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Phenylephrine impairs host defence mechanisms to infection: a combined laboratory study in mice and translational human study

Roeland F. Stolk[1,2,3], Flavia Naumann[1,2], Eva van der Pasch[1,2], Joost Schouwstra[1,2], Steffi Bressers[1,2], Antonius E. van Herwaarden[4], Jelle Gerretsen[1,2], Roel Schambergen[1,2], Mike Ruth[2,5], Hans G. van der Hoeven[1,2], Henk J. van Leeuwen[1,3], Peter Pickkers[1,2] and Matthijs Kox[1,2,*]

[1]Department of Intensive Care Medicine, Radboud University Medical Center, Nijmegen, The Netherlands, [2]Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, The Netherlands, [3]Department of Intensive Care Medicine, Hospital Rijnstate, Arnