



# Neutrophils in critical illness

Braedon McDonald<sup>1</sup>

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## Abstract

During critical illness, dramatic alterations in neutrophil biology are observed including abnormalities of granulopoiesis and lifespan, cell trafficking and antimicrobial effector functions. As a result, neutrophils transition from powerful antimicrobial protectors into dangerous mediators of tissue injury and organ dysfunction. In this article, the role of neutrophils in the pathogenesis of critical illness (sepsis, trauma, burns and others) will be explored, including pathological changes to neutrophil function during critical illness and the utility of monitoring aspects of the neutrophil phenotype as biomarkers for diagnosis and prognostication. Lastly, we review findings from clinical trials of therapies that target the harmful effects of neutrophils, providing a bench-to-bedside perspective on neutrophils in critical illness.

**Keywords** Neutrophils · Critical illness · Sepsis · Immunotherapy

## Introduction

Critical illness and organ dysfunction can be precipitated by a variety of insults (sepsis, trauma, burns, ischemia-reperfusion injury and many others) but a common feature of disease pathogenesis is inflammation and immune-mediated pathology. Neutrophils are central players in the inflammatory pathogenesis of organ failure in critical illness. Armed with powerful antimicrobial effector functions, neutrophils are both essential guardians of host defense and dangerous mediators of tissue damage during states of unchecked inflammation. Abnormalities of neutrophil function have been identified in diseases of the critically ill that predispose to immune-mediated organ dysfunction and weaken host defenses, resulting in susceptibility to nosocomial infections. Here, the role of neutrophils in the pathogenesis of critical illness and utility of targeting neutrophils therapeutically, will be reviewed.

## The function of neutrophils in critical illness

### Lifespan

The number of circulating neutrophils is commonly elevated in patients with sepsis and other states of critical illness. Sepsis is a state of systemic inflammation, dysregulated host response and organ dysfunction that is caused by infection (Singer et al. 2016). In the setting of sepsis and systemic inflammation, neutrophils are released from bone marrow as well as intravascular stores and their survival in the bloodstream is increased several fold (Colotta et al. 1992). Cytokines such as TNF $\alpha$ , IL-1 and IL-6, as well as bacterial products can stimulate granulopoiesis in the bone marrow through the generation of granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF). Furthermore, downregulation of chemokines responsible for retaining neutrophils in the bone marrow (CXCL12), together with simultaneous upregulation of chemokines that promote neutrophil egress (CXCL1), result in rapid release of neutrophils into the blood (Eash et al. 2010; Delano et al. 2011). Mediators of systemic inflammation (cytokines and bacterial products) also prolong the lifespan of neutrophils in the circulation by inhibiting apoptosis (Colotta et al. 1992). During sepsis, neutrophils become resistant to cell death as a result of downregulated expression of pro-apoptotic caspases (Taneja et al. 2004; Guo et al. 2006). In addition to prolonging neutrophil lifespan, reduced

✉ Braedon McDonald  
braedon.mcdonald@icloud.com

<sup>1</sup> Department of Critical Care Medicine, Cumming School of Medicine, University of Calgary, HRIC 4C51, 3230 Hospital Dr. NW, Calgary, AB T2N 4N1, Canada

apoptosis also inhibits the homeostatic mechanisms that regulate circulating neutrophil counts, as this process is dependent on negative feedback from macrophages that have engulfed apoptotic neutrophils from the circulation (Stark et al. 2005). Together, these mechanisms work synergistically to increase the number of circulating neutrophils and to prolong their lifespan. Teleologically, it is thought that this response has evolved to ensure adequate supply of neutrophils to sites of infection. However, as described below, during the extremes of critical illness, neutrophils acquire functional abnormalities that impair their ability to fight infection while causing tissue injury and organ dysfunction.

## Trafficking

Multiple abnormalities of neutrophil trafficking have been identified in sepsis, burns, trauma and other diseases of the critically ill (Dong et al. 1993; Butler et al. 2010; Phillipson and Kubes 2011). In response to localized infections, neutrophils are rapidly and precisely recruited to the site of infection where they kill pathogens and aid in tissue healing. In contrast, a state of “neutrophil paralysis” is observed during sepsis, in which trafficking to infected tissues is impaired as a result of altered adhesion molecule expression and chemotaxis signaling (Heit et al. 2002; Alves-Filho et al. 2010). Instead, neutrophils are re-directed to the microvasculature of internal organs including the lung, liver and kidneys, resulting in neutrophil-mediated organ damage (Phillipson and Kubes 2011). The molecular mechanisms that mediate these abnormalities of neutrophil trafficking during sepsis (and other states of critical illness) have been recently reviewed by others (Phillipson and Kubes 2011; Kolaczkowska and Kubes 2013; Sônego et al. 2016). Overall, this state of “neutrophil paralysis” acquired during sepsis results in immune-mediated organ damage as neutrophils are inappropriately sequestered within internal organs (e.g., acute lung injury, acute kidney injury, acute hepatic dysfunction). The reasons for this trafficking behavior are

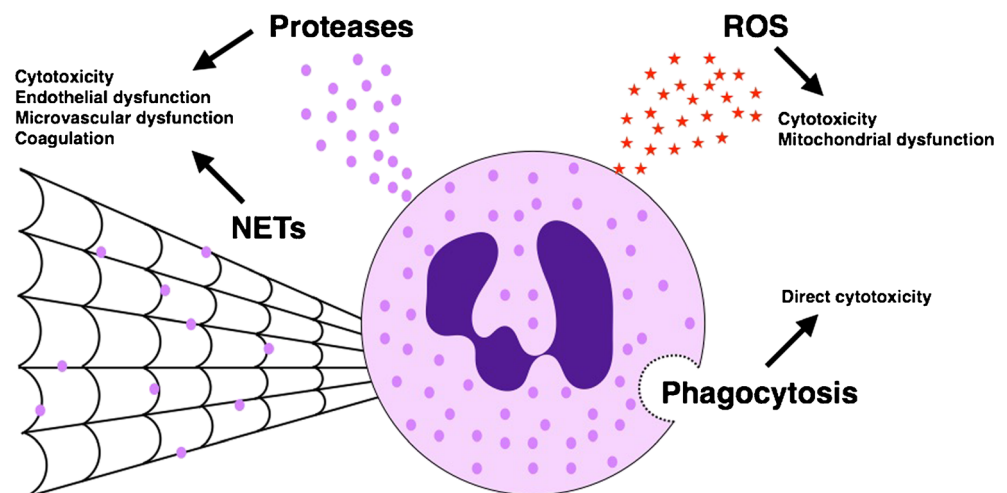
incompletely understood and may represent a clever immune evasion mechanism by bacteria to misdirect neutrophils away from foci of infection. Alternatively, there is evolving evidence that this may be a coordinated host defense mechanism to position neutrophils within the body’s most dense microvascular beds to protect against blood-borne dissemination of bacteria during severe infection (McDonald et al. 2012; Yipp et al. 2017).

