations to the understanding of neutrophils in sepsis

Elucidation of the functional status of neutrophils in patients with sepsis is hampered by insufficient studies in some areas (eg, phagocytosis) and conflicting data in others (eg, expression of adhesion molecules). The apparent inconsistencies probably relate to inadequate stratification of patients, variable drug intervention (eg, steroids), and differences in experimental design. A meaningful assessment of neutrophil behaviour in sepsis needs longitudinal studies of individual patients, since neutrophils have a short half-life in the circulation, and assessment at one timepoint provides little understanding of a disorder that persists for days or weeks. Aspects of the typical neutrophil lifecycle are still not fully understood. For example, little is known of the mechanisms by which G-CSF and GM-CSF are generated and release neutrophils from the bone marrow, and whether extravasation into organs that accommodate apoptosis depends on the same surface adhesion molecules and chemotactic receptors as those that promote trafficking into sites of infection and inflammation.

Animal models have allowed the identification of specific mediators and adhesion molecules implicated in experimentally induced sepsis, but conclusions so far have yet to have clinical effect. Antibodies against TNFα and interferon γ protect baboons[138] and mice[139] against bacterial insult, whereas antagonising of TNFα is ineffective in patients with sepsis,[140,141] and is even deleterious.[142] Similarly, the use of interferon γ enhances neutrophil function[143] but does not improve patients' outcomes.[144] Antagonising the activities of interleukin 1 and platelet-activating factor has not shown any clinical benefit (table 3).[145,146] A fact often overlooked is that, in human beings, neutrophils are the main population of leucocytes in the blood, whereas in mice, commonly used as animal

	Patients' outcome	Effect (anticipated or unexpected on neutrophil function)	Reference
Anti-TNFα antibodies	No improvement	Reduces activation by TNFα; decreases expression of endothelial adhesion molecules	140,141
TNF Fc fusion protein	Worse	Reduces activation by TNFα; decreases expression of endothelial adhesion molecules	142
Interleukin 1 receptor antagonist	No improvement	Decreases expression of endothelial adhesion molecules	145
Interferon γ	No improvement	Increases respiratory burst, phagocytosis, cytotoxicity; upregulates β2 integrins, CD64, and certain chemokine receptors[136]	144
PAF receptor antagonist	No improvement	Reduces activation by PAF	146
*Recombinant G-CSF	No improvement	Accelerates differentiation and maturation of neutrophil precursors and production of neutrophils; activates mature neutrophils	53,54
Fluconazole (antifungal drug)	Improvement	Enhances bacteriocidal activity and inhibits endothelial adhesion and migration	147
Low-dose hydrocortisone	Improvement	Decreases expression of CD11b and CD64; no effect on respiratory burst and phagocytosis	148,149
Activated protein C	Improvement	Inhibits chemotaxis and binding to blood vessel walls, and decreases expression of vascular adhesion molecules	150–152

PAF=platelet-activating factor. *Only treatment used to specifically target neutrophils.

Table 3: **Therapeutic compounds used to treat patients with sepsis: potential modification of neutrophil behaviour**

models of sepsis, neutrophils are a minor population. The β1 integrin, CD49d, which is found on a small population of cells in patients with sepsis[73] and is virtually absent from the neutrophils of healthy individuals, is constitutively expressed on murine neutrophils.[153] Pharmacological intervention in inbred strains of animals often uses prophylactic treatment, which is less relevant in patients with sepsis. Gene knockout mice allow individual molecules in the pathology of sepsis to be assessed, but the overall importance of these findings is occasionally confounded by enforced compensatory mechanisms. This point is shown by CD62E/CD62P selectin double-knockout mice that unexpectedly have far more neutrophils entering the lung in response to bacterial infection than wild-type mice.[154]

Neutrophil-endothelial interactions and sepsis-related organ failure

In severe sepsis, there seems to be a functional dichotomy of neutrophils with respect to responsiveness to bacterial infections. In non-pulmonary tissue, the extravasation of neutrophils into sites of infection is impeded possibly because of excessive endothelial binding and reduced chemotactic responsiveness, by contrast with the intense infiltration of these cells into infected pulmonary tissue (figure 3). The sequestration of neutrophils could be a key stage in the initiation of multiple organ failure.[155] Binding of neutrophils to blood vessel walls might be mediated by an abnormal expression of adhesion molecules different from those promoting attachment to pulmonary endothelium, or by molecules that have a high avidity for their corresponding endothelial ligands. This augmented adherence, further encouraged by a downregulation of chemotactic receptors, could produce microvascular occlusions with subsequent tissue hypoperfusion and hypoxia.[10,30] A relevant finding is that the impaired reactive hyperaemia of patients with sepsis and the septic shock deficits in tissue injury are both linked to impaired tissue perfusion and also to increased morbidity and mortality.[156,157] Should neutrophil-endothelial binding occurs at microvascular sites supporting fibrin deposition, then further binding with leuco-aggregation has the potential to take place. Moreover, if neutrophils primed by circulating factors were to encounter additional priming agents on the blood vessel wall, these neutrophils might release lytic factors that could damage endothelial cells and increase vascular permeability (figure 3).

