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Neutrophils mitigate the systemic host response during endotoxemia in mice

Anne Jan van der Meer,[1,†] Chao Ding,[1,2,†] Arie Johan Hoogendijk,[1] Alex F. de Vos,[1] Louis Boon,[3] Sacha S. Zeerleder[4,5,6] and Tom van der Poll[1,7]

[1]*Center of Experimental & Molecular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands,* [2]*Department of Gastric Surgery, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, China,* [3]*Bioceros, Utrecht, the Netherlands,* [4]*Department of Haematology and Central Haematology Laboratory, Inselspital, Bern University Hospital, University of Bern, Bern,* [5]*Department for BioMedical Research, Bern University Hospital, University of Bern, Bern, Switzerland,* [6]*Department of Immunopathology, Sanquin Research, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, and and* [7]*Division of Infectious Diseases Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands*

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[†]These authors contributed equally.
Correspondence: Anne Jan van der Meer, Academic Medical Center, Meibergdreef 9, G2-130, 1105 AZ Amsterdam, the Netherlands. Email: ajvdrmeer@gmail.com
Senior author: Tom van der Poll, Email: t.vanderpoll@amc.uva.nl

Summary

Recent studies have suggested that neutrophils can exert anti-inflammatory effects. To determine the role of neutrophils in the acute response to lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, we challenged neutrophil-depleted and control mice with LPS and analyzed the plasma concentrations of biomarkers indicative of the cytokine and chemokine network, activation of coagulation and the vascular endothelium, and cellular injury. We here show that neutrophils serve an anti-inflammatory role upon LPS administration, as reflected by sustained elevations of multiple cytokines and chemokines, and enhanced release of nucleosomes in mice depleted of neutrophils, compared with control mice.

Keywords: chemokines; cytokines; inflammation; neutrophil.

Introduction

Neutrophils are traditionally considered to be important players in acute inflammation with a key function in the elimination of extracellular pathogens.[1] Recent investigations have indicated that the role of neutrophils in immunity stretches beyond a limited set of pro-inflammatory actions in the innate immune response.[2] Neutrophils have been implicated in chronic inflammatory conditions and the regulation of adaptive immune responses, and a subset of neutrophils may even exert anti-inflammatory effects.[1,2] In addition, neutrophils may cause a misbalance in hemostasis through activation of coagulation and concurrent inactivation of natural anticoagulants.[3]

Intravenous administration of lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, has been used as a model of acute systemic inflammation in humans and rodents, and is accompanied by a strong increase in neutrophil counts in peripheral blood.[4] Intravenous LPS injection in humans was associated with the appearance of a neutrophil subset in

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the circulation that was capable of suppressing T-cell proliferation and less able to adhere to endothelial cells.[5,6] Significant numbers of these 'anti-inflammatory' neutrophils were detected several hours after LPS administration and these anti-inflammatory neutrophils were also found in patients with a severe injury. Depletion of neutrophils in mice before LPS injection resulted in higher plasma levels of interleukin-1β (IL-1β) when compared with animals not depleted of neutrophils.[7] The aim of the present study was to determine the role of these anti-inflammatory neutrophils in the systemic inflammatory, vascular and procoagulant responses to LPS.

Material and methods

Female C57BL/6 mice were purchased from Charles River Inc. (Maastricht, the Netherlands). Mice were housed under specific pathogen-free conditions receiving food and water *ad libitum*. Experiments were carried out in accordance with the Dutch Experiments on Animals Acts and approved by the Animal Care and Use Committee of the University of Amsterdam (permit number DIX102291). Mice were studied at 12 weeks of age. Endotoxemia was induced by intraperitoneal injection of LPS (from *Escherichia coli* 055:B5; Sigma-Aldrich, St Louis, MO; 10 mg/kg in 200 µl 0·9% NaCl). Mice were depleted of neutrophils by intraperitoneal injection with anti-mouse-GR1 (clone RB6.8C5; rat IgG IgG2b, κ; 100 µg) 24 hr before LPS administration.[8] Control mice were given the equivalent amount of isotype control antibody anti-phytochrome (clone AFRC Mac 5.1; rat IgG IgG2b, κ). Ethylenediaminetetraacetic acid anticoagulated plasma was obtained before, and 4 and 24 hr after LPS injection (n = 8 mice per group at each time-point). White blood cell counts were determined in a hemocytometer (Beckman Coulter, Fullerton, CA), and differential cell counts were performed on cytospin preparations stained with Giemsa stain (Diff-Quick; Dade Behring, Düdingen, Switzerland).