## Effector functions

Neutrophils are armed with a powerful arsenal of weapons designed to capture and kill invading microbes. However, when these weapons are unleashed in an overabundant or indiscriminatory manner, they can cause collateral damage to cells and tissues of the host (Fig. 1). Furthermore, defective neutrophil function in critically ill patients also increases the risk of nosocomial infections. Studies to date have identified multiple abnormalities of neutrophil effector functions during critical illness.

**Phagocytosis** Variability exists in published reports of the effects of critical illness on the ability of neutrophils to phagocytose bacteria, with some studies finding impaired phagocytosis while others finding no difference from healthy controls. Much of the observed variability likely stems from differences in the neutrophil populations being studied, as it is now known that neutrophils exist in a variety of phenotypically unique subsets (Kolaczkowska and Kubes 2013; Tak et al. 2017). For example, circulating neutrophils from patients with septic shock were able to engulf bacteria in quantities that were no different from healthy patient neutrophils (Demaret et al. 2015). In contrast, peritoneal neutrophils from septic mice displayed profound defects in phagocytosis (Chiswick et al. 2015). These findings may reflect the phenotypic difference between unique populations of neutrophils found in these different compartments (Kolaczkowska and Kubes 2013; Tak

**Fig. 1** Pathological neutrophil effector mechanisms in critical illness. Multiple neutrophil effector mechanisms contribute to tissue damage and organ dysfunction during critical illness including neutrophil extracellular traps (NETs), neutrophil proteases, reactive oxygen species (ROS) and phagocytosis. See text for details



et al. 2017). Lastly, in addition to abnormalities of phagocytosis, studies have reported defects in phagosome maturation and intracellular killing during sepsis (Chiswick et al. 2015; Lelifeld et al. 2016).

**Reactive oxygen species** Oxidative killing mechanisms represent an essential component of the anti-microbial armamentarium. During critical illness, uncontrolled reactive oxygen species (ROS) produced by neutrophil oxidative bursts results in damage to cell membranes and organelles, causing structural and metabolic dysfunction that predisposes to cell death (Mittal et al. 2014). Neutrophils from patients with trauma, burns, ARDS, sepsis, acute liver failure and others have been shown to produce abnormal levels of ROS compared to neutrophils from healthy controls (Simms and D’Amico 1991; Santos et al. 2012; Liao et al. 2013; Taylor et al. 2013). Furthermore, hyperactive ROS production by neutrophils was associated with an increased risk of death in sepsis (Santos et al. 2012). Therefore, uncontrolled ROS production by neutrophils is thought to represent a central mechanism of cellular toxicity and organ dysfunction in critical illness (Brown et al. 2006).

**Proteases** Activated neutrophils release serine proteases that cause tissue injury via multiple mechanisms. First, neutrophil proteases can cause direct cytotoxicity to host cells (Korkmaz et al. 2010). Furthermore, the release of neutrophil elastase, cathepsin G and proteinase-3 from activated neutrophils exacerbates local and systemic inflammatory responses through amplification of cytokine production and signaling, as well as effects on adhesion molecule and chemoattractant function in leukocyte recruitment (Pham 2006). Neutrophil proteases can also activate the coagulation and complement systems, both of which fuel the pathogenesis of sepsis and other states of critical illness (Massberg et al. 2010; Kolev et al. 2014). Lastly, neutrophil proteases are essential components of neutrophil extracellular traps (NETs), contributing to both production and antimicrobial function of NETs (Papayannopoulos et al. 2010).

**Neutrophil extracellular traps** High levels of NETs have been identified in the bloodstream and tissues of patients with sepsis and trauma (Liu et al. 2014; Itagaki et al. 2015; Czaikoski et al. 2016). In sepsis, NETs protect against bacterial dissemination by capturing and clearing bacteria from the bloodstream (McDonald et al. 2012). However, NETs also cause pathology through their ability to induce cellular injury and microvascular dysfunction. Studies of animal models of sepsis have shown that NET constituents such as histones and neutrophil serine proteases are cytotoxic to host cells and that inhibiting NETs (or neutralization of histones) results in decreased organ damage and improved survival (Xu et al. 2009; McDonald et al. 2012; Martinod et al. 2015; Czaikoski et al.

2016). More recently, NETs were identified as a key precipitant of disseminated intravascular coagulation in sepsis, leading to widespread microvascular hypoperfusion and multi-organ dysfunction (Yang et al. 2017; Delabranche et al. 2017; McDonald et al. 2017). Overall, NETs are perhaps the most potent and destructive effector mechanism produced by neutrophils, which makes them attractive therapeutic targets in sepsis and other diseases (see below).

## Neutrophils as a biomarker in critical illness

Neutrophils have been attractive targets for biomarker investigations in critical illness. A variety of neutrophil characteristics have been studied and used clinically to aid in diagnosis, monitoring and prognostication in various diseases of the critically ill.

**Circulating neutrophil counts** One of the most commonly tested biomarkers in critically ill patients is the total white-blood cell (or leukocyte) count. The majority of circulating leukocytes are neutrophils and their abundance and maturation state (i.e., the presence of immature band cells) serve as markers of systemic inflammation (see above). In fact, leukocyte counts and the degree of bandemia are criteria for the diagnosis of the systemic inflammatory response syndrome (SIRS), which, until recently (Singer et al. 2016), was essential for the diagnosis of sepsis. However, neutrophil and total leukocyte counts only serve as adjuncts in the assessment of critically ill patients, because they lack sufficient sensitivity and specificity to serve as accurate diagnostic biomarkers in critical illness (Mare et al. 2015; Kaukonen et al. 2015).

**CD64** The Fc $\gamma$  receptor 1, CD64, mediates phagocytosis of opsonized bacteria by neutrophils. Surface expression of CD64 on neutrophils is upregulated 10-fold in response to pro-inflammatory stimuli such as cytokines and bacterial products. As such, quantitative analysis of CD64 expression on neutrophils has been investigated as a diagnostic biomarker for the early recognition of sepsis. High levels of CD64 expression have been shown to distinguish bacterial from viral infections with a specificity of up to 91% (Leino et al. 1997; Cid et al. 2010; Li et al. 2013; Dimoula et al. 2014). CD64 expression also carries prognostic significance in sepsis, as high levels of neutrophil CD64 are associated with an increased risk of in-hospital and 28-day mortality (Livaditi et al. 2006; Dimoula et al. 2014). Despite these promising data, the practicality of measuring neutrophil CD64 expression by flow-cytometry is limited in routine clinical practice and therefore has not been adopted into widespread use.

**Triggering receptor expressed on myeloid cells 1** TREM-1 is a surface molecule of the immunoglobulin superfamily that is expressed in both membrane-bound and soluble forms by

neutrophils and other myeloid cells. Functionally, membrane-bound TREM-1 amplifies inflammatory responses to bacterial and fungal products (Bouchon et al. 2001). In contrast, soluble TREM-1 acts in an inhibitory fashion by scavenging ligands and antagonizing the activity of membrane-bound TREM-1 (Baruah et al. 2015). Expression levels of both membrane-bound and soluble TREM-1 are dramatically upregulated in response to microbial products. As a diagnostic biomarker, expression levels of TREM-1 can help distinguish infection/sepsis from other causes of SIRS, to help rapidly identify patients who will benefit from early antibiotic administration and source control measures. In patients with suspected pneumonia, detection of sTREM-1 in bronchoalveolar lavage fluid identified bacterial and fungal pneumonia with a sensitivity and specificity of 98% and 90%, respectively (Gibot et al. 2004). However, in patients presenting with positive SIRS criteria, high plasma sTREM-1 levels identified infectious etiologies with a modest accuracy (sensitivity and specificity of 79% and 80%, respectively) (Wu et al. 2012). Therefore, although initially promising, assays of sTREM-1 have not been adopted into widespread clinical use.

**Cell-free DNA** Circulating levels of cfDNA can be readily detected in plasma samples using PCR-based methods or spectrophotometric assays. There is an expanding literature on the utility of cfDNA as a diagnostic and prognostic biomarker in sepsis, burns and trauma. Multiple studies have identified associations between high levels of plasma cfDNA and organ dysfunction, ICU-acquired complications and mortality (Jacobs and Wong 2016). It has been hypothesized that neutrophils may represent an important source of cfDNA in critically ill patients through the release of NETs. However, studies suggesting that NETs are the source of cfDNA have largely relied on methodologies that do not discriminate sufficiently between NETs and other possible sources of extracellular DNA (e.g., necrosis and apoptosis of other cell types) (Margraf et al. 2008). The identification of NETs as a source of cfDNA requires intricate assays that detect complexes formed by DNA and neutrophil proteins (e.g., DNA-MPO, DNA-citrullinated H3) and studies using such robust assays are lacking in critically ill populations (Kessenbrock et al. 2009; Cadrillier et al. 2012; Thålin et al. 2017). Therefore, while cfDNA has certainly proven a useful biomarker in multiple diseases of the critically ill, there are insufficient data to consider this a neutrophil-based biomarker.

## Therapeutic manipulation of neutrophils in critical illness

Neutrophils are the targets (directly and indirectly) of a number of treatment strategies that have been trialed in critically ill patients (Table 1). In this section, the biological rationale and

**Table 1** Therapeutic modulation of neutrophils in critical illness

Treatment	Effects on neutrophils	Patient outcomes
Steroids	Inhibition of leukocyte trafficking, oxidative bursts, downregulation of protease production	Reduced duration of shock, no improvement in 28-day mortality in septic shock
G-CSF	Increased neutrophil counts, priming of oxidative bursts and NETs production, increased responsiveness to endotoxin	No improvement in organ function, length of stay, survival
GM-CSF	Increased neutrophil counts, increased responsiveness to endotoxin and cytokines	Reduced organ failure scores in patient with low mHLA-DR expression
Anti-TNF $\alpha$	Decreased neutrophil trafficking to site of inflammation	No improvement in survival
Anti-E-selectin	Inhibition of neutrophil rolling phase	No improvement in survival
Anti-E- and L-selectin	Inhibition of neutrophil rolling phase	Increased mortality in primate studies
Anti-ICAM-1	Inhibition of neutrophil adhesion and intravascular crawling	Increased mortality in primate studies
Extracorporeal leukofiltration	Neutrophil depletion	Improved pulmonary and renal function post-cardiopulmonary bypass
Granulocyte transfusions	Increased neutrophil counts in neutropenic patients	Case reports of success in refractory neutropenia with infections
Sivelestat (NE inhibitor)	Inhibition of neutrophil elastase, possible inhibition of NETs	No improvement in survival in ARDS, sepsis, multi-organ failure
N-acetylcysteine	Antagonism of ROS	No improvement in survival in ARDS
Anti-oxidant cocktail (see text)	Antagonism of ROS	No improvement in survival
Recombinant human DNase	Degradation of NETs	Untested in humans



clinical effectiveness of various therapeutic strategies to manipulate neutrophil function will be reviewed.