Alternatively, organ failure could ensue from neutrophils having a subtle effect on endothelial cell function that might well relate to other pathological mechanisms proposed for sepsis. Neutrophils are an important source of proinflammatory cytokines[10] whose expression is controlled by NFκB, which is highly expressed in patients with sepsis.[87,101] Secretion of cytokines by neutrophils confined to blood vessel walls could change the non-thrombogenic properties of endothelial cells to a procoagulant state with the initiation of disseminated intravascular coagulation,[8] and also induce the production of nitric oxide in both endothelial and smooth muscle cells.[4] In addition to inducing the hypotension of septic shock,[158] the release of nitric oxide could impair tissue metabolism via inhibition of mitochondrial enzymes;[159] an effect accentuated by the further generation of nitric oxide by the neutrophils themselves.[160] In the lung, organ failure stems from alveolar denudation, basement membrane destruction,[4] and damage of the typical alveolar fluid-clearance mechanism.[10] Large numbers of primed neutrophils entering the alveolar tissue and spaces seem to secrete proteolytic enzymes and oxygen radicals in response to untoward activation by local inflammatory factors or bacterial products (figure 3).

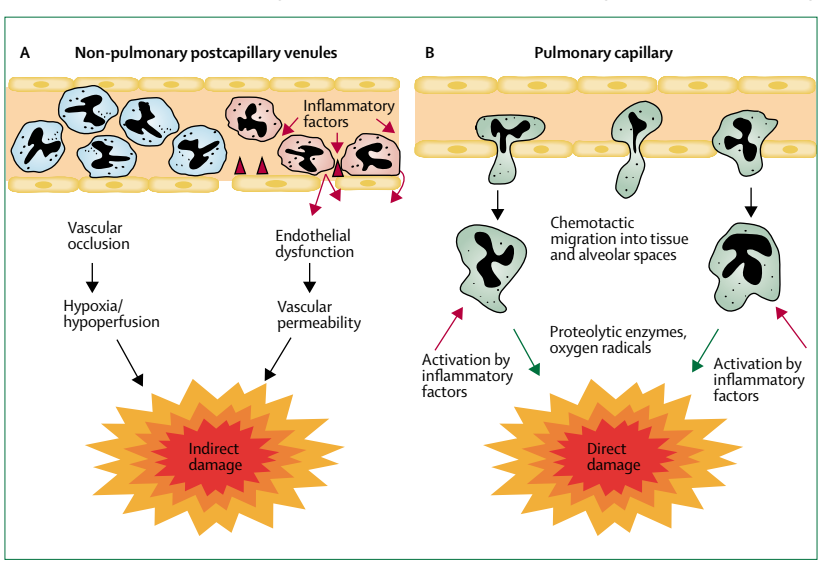

Figure 3: **Proposed mechanisms of neutrophil-mediated organ damage in sepsis**
(A) Non-pulmonary tissue might be indirectly damaged from the augmented binding of neutrophils to blood vessel walls. The neutrophils (blue) adhere so strongly to the endothelium of postcapillary venules that they produce vascular occlusions leading to tissue hypoxia and hypoperfusion. Alternatively, neutrophils (red) primed by circulating inflammatory factors bind avidly to endothelium, and become readily activated by chemokines (triangles) expressed on the endothelial surface in response to an underlying inflammatory or infective lesion. In response to this untoward activation, the neutrophils release lytic factors that induce endothelial dysfunction, the opening of intercellular junctions, and increase vascular permeability. (B) In the lung, infiltrating neutrophils induce direct damage. Neutrophils bind to the capillary endothelium and, in response to chemotactic stimuli generated by micro-organisms associated with pulmonary infection, migrate into the surrounding parenchyma and alveolar spaces. When activated, the infiltrating neutrophils induce direct tissue damage through the extracellular release of proteolytic enzymes and oxygen radicals. Both mechanisms of organ damage could occur simultaneously if damage to blood vessel walls by highly adherent neutrophils promotes the binding and tissue extravasation of additional neutrophils.

Therapeutic strategies

Neutrophil adhesion molecules

If the untoward binding of neutrophils to endothelium is relevant to the induction of organ dysfunction in sepsis, is clinical benefit likely to accrue from inhibition of this interaction? Antagonism of CD18 reverses the lung vascular injury induced by neutrophils in experimentally induced sepsis.[161] Similar approaches have not been undertaken in the clinical setting but use of anti-CD18 antibodies for patients with traumatic shock[162] or with myocardial infarction[163] have been disappointing, possibly because of the inappropriate targeting of CD18 and the

neutrophils heavily relying on CD18-independent (eg, β1 integrins) adhesion Other members of the β1 integrin family might also be contributing to neutrophil-endothelial binding in sepsis in addition to molecules that hitherto have not been implicated in the adhesion process (eg, CD64) or which remain to be identified.[32]

We are currently examining the leucocyte expression of the epidermal-growth-factor-like-seven transmembrane spanning family, whose molecular structure suggests a role in adhesion and signalling.[164] We note that one member of this family (EMR2), which is present on a few normal neutrophils, is expressed on about 50% of neutrophils with sepsis (unpublished data). By focusing specifically on neutrophils in patients with sepsis, future studies might identify other adhesion-like molecules in which increased expression is a specific feature of infection or inflammation. If such molecules were to become candidates for therapeutic targeting, what stage of the disease should this intervention take place?