Tumor necrosis factor (TNF), IL-10 and interferon-γ (IFN-γ) were determined using a commercially available cytometric beads array multiplex assay (BD Biosciences, Breda, the Netherlands). IL-6, CXCL1, CXCL2, soluble vascular cell adhesion molecule 1 (VCAM-1), soluble E-selectin (all R&D Systems, Abingdon, UK), thrombin–antithrombin complexes (TATc; Affinity Biologicals INC, Ancaster, Canada) and nucleosomes[9] were determined by enzyme-linked immunosorbent assay. Plasma creatinine and aspartate aminotransferase (AST) were measured using a c702 Roche Diagnostics (Roche Diagnostics BV, Almere, the Netherlands). Groups were compared by Kruskal–Wallis test followed by Mann–Whitney U test where appropriate. $P < 0{\cdot}05$ was considered statistically significant.

Results and discussion

Lipopolysaccharide injection induced a brisk rise in neutrophil counts in peripheral blood in mice treated with the control antibody (Fig. 1). Mice treated with anti-GR1 antibody showed a persistent neutropenia. Cytokine and chemokine responses were more sustained in neutrophil-depleted mice; all cytokines (TNF, IL-6, IL-10, IFN-γ) and chemokines (CXCL1, CXCL2) measured were significantly higher in mice with neutropenia 24 hr after injection of LPS (Fig. 1).

To obtain insight into the role of neutrophils in the procoagulant and endothelial cell response to LPS we measured the plasma levels of TATc, soluble VCAM-1 and soluble E-selectin (Fig. 2). The plasma concentration of TATc is a measure of the generation of thrombin and its subsequent binding of its natural inhibitor antithrombin, thereby reflecting activation of the coagulation system. Baseline TATc concentrations were lower in neutrophil-depleted mice when compared with control mice; however, the LPS induced increase in plasma TATc levels did not differ between groups. Likewise, neutrophil depletion did not modify the LPS-induced increase in plasma soluble VCAM-1 levels, although at 24 hr post-LPS soluble VCAM-1 concentrations tended to be lower in neutrophil-depleted mice. Remarkably, neutrophil depletion strongly reduced plasma soluble E-selectin concentrations; this effect was already detectable before LPS injection and the LPS-induced rise in soluble E-selectin levels was virtually absent in neutrophil-depleted mice.

Nucleosomes, which are segments of DNA wound around histone protein cores, can be released from dead cells by the activity of endonucleases and factor VII-activating protease.[10] Nucleosomes detected in the circulation have been used as a marker of inflammation.[11] Nucleosomes were not detectable in plasma before LPS injection (Fig. 2). The LPS induced an increase in plasma nucleosome concentrations in control mice, but notably the increase in neutrophil-depleted mice was much higher. Plasma markers of organ injury did not show differences between neutrophil-depleted and control mice: creatinine (renal function) and alanine aminotransferase (liver injury) did not increase upon LPS administration in either group (data not shown), whereas AST (indicative of hepatocellular injury) similarly increased in both groups after the induction of endotoxemia (Fig. 2), showing the specificity of the strong increase in plasma nucleosome concentration in anti-GR1-treated mice. Notably, the LPS-induced vascular and injurious responses had not yet declined at 24 hr; but our study does not provide insight into possible effects of neutrophil depletion beyond 24 hr after LPS administration.

Neutrophils are at the front line of the innate immune system and essential elements in host defense,

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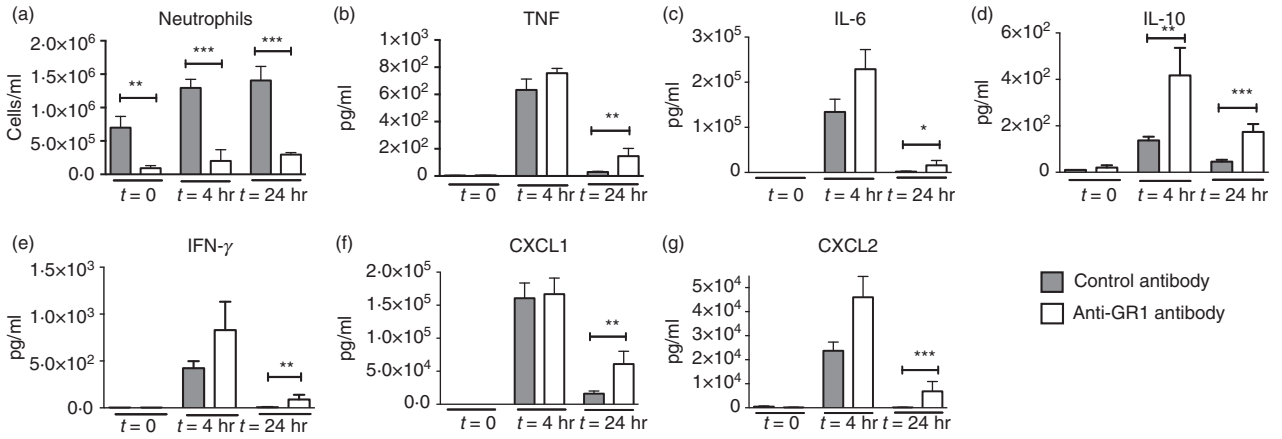

Figure 1. Neutrophil depletion is associated with sustained elevated cytokine and chemokine levels after intraperitoneal lipopolysaccharide (LPS) injection. Neutrophil counts (a), cytokines [tumor necrosis factor (TNF), interleukin-6 (IL-6), IL-10 and interferon-γ (IFN-γ)] (b–e) and chemokines (CXCL1 and CXCL2) (f, g) plasma levels, 0, 4 and 24 hr after intraperitoneal LPS injection in mice treated with a control or a neutrophil-depleting antibody. Data are mean ± SE of eight mice per group. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ and ***$P < 0{\cdot}001$ versus mice receiving a control antibody at the same time-point.