## Steroids

Corticosteroid therapy induces widespread anti-inflammatory effects, ranging from inhibition of inflammatory mediators (pro-inflammatory cytokines, iNOS and NO production and lipid mediators) to direct modulation of immune cell function. The administration of systemic glucocorticoids increases circulating neutrophil counts through at least three mechanisms: (1) demargination of intravascular neutrophils, (2) increased release from bone marrow and (3) decreased neutrophil apoptosis leading to prolonged survival (Fay et al. 2016; Cain and Cidlowski 2017). However, corticosteroids also blunt neutrophil function at sites of inflammation. When administered at high doses, corticosteroids impair neutrophil trafficking through both reduced responsiveness to chemoattractants and downregulation of adhesion molecule expression (Jilma et al. 1997; Cain and Cidlowski 2017). Furthermore, supraphysiological doses of steroids decrease oxidative bursts and impair phagocytosis and other effector mechanisms (Kaufmann et al. 2008). Aside from neutrophils, corticosteroids have also been shown to impart broad-spectrum immune-suppressing effects on almost all immune cells (Cain and Cidlowski 2017). Despite the long history and extensive study of corticosteroids in septic shock and other diseases in the ICU, the mechanisms underlying the immunomodulatory properties of steroids remain incompletely understood, largely due to the immense complexity of biological activity.

Clinically, the role of corticosteroids in critical illnesses like sepsis is an ongoing area of research. Available evidence from large randomized controlled trials has revealed that corticosteroids decrease markers of systemic inflammation and reduce the duration of shock (vasopressor dependency) in patients with sepsis but do not improve survival (Sprung et al. 2008; Gibbison et al. 2017).

## Immunotherapy

A wide range of immunomodulatory therapies have been studied in critically ill patients, primarily those with septic shock (Gotts and Matthay 2016). While many of these therapies indirectly affect neutrophil function, two of the more promising immunotherapies, G-CSF and GM-CSF, directly target neutrophil biology. G-CSF is a cytokine that promotes maturation and release of neutrophils from the bone marrow, as well as activation of the neutrophil effector mechanism. Recombinant human G-CSF has been used for years in the field of oncology to prevent and treat chemotherapy-induced neutropenia (Hartmann et al. 1997). In patients with sepsis, G-CSF increases the number of circulating neutrophils, augments neutrophil responsiveness to endotoxin and cytokines

and primes neutrophil effector functions, as well as additional effects on monocytes, dendritic cells and lymphocytes (Flohé et al. 1999). However, the cumulative data available from multiple randomized controlled trials suggest that G-CSF does not improve outcomes (organ dysfunction scores, length of stay, duration of mechanical ventilation, or changes in 28-day mortality) in patients with sepsis (Bo et al. 2011).

Another attractive target for neutrophil immunotherapy in sepsis and other critical illnesses is GM-CSF. Administration of recombinant GM-CSF promotes the proliferation and release of myeloid cells from the bone marrow and activates circulating neutrophils, monocytes/macrophages and dendritic cells (Mathias et al. 2015). Given its pleiotropic effects, multiple studies have tested the hypothesis that GM-CSF may reverse the “immunoparalysis” that is observed in many septic patients, including augmentation of neutrophil function. A number of small clinical trials have observed increased levels of circulating neutrophils and enhanced neutrophil effector functions in septic patients treated with GM-CSF but have found mixed results in terms of clinical outcomes (Mathias et al. 2015). The most promising results were observed in patients with sepsis and biomarkers of established “immunoparalysis” (low HLA-DR expression on monocytes), where GM-CSF was shown to reverse sepsis-induced immunosuppression and reduce organ failure (Meisel et al. 2009). Given these promising preliminary results, a larger trial is underway to further investigate the utility of GM-CSF for immunomodulation in septic patients with low mHLA-DR expression (NCT-02361528, [clinicaltrials.gov](http://clinicaltrials.gov)).

## Anti-adhesion molecule therapy

Given that neutrophils must transit out of the circulation and into tissues in order to mediate tissue damage (Kolaczowska and Kubes 2013), investigators have tested the effects of blocking neutrophil adhesion molecules to prevent organ dysfunction and death in critically ill patients. Therapies aimed at blocking the rolling phase of neutrophil recruitment have shown mixed results. An early pilot trial of anti-E-selectin monoclonal antibody (CY1787) in patients with septic shock reported a small signal towards reduced organ dysfunction and shock (Friedman et al. 1996). In contrast, a study in septic baboons found that anti-E- and L-selectin antibodies caused harm (worsening shock, organ failure and increased mortality) (Carraway et al. 1998). Similarly, blocking the adhesion phase of neutrophil recruitment using antibodies against integrins (e.g., CD18) or their ligands (ICAM-1) also resulted in increased mortality (Welty-Wolf et al. 2001). Overall, these early trials of anti-adhesion molecule therapy suggested that blocking cell recruitment may actually be harmful in critically ill patients with sepsis.

The failure of these early studies to show a reduction in organ damage and mortality may have been due to a limited

appreciation of the intricacies of leukocyte trafficking and the widespread immunological effects of blocking adhesion molecules. We now know that adhesion molecules are involved in multiple aspects of immune function (lymphocyte activation, complement and coagulation, platelet function, etc.) and therefore broad-spectrum anti-adhesion therapies may have had unintended consequences in these early studies. Furthermore, early studies failed to appreciate that leukocyte recruitment occurs in an organ-specific manner, involving unique combinations of adhesion molecules and chemokines (Kolaczowska and Kubes 2013). A greater appreciation of these intricacies of leukocyte trafficking has enabled the development of second-generation anti-adhesion molecule therapies for the treatment of organ-specific diseases like psoriasis, inflammatory bowel disease and multiple sclerosis (Ley et al. 2016).

Overall, the complex multi-organ involvement of critical illness makes anti-adhesion molecule therapy difficult to implement in a safe and targeted manner. However, therapeutic inhibition of adhesion molecules may yet prove beneficial to combat other aspects of critical illness pathogenesis. For example, anti-adhesion molecule therapy is used to disrupt platelet adhesion and aggregation in thrombotic diseases like acute coronary syndrome and stroke (Capodanno et al. 2013). In animal models of sepsis, blocking adhesion molecules that mediate platelet–neutrophil interactions reduced markers of organ dysfunction (McDonald et al. 2012; Rossaint and Zarbock 2015). Therefore, targeting the pathological effects of platelets and thrombosis with anti-adhesion molecule therapy remains an active area of research in multiple diseases of the critically ill.