Delivery before the first stages of organ failure is probably impractical because most patients with signs and symptoms of infection will have already developed some organ dysfunction by the time of admission to the intensive care unit. Thus, any therapeutic strategy will probably need to aim at disrupting the adhesion of neutrophils already sequestrated in the microvasculature, as shown by anti-integrin antibodies dislodging neutrophils bound to endothelium,[165] or the prevention of additional binding interactions that exacerbate organ dysfunction. If this strategy was successful, implementation should take place as early as possible in the course of the patient's illness, as recently emphasised in the Surviving Sepsis Campaign, which describes care bundles, including use of antibiotics and goal-directed therapy that should begin in the emergency care department and before the patient has deteriorated to the point of needing intensive care.[166]

Genetics and cell signalling

Susceptibility to systemic infection and inflammation might be due to polymorphisms in the expression of neutrophil adhesion molecules. Allelic polymorphisms in the genes for TNFα[9] and for members of the interleukin 1 family[167] are reported to increase the risk of mortality in patients with sepsis. Since polymorphisms in Fc receptors for IgG seem to be associated with meningococcal disease outcome,[168] a similar association might exist between sepsis and CD64, the high-affinity IgG receptor whose expression is upregulated on neutrophils from patients with sepsis[84] and that is associated with endothelial adhesion.[123]

An alternative approach to modify neutrophil function in sepsis is to discriminate between the signalling pathways that initiate homoeostatic functions from those that evoke tissue injury.[169] In experimental models of sepsis, organ damage by neutrophils is dependent on activation of the signal transducer and activator of transcription (STAT) 4 and 6,[170] whereas STAT3 is needed for the anti-inflammatory activities of interleukin 10[171] and phosphoinositide 3-kinase is implicated in anti-apoptotic and chemotactic processes.[172] Hopefully, the design of drugs that specifically inhibit the pro-inflammatory activities of neutrophils will not be limited by the widespread cellular distribution of the transduction pathways and by their complex interactions with one another.

Current approaches

An additional insight into neutrophil behaviour in sepsis might emerge from examination of the mode of action of successful therapeutic agents (table 3). For example, an improvement in the survival of patients with septic shock by fluconazole is attributed to modification of neutrophil function,[147] whereas the beneficial activity of low-dose hydrocortisone[148] could be related to an inhibition of neutrophil activation.[149] Use of the recombinant form of activated protein C to patients with severe sepsis has improved survival.[173] The effect was ascribed to a modification of the coagulation and inflammatory systems. The potential anti-inflammatory properties of activated protein C include impairment of neutrophil-endothelial cell interaction[150] and a downregulation of vascular adhesion molecules.[151] In a human model of endotoxin-induced pulmonary inflammation, activated protein C was shown to suppress local pulmonary coagulation[174] and to inhibit the migration of neutrophils into the lung.[152] These findings also support evidence that neutrophils have a greater contribution to the coagulation and fibrinolytic systems than hitherto suspected. Neutrophils are directly implicated in the pathophysiology of disseminated intravascular coagulation,[175] the protein C pathway impinges on neutrophil-endothelial cell interactions,[176] and the soluble endothelial protein C receptor binds to activated neutrophils.[177]

Conclusions

During sepsis, the immune response is deemed to be suppressed, as shown by the hyporeactivity of lymphocytes and by the depletion of their numbers due to increased apoptosis. By contrast, circulating numbers of neutrophils are often increased, survival is extended, and functional responses (apart from chemotaxis) are enhanced (table 2).

In sepsis, neutrophils engage in repelling invading pathogens while simultaneously inducing collateral damage in which organ function is the casualty. At present, these two neutrophil forces cannot be differentiated from one another; therefore, suppression of neutrophil functions associated with organ damage could impede the clearance of pathogenic organisms. Underlying this predicament is the central question of which is the greater threat to patients' survival, the infections themselves or the immune assault on the organs? Notably, the systemic inflammatory response to non-infectious conditions such as trauma, pancreatitis, and cardiopulmonary bypass surgery, often leads to organ failure with an immunopathology similar to that of

sepsis. Furthermore, in the intensive care unit, all patients with severe sepsis are quickly treated with antibiotics to control bacterial infections, thereby possibly compensating for neutrophil deficiencies.[3] Against this background, we suggest that strategies to antagonise components of the immune system that promote organ failure would be beneficial. Although previous immunosuppressive therapies (eg, antagonising TNFα activity, high-dose corticosteroids) have not improved the outcome of patients with sepsis, we believe that selectively targeting the interaction of subsets of neutrophils to blood vessel walls in organs susceptible to dysfunction is worthy of further therapeutic consideration.

Conflict of interest statement

We declare that we have no conflict of interest.

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