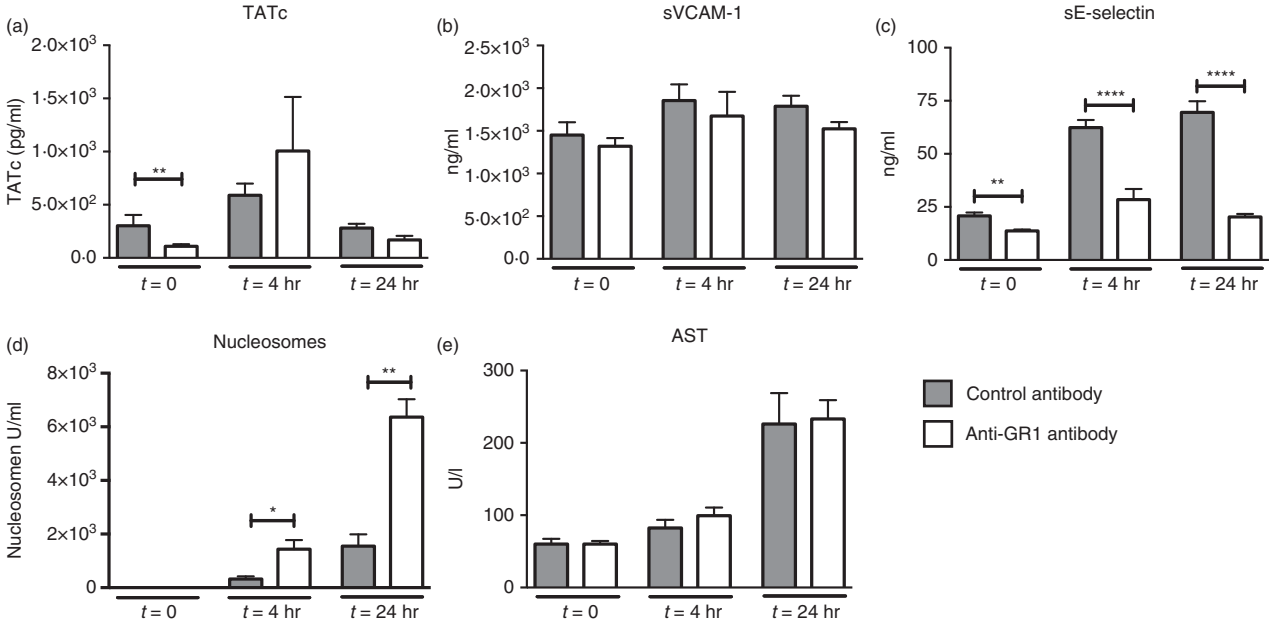

Figure 2. Influence of neutrophil depletion on coagulation activation, endothelial cell activation and cell injury after lipopolysaccharide (LPS) injection. Thrombin–antithrombin complexes (TATc) (a), soluble vascular cell adhesion molecule 1 (sVCAM-1) (b) and sE-selectin (c), nucleosomes (d) and aspartate aminotransferase (AST) (e) plasma levels 0, 4 and 24 hr after intraperitoneal LPS injection in mice treated with control and a neutrophil-depleting antibody. Data are mean ± SE of eight mice per group. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ and ****$P < 0{\cdot}0001$ versus mice receiving a control antibody at the same time-point.

especially against extracellular pathogens.[1,2] Excessive activity of neutrophils may cause tissue injury in non-infectious conditions and severe infection, exemplifying the strong pro-inflammatory nature of these cells. Recent evidence has indicated, however, that neutrophils can also exert immune modulating and anti-inflammatory effects.[1,2] We have studied the role of neutrophils in the systemic host reaction elicited by LPS by comparing cytokine, procoagulant, vascular and damage responses in mice with or without depletion of neutrophils. Our main finding was that neutrophil depletion was associated with exaggerated responses to LPS, suggesting anti-inflammatory activity of neutrophils under these conditions.

Neutrophil depletion resulted in an enhanced cytokine and chemokine response to intravenous LPS, which

involved both pro-inflammatory mediators and the anti-inflammatory cytokine IL-10. In accordance, LPS-induced TNF and IL-1β production by human peripheral blood mononuclear cells was lowered in the presence of neutrophils.[7] This inhibitory effect of neutrophils was partially reversed by the addition of α1-antitrypsin, suggesting the involvement of a protease.[7] Indeed, elastase was shown to degrade TNF and IL-1β; however, these data are unlikely to explain our present results, considering that elastase cannot degrade IL-10,[7] whereas IL-10 levels were consistently higher in neutrophil-depleted mice.

Neutrophils can also influence activation of the coagulation system by producing neutrophil extracellular traps, which can promote tissue-factor-mediated coagulation, and through degranulation with release of elastase, which can inactivate natural anticoagulants such as tissue factor pathway inhibitor and thrombomodulin.[3] These procoagulant effects mediated by neutrophils might explain at least in part the lower plasma TATc levels in neutrophil-depleted mice before LPS injection. The current finding that neutrophil depletion did not modify the LPS-induced rise in plasma TATc levels suggests that neutrophils do not contribute to the initiation of coagulation during endotoxemia. Although these data do not exclude a role for neutrophils in microvascular thrombosis during infection and sepsis, in our model they do not play a major role. Moreover, our results do not exclude a role for neutrophils extracellular traps (NETs) in LPS-induced systemic inflammation.

During inflammation neutrophils adhere to the vascular endothelium, which is associated with pro-inflammatory changes in both neutrophils and endothelial cells.[1] Neutrophil depletion strongly reduced soluble E-selectin levels, while not affecting the concentrations of soluble VCAM-1. Both proteins are shed by endothelial cells and their plasma concentrations have been used as markers of endothelial cell activation.[12] Although the release of soluble VCAM-1 from endothelial cells can be induced by several ADAM (a disintegrin and metalloprotease) metallopeptidases (ADAM8, ADAM9 and ADAM17), the protease(s) responsible for the shedding of E-selectin remain to be discovered.[13,14] Besides ADAMs, members of two additional protease families are important for proteolytic cleavage of cell surface transmembrane proteins with release of a soluble extracellular domain fragment: metalloproteinases and soluble neutrophil-derived serine proteinases.[13] It is tempting to speculate that the release of soluble E-selectin was reduced in neutrophil-depleted mice because of the lack of a neutrophil-derived protease.

Nucleosomes are released from dying and injured cells into the extracellular environment. The plasma concentrations of nucleosomes are elevated in a variety of diseases, including cancers, stroke, trauma and sepsis[15] We here show that LPS injection induces a rise in the plasma levels of nucleosomes. This increase was greater in neutrophil-depleted mice, suggesting that neutrophils limit cell death during endotoxemia and that neutrophils are not a predominant source of nucleosomes during LPS-induced injury. Unaltered plasma levels of creatinine, and the similarly modestly elevated plasma levels of AST, in both neutrophil-depleted and control mice suggest that the differential release of nucleosomes in both groups does not originate from kidneys or hepatocytes.