### Extracorporeal therapies

An extreme approach to mitigating the potentially harmful effects of neutrophils in critical illness is to remove them from the circulation. Extracorporeal leukocyte removal by apheresis is not commonly used for therapeutic purposes, aside from rare conditions caused by hyperleukocytosis (e.g., leukostasis in acute leukemia and pertussis-associated hyperleukocytosis). Experimental use of extracorporeal leukofiltration during cardiopulmonary bypass decreased SIRS markers (TNF $\alpha$  and other cytokines), improved renal function and reduced inflammatory lung pathology in patients undergoing cardiac surgery (Treacher et al. 2001; Kiliç et al. 2009). However, there are very limited data on the efficacy and safety of extracorporeal neutrophil removal in the treatment of critical illness.

Other forms of extracorporeal treatments have been studied extensively (e.g., hemodialysis, hemoperfusion, extracorporeal endotoxin removal, extracorporeal membrane oxygenation). These modalities have widespread effects on physiology and host defense aside from neutrophil function and therefore are beyond the scope of this review.

### Granulocyte transfusions

As outlined above, neutrophil dysfunction (neutropenia or defects in effector mechanisms) leads to an increased risk of infections and exacerbation of critical illness. Therefore, some have hypothesized that administration of normal donor granulocytes may benefit selected populations of critically ill patients with neutrophil dysfunction. While uncommon in routine clinical practice, granulocyte transfusions have been utilized with some success in patients with refractory neutropenia and difficult-to-treat infections (Alavi et al. 1977; Price et al. 2015). Within pediatric intensive care units, granulocyte transfusions have been used to support neutrophil counts in septic neonates (Mohan and Brocklehurst 2003). Neonates possess immature granulopoiesis mechanisms and are therefore highly susceptible to severe neutropenia during sepsis. However, insufficient evidence exists to support a clinical benefit of granulocyte transfusion in neonates with sepsis (Mohan and Brocklehurst 2003).

### Anti-effector mechanism therapies

**Neutrophil elastase inhibitors** Inhibitors of neutrophil elastase have been most extensively studied in acute respiratory distress syndrome (ARDS). Animal models of acute lung injury have consistently demonstrated a pathological role for neutrophil elastase and inhibitors of neutrophil elastase are protective in models of acute lung injury caused by a variety of insults (Grommes and Soehnlein 2011). Based on the strength of these pre-clinical data, neutrophil elastase inhibition has been studied in patients with ARDS. A recent meta-analysis of 8 randomized controlled trials reported a small improvement in lung function in patients treated with sivelestat (small-molecule NE inhibitor) but no improvement in mortality or duration of mechanical ventilation (Iwata et al. 2010). As such, NE inhibitors are not routinely employed in the management of ARDS in North America and Europe.

**Anti-oxidant therapy** As described above, neutrophils are a major source of ROS during systemic inflammatory diseases. Oxidative stress contributes to mitochondrial and cellular dysfunction and, thus, is an attractive therapeutic target. The most extensively studied anti-oxidant therapy is *N*-acetylcysteine (NAC), which scavenges ROS and promotes replenishment of intracellular glutathione stores. Multiple randomized controlled trials have been conducted comparing NAC versus placebo in patients with sepsis, multi-organ failure and ARDS but none have demonstrated a meaningful benefit to patient outcomes (Vincent et al. 2002). Therefore, NAC use is limited in the ICU to a select number of conditions where its anti-oxidant effects have proven beneficial, including acute acetaminophen overdose and non-acetaminophen-related acute liver failure (Darweesh et al. 2017).

Anti-oxidant vitamins and mineral supplementation have also been investigated in critically ill patients. For example, selenium is an essential co-factor in the normal function glutathione peroxidase, a powerful intracellular ROS scavenger. Other vitamins and trace minerals, such as zinc, beta-carotene, vitamin E and vitamin C, also possess anti-oxidant activity in vivo. However, a large randomized controlled trial of 1223 critically ill patients with multi-organ failure on mechanical ventilation showed no differences in mortality or organ failure scores between patients treated with an anti-oxidant cocktail (selenium, zinc, beta-carotene, and vitamins E and C) or placebo (Heyland et al. 2013).

**Anti-NETs therapy** Overexuberant release of NETs during sepsis and other systemic inflammatory diseases contributes to a wide variety of pathological processes, including cell and tissue damage, intravascular coagulation and microvascular dysfunction (Clark et al. 2007; McDonald et al. 2012, 2017; Martinod et al. 2015; Czaikoski et al. 2016). A number of anti-NETs therapies have been shown to improve organ function and survival in septic animals, including intravenous DNase infusions, antibodies/inhibitors against components of NETs (histones, proteases) and inhibitors of peptidylarginine deiminase 4 (PAD4, an enzyme required for NETs production) (McDonald et al. 2012, 2017; Martinod et al. 2015; Czaikoski et al. 2016). To date, there have been no human trials of anti-NET therapies. Given the promising pre-clinical data from animal models, human studies are eagerly awaited.

## Conclusions and future perspectives

As both pivotal guardians of host defense and dangerous mediators of organ damage, neutrophils represent a true paradox in sepsis and other critical illnesses. The ideal therapeutic tool would enable selective inhibition of pathological neutrophil functions without impairing host defense and tissue repair; however, no such agent has been discovered to date. Furthermore, the tremendous heterogeneity amongst critically ill patients makes it difficult to identify individuals that may respond to targeted immunomodulatory therapies. The future success of immunomodulatory therapies in diseases like sepsis will undoubtedly require a “personalized medicine” approach to clinical trial design, using biomarkers and other phenotyping tools to identify patients with pathology that is amenable to targeted therapy. Fortunately, our expanding understanding of the molecular mechanisms of neutrophil function, as well as their roles in the pathogenesis of critical illness, continues to move us closer to this goal.

## References

- Alavi JB, Root RK, Djerassi I et al (1977) A randomized clinical trial of granulocyte transfusions for infection in acute leukemia. *N Engl J Med* 296:706–711. <https://doi.org/10.1056/NEJM197703312961302>
- Alves-Filho JC, Spiller F, Cunha FQ (2010) Neutrophil paralysis in sepsis. *Shock* 34(Suppl 1):15–21. <https://doi.org/10.1097/SHK.0b013e3181e7e61b>
- Baruah S, Keck K, Vrenios M et al (2015) Identification of a novel splice variant Isoform of TREM-1 in human Neutrophil granules. *J Immunol* 195:5725–5731. <https://doi.org/10.4049/jimmunol.1402713>
- Bo L, Wang F, Zhu J et al (2011) Granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) for sepsis: a meta-analysis. *Crit Care (London)* 15:R58. <https://doi.org/10.1186/cc10031>
- Bouchon A, Facchetti F, Weigand MA, Colonna M (2001) TREM-1 amplifies inflammation and is a crucial mediator of septic shock. *Nature* 410:1103–1107. <https://doi.org/10.1038/35074114>
- Brown KA, Brain SD, Pearson JD et al (2006) Neutrophils in development of multiple organ failure in sepsis. *Lancet* 368:157–169. [https://doi.org/10.1016/S0140-6736\(06\)69005-3](https://doi.org/10.1016/S0140-6736(06)69005-3)
- Butler KL, Ambravaneswaran V, Agrawal N et al (2010) Burn injury reduces neutrophil directional migration speed in microfluidic devices. *PLoS ONE* 5:e11921. <https://doi.org/10.1371/journal.pone.0011921>
- Cain DW, Cidlowski JA (2017) Immune regulation by glucocorticoids. *Nat Rev Immunol* 17:233–247. <https://doi.org/10.1038/nri.2017.1>
- Capodanno D, Ferreira JL, Angiolillo DJ (2013) Antiplatelet therapy: new pharmacological agents and changing paradigms. *J Thromb Haemost* 11(Suppl 1):316–329. <https://doi.org/10.1111/jth.12219>
- Carraway MS, Welty-Wolf KE, Kantrow SP et al (1998) Antibody to E- and L-selectin does not prevent lung injury or mortality in septic baboons. *Am J Respir Crit Care Med* 157:938–949. <https://doi.org/10.1164/ajrccm.157.3.9707129>
- Caudrillier A, Kessenbrock K, Gilliss BM et al (2012) Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest* 122:2661–2671. <https://doi.org/10.1172/JCI61303>
- Chiswick EL, Mella JR, Bernardo J, Remick DG (2015) Acute-phase deaths from Murine Polymicrobial sepsis are characterized by innate immune suppression rather than exhaustion. *J Immunol* 195:3793–3802. <https://doi.org/10.4049/jimmunol.1500874>
- Cid J, Aguinaco R, Sánchez R et al (2010) Neutrophil CD64 expression as marker of bacterial infection: a systematic review and meta-analysis. *J Inf Secur* 60:313–319. <https://doi.org/10.1016/j.jinf.2010.02.013>
- Clark SR, Ma AC, Tavener SA et al (2007) Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* 13:463–469. <https://doi.org/10.1038/nm1565>
- Colotta F, Re F, Polentarutti N et al (1992) Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. *Blood* 80:2012–2020
- Czaikoski PG, Mota JM, Nascimento DC et al (2016) Neutrophil extracellular traps induce organ damage during experimental and clinical sepsis. *PLoS ONE* 11:e0148142. <https://doi.org/10.1371/journal.pone.0148142>
- Darweesh SK, Ibrahim MF, El-Tahawy MA (2017) Effect of N-Acetylcysteine on mortality and liver transplantation rate in non-acetaminophen-induced acute liver failure: a multicenter study. *Clin Drug Investig* 37:473–482. <https://doi.org/10.1007/s40261-017-0505-4>
- Delabranche X, Stiel L, Severac F et al (2017) Evidence of Netosis in septic shock-induced disseminated intravascular coagulation. *Shock* 47:313–317. <https://doi.org/10.1097/SHK.0000000000000719>