Neutrophils present a large phenotypic heterogeneity and functional diversity, which partially relate to their location (blood or tissues). Our study does not provide conclusive information on which neutrophil subset drives the anti-inflammatory effects exposed here by neutrophil depletion. In addition, our study was limited to markers of systemic inflammatory, vascular and procoagulant responses and did not investigate the effect of neutrophil depletion on mortality.

The data presented here suggest that neutrophilic leukocytosis induced by intravenous LPS at least in part serves an anti-inflammatory role, as reflected by sustained elevations in the plasma concentrations of multiple cytokines and chemokines, and enhanced release of nucleosomes in the circulation during endotoxemia in mice depleted of neutrophils compared with non-depleted control mice.

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Authorship

AJM and CD participated in the design of the study, performed the research and did the analysis and interpretation of data and the writing of the manuscript. AJH participated in the design of the study, collection and processing of samples and analysis and interpretation of data. AFV participated in the design of the study and analysis and interpretation of data. LB participated in the design of the study and contributed essential reagents. SSZ participated in the design of the study and analysis and interpretation of data. TvdP was responsible for study design, data interpretation and manuscript writing.

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All authors read, commented on and approved the final manuscript.

Disclosures

The authors declare no conflict of interest.

References

1 Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol* 2013; **13**:159–75.

2 Jaillon S, Galdiero MR, Del Prete D, Cassatella MA, Garlanda C, Mantovani A. Neutrophils in innate and adaptive immunity. *Semin Immunopathol* 2013; **35**:377–94.

3 Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol* 2013; **13**:34–45.

4 van der Poll T. Preclinical sepsis models. *Surg Infect (Larchmt)* 2012; **13**:287–92.

5 Kamp VM, Pillay J, Lammers J-WJ, Pickkers P, Ulfman LH, Koenderman L. Human suppressive neutrophils $CD16^{bright}/CD62L^{dim}$ exhibit decreased adhesion. *J Leukoc Biol* 2012; **92**:1011–20.

6 Pillay J, Kamp VM, van Hoffen E, Visser T, Tak T, Lammers JW, *et al.* A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. *J Clin Invest* 2012; **122**:327–36.

7 Gresnigt MS, Joosten LAB, Verschueren I, van der Meer JW, Netea MG, Dinarello CA, *et al.* Neutrophil-mediated inhibition of proinflammatory cytokine responses. *J Immunol* 2012; **189**:4806–15.

8 Leendertse M, Willems RJL, Giebelen IAJ, Roelofs JJTH, Bonten MJM, van der Poll T. Neutrophils are essential for rapid clearance of *Enterococcus faecium* in mice. *Infect Immun* 2009; **77**:485–91.

9 van der Windt GJW, Schouten M, Zeerleder S, Florquin S, van der Poll T. CD44 is protective during hyperoxia-induced lung injury. *Am J Respir Cell Mol Biol* 2011; **44**:377–83.

10 Zeerleder S, Zwart B, te Velthuis H, Stephan F, Manoe R, Rensink I *et al.* Nucleosome-releasing factor: a new role for factor VII-activating protease (FSAP). *FASEB J* 2008; **22**:4077–84.

11 Zeerleder S, Zwart B, Wuillemin WA, Aarden LA, Groeneveld AB, Caliezi C, *et al.* Elevated nucleosome levels in systemic inflammation and sepsis. *Crit Care Med* 2003; **31**:1947–51.

12 Opal SM, van der Poll T. Endothelial barrier dysfunction in septic shock. *J Intern Med* 2015; **277**:277–93.

13 Garton KJ, Gough PJ, Raines EW. Emerging roles for ectodomain shedding in the regulation of inflammatory responses. *J Leukoc Biol* 2006; **79**:1105–16.

14 Dreymueller D, Pruessmeyer J, Groth E, Ludwig A. The role of ADAM-mediated shedding in vascular biology. *Eur J Cell Biol* 2012; **91**:472–85.

15 Holdenrieder S, Stieber P. Clinical use of circulating nucleosomes. *Crit Rev Clin Lab Sci* 2009; **46**:1–24.