- Delano MJ, Kelly-Scumpia KM, Thayer TC et al (2011) Neutrophil mobilization from the bone marrow during polymicrobial sepsis is dependent on CXCL12 signaling. *J Immunol* 187:911–918. <https://doi.org/10.4049/jimmunol.1100588>
- Demaret J, Venet F, Friggeri A et al (2015) Marked alterations of neutrophil functions during sepsis-induced immunosuppression. *J Leukoc Biol* 98:1081–1090. <https://doi.org/10.1189/jlb.4A0415-168RR>
- Dimoula A, Pradier O, Kassenger Z et al (2014) Serial determinations of neutrophil CD64 expression for the diagnosis and monitoring of sepsis in critically ill patients. *Clin Infect Dis* 58:820–829. <https://doi.org/10.1093/cid/cit936>
- Dong YL, Abdullah K, Yan TZ et al (1993) Effect of thermal injury and sepsis on neutrophil function. *J Trauma* 34:417–421
- Eash KJ, Greenbaum AM, Gopalan PK, Link DC (2010) CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *J Clin Invest* 120:2423–2431. <https://doi.org/10.1172/JCI141649>
- Fay ME, Myers DR, Kumar A et al (2016) Cellular softening mediates leukocyte demargination and trafficking, thereby increasing clinical blood counts. *Proc Natl Acad Sci U S A* 113:1987–1992. <https://doi.org/10.1073/pnas.1508920113>
- Flohé S, Börgermann J, Domínguez FE et al (1999) Influence of granulocyte-macrophage colony-stimulating factor (GM-CSF) on whole blood endotoxin responsiveness following trauma, cardiopulmonary bypass, and severe sepsis. *Shock* 12:17–24
- Friedman G, Jankowski S, Shahla M et al (1996) Administration of an antibody to E-selectin in patients with septic shock. *Crit Care Med* 24:229–233
- Gibbison B, López-López JA, Higgins JPT et al (2017) Corticosteroids in septic shock: a systematic review and network meta-analysis. *Crit Care (London)* 21:78. <https://doi.org/10.1186/s13054-017-1659-4>
- Gibot S, Cravoisy A, Levy B et al (2004) Soluble triggering receptor expressed on myeloid cells and the diagnosis of pneumonia. *N Engl J Med* 350:451–458. <https://doi.org/10.1056/NEJMoa031544>
- Gotts JE, Matthay MA (2016) Sepsis: pathophysiology and clinical management. *BMJ* 353:i1585
- Grommes J, Soehnlein O (2011) Contribution of neutrophils to acute lung injury. *Mol Med* 17:293–307. <https://doi.org/10.2119/molmed.2010.00138>
- Guo R-F, Sun L, Gao H et al (2006) In vivo regulation of neutrophil apoptosis by C5a during sepsis. *J Leukoc Biol* 80:1575–1583. <https://doi.org/10.1189/jlb.0106065>
- Hartmann LC, Tschetter LK, Habermann TM et al (1997) Granulocyte colony-stimulating factor in severe chemotherapy-induced afebrile neutropenia. *N Engl J Med* 336:1776–1780. <https://doi.org/10.1056/NEJM199706193362502>
- Heit B, Tavener S, Raharjo E, Kubes P (2002) An intracellular signaling hierarchy determines direction of migration in opposing chemotactic gradients. *J Cell Biol* 159:91–102. <https://doi.org/10.1083/jcb.200202114>
- Heyland D, Muscedere J, Wischmeyer PE et al (2013) A randomized trial of glutamine and antioxidants in critically ill patients. *N Engl J Med* 368:1489–1497. <https://doi.org/10.1056/NEJMoa1212722>
- Itagaki K, Kaczmarek E, Lee YT et al (2015) Mitochondrial DNA released by trauma induces neutrophil extracellular traps. *PLoS ONE* 10:e0120549. <https://doi.org/10.1371/journal.pone.0120549>
- Iwata K, Doi A, Ohji G et al (2010) Effect of neutrophil elastase inhibitor (sivelestat sodium) in the treatment of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS): a systematic review and meta-analysis. *Intern Med* 49:2423–2432
- Jacobs L, Wong HR (2016) Emerging infection and sepsis biomarkers: will they change current therapies? *Expert Rev Anti-Infect Ther* 14:929–941. <https://doi.org/10.1080/14787210.2016.1222272>
- Jilma B, Voltmann J, Albinni S et al (1997) Dexamethasone down-regulates the expression of L-selectin on the surface of neutrophils and lymphocytes in humans. *Clin Pharmacol Ther* 62:562–568. [https://doi.org/10.1016/S0009-9236\(97\)90052-7](https://doi.org/10.1016/S0009-9236(97)90052-7)
- Kaufmann I, Briegel J, Schliephake F et al (2008) Stress doses of hydrocortisone in septic shock: beneficial effects on opsonization-dependent neutrophil functions. *Intensive Care Med* 34:344–349. <https://doi.org/10.1007/s00134-007-0868-8>
- Kaukonen K-M, Bailey M, Pilcher D et al (2015) Systemic inflammatory response syndrome criteria in defining severe sepsis. *N Engl J Med* 372:1629–1638. <https://doi.org/10.1056/NEJMoa1415236>
- Kessenbrock K, Krumbholz M, Schönemärck U et al (2009) Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med* 15:623–625. <https://doi.org/10.1038/nm.1959>
- Kiliç D, Gunaydin S, Kisa U et al (2009) Clinical efficacy of leukofiltration on cardiopulmonary bypass related inflammatory response: fact or foe? *Inflamm Res* 58:292–297. <https://doi.org/10.1007/s00011-008-7244-1>
- Kolaczowska E, Kubes P (2013) Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol* 13:159–175. <https://doi.org/10.1038/nri3399>
- Kolev M, Le Friec G, Kemper C (2014) Complement-tapping into new sites and effector systems. *Nat Rev Immunol* 14:811–820. <https://doi.org/10.1038/nri3761>
- Korkmaz B, Horwitz MS, Jenne DE, Gauthier F (2010) Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. *Pharmacol Rev* 62:726–759. <https://doi.org/10.1124/pr.110.002733>
- Leino L, Sorvajärvi K, Katajisto J et al (1997) Febrile infection changes the expression of IgG Fc receptors and complement receptors in human neutrophils in vivo. *Clin Exp Immunol* 107:37–43
- Leliefeld PHC, Wessels CM, Leenen LPH et al (2016) The role of neutrophils in immune dysfunction during severe inflammation. *Crit Care (London)* 20:73. <https://doi.org/10.1186/s13054-016-1250-4>
- Ley K, Rivera-Nieves J, Sandborn WJ, Shattil S (2016) Integrin-based therapeutics: biological basis, clinical use and new drugs. *Nat Rev Drug Discov* 15:173–183. <https://doi.org/10.1038/nrd.2015.10>
- Li S, Huang X, Chen Z et al (2013) Neutrophil CD64 expression as a biomarker in the early diagnosis of bacterial infection: a meta-analysis. *Int J Infect Dis* 17:e12–e23. <https://doi.org/10.1016/j.ijid.2012.07.017>
- Liao Y, Liu P, Guo F et al (2013) Oxidative burst of circulating neutrophils following traumatic brain injury in human. *PLoS ONE* 8:e68963. <https://doi.org/10.1371/journal.pone.0068963>
- Liu F-C, Chuang Y-H, Tsai Y-F, H-P Y (2014) Role of neutrophil extracellular traps following injury. *Shock* 41:491–498. <https://doi.org/10.1097/SHK.0000000000000146>
- Livadioti O, Kotanidou A, Psarra A et al (2006) Neutrophil CD64 expression and serum IL-8: sensitive early markers of severity and outcome in sepsis. *Cytokine* 36:283–290. <https://doi.org/10.1016/j.cyt.2007.02.007>
- Mare TA, Treacher DF, Shankar-Hari M et al (2015) The diagnostic and prognostic significance of monitoring blood levels of immature neutrophils in patients with systemic inflammation. *Crit Care (London)* 19:57. <https://doi.org/10.1186/s13054-015-0778-z>
- Margraf S, Lögters T, Reipen J et al (2008) Neutrophil-derived circulating free DNA (cf-DNA/NETs): a potential prognostic marker for post-traumatic development of inflammatory second hit and sepsis. *Shock* 30:352–358. <https://doi.org/10.1097/SHK.0b013e31816a6bb1>
- Martinod K, Fuchs TA, Zitomersky NL et al (2015) PAD4-deficiency does not affect bacteremia in polymicrobial sepsis and ameliorates endotoxemic shock. *Blood* 125:1948–1956. <https://doi.org/10.1182/blood-2014-07-587709>
- Massberg S, Grahl L, Bruehl von M-L et al (2010) Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat Med*. <https://doi.org/10.1038/nm.2184>



- Mathias B, Szpila BE, Moore FA et al (2015) A review of GM-CSF therapy in sepsis. *Medicine* (Baltimore) 94:e2044. <https://doi.org/10.1097/MD.0000000000002044>
- McDonald B, Urrutia R, Yipp BG et al (2012) Intravascular Neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe* 12:324–333. <https://doi.org/10.1016/j.chom.2012.06.011>
- McDonald B, Davis RP, Kim S-J et al (2017) Platelets and neutrophil extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. *Blood* 129:1357–1367. <https://doi.org/10.1182/blood-2016-09-741298>
- Meisel C, Scheffold JC, Pschowski R et al (2009) Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. *Am J Respir Crit Care Med* 180:640–648. <https://doi.org/10.1164/rccm.200903-0363OC>
- Mittal M, Siddiqui MR, Tran K et al (2014) Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* 20:1126–1167. <https://doi.org/10.1089/ars.2012.5149>
- Mohan P, Brocklehurst P (2003) Granulocyte transfusions for neonates with confirmed or suspected sepsis and neutropaenia. *Cochrane database of systematic reviews* (Online) 80:CD003956. <https://doi.org/10.1002/14651858.CD003956>
- Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A (2010) Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol* 191:677–691. <https://doi.org/10.1083/jcb.201006052>
- Pham CTN (2006) Neutrophil serine proteases: specific regulators of inflammation. *Nat Rev Immunol* 6:541–550. <https://doi.org/10.1038/nri1841>
- Phillipson M, Kubes P (2011) The neutrophil in vascular inflammation. *Nat Med* 17:1381–1390. <https://doi.org/10.1038/nm.2514>
- Price TH, Boeckh M, Harrison RW et al (2015) Efficacy of transfusion with granulocytes from G-CSF/dexamethasone-treated donors in neutropenic patients with infection. *Blood* 126:2153–2161. <https://doi.org/10.1182/blood-2015-05-645986>
- Rossaint J, Zarbock A (2015) Platelets in leucocyte recruitment and function. *Cardiovasc Res* 107:386–395. <https://doi.org/10.1093/cvr/cvv048>
- Santos SS, Brunialti MKC, Rigato O et al (2012) Generation of nitric oxide and reactive oxygen species by neutrophils and monocytes from septic patients and association with outcomes. *Shock* 38:18–23. <https://doi.org/10.1097/SHK.0b013e318257114e>
- Simms HH, D'Amico R (1991) Increased PMN CD11b/CD18 expression following post-traumatic ARDS. *J Surg Res* 50:362–367
- Singer M, Deutschman CS, Seymour CW, et al (2016) The third international consensus definitions for sepsis and septic shock (Sepsis-3). Pp 801–810
- Sónego F, Castanheira FVES, Ferreira RG et al (2016) Paradoxical roles of the Neutrophil in sepsis: protective and deleterious. *Front Immunol* 7:155. <https://doi.org/10.3389/fimmu.2016.00155>
- Sprung CL, Annane D, Keh D et al (2008) Hydrocortisone therapy for patients with septic shock. *N Engl J Med* 358:111–124. <https://doi.org/10.1056/NEJMoa071366>
- Stark MA, Huo Y, Burcin TL et al (2005) Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. *Immunity* 22:285–294. <https://doi.org/10.1016/j.immuni.2005.01.011>
- Tak T, Wijten P, Heeres M et al (2017) Human CD62L(dim) neutrophils identified as a separate subset by proteome profiling and in vivo pulse-chase labeling. *Blood* 129:3476–3485. <https://doi.org/10.1182/blood-2016-07-727669>
- Taneja R, Parodo J, Jia SH et al (2004) Delayed neutrophil apoptosis in sepsis is associated with maintenance of mitochondrial transmembrane potential and reduced caspase-9 activity. *Crit Care Med* 32:1460–1469
- Taylor NJ, Nishtala A, Manakkat Vijay GK et al (2013) Circulating neutrophil dysfunction in acute liver failure. *Hepatology* 57:1142–1152. <https://doi.org/10.1002/hep.26102>
- Thälín C, Daleskog M, Göransson SP et al (2017) Validation of an enzyme-linked immunosorbent assay for the quantification of citrullinated histone H3 as a marker for neutrophil extracellular traps in human plasma. *Immunol Res* 65:706–712. <https://doi.org/10.1007/s12026-017-8905-3>
- Treacher DF, Sabbato M, Brown KA, Gant V (2001) The effects of leucodepletion in patients who develop the systemic inflammatory response syndrome following cardiopulmonary bypass. *Perfusion* 16(Suppl):67–73. <https://doi.org/10.1177/026765910101600i110>
- Vincent J-L, Sun Q, Dubois M-J (2002) Clinical trials of immunomodulatory therapies in severe sepsis and septic shock. *Clin Infect Dis* 34:1084–1093. <https://doi.org/10.1086/339549>
- Welty-Wolf KE, Carraway MS, Huang YC et al (2001) Antibody to intercellular adhesion molecule 1 (CD54) decreases survival and not lung injury in baboons with sepsis. *Am J Respir Crit Care Med* 163:665–673. <https://doi.org/10.1164/ajrccm.163.3.2004191>
- Wu Y, Wang F, Fan X et al (2012) Accuracy of plasma sTREM-1 for sepsis diagnosis in systemic inflammatory patients: a systematic review and meta-analysis. *Crit Care (London)* 16:R229. <https://doi.org/10.1186/cc11884>
- Xu J, Zhang X, Pelayo R et al (2009) Extracellular histones are major mediators of death in sepsis. *Nat Med* 15:1318–1321. <https://doi.org/10.1038/nm.2053>
- Yang S, Qi H, Kan K et al (2017) Neutrophil extracellular traps promote Hypercoagulability in patients with sepsis. *Shock* 47:132–139. <https://doi.org/10.1097/SHK.0000000000000741>
- Yipp BG, Kim JH, Lima R et al (2017) The lung is a host Defense niche for immediate Neutrophil-mediated vascular protection. *Sci Immunol* 2:eaam8929. <https://doi.org/10.1126/sciimmunol.aam8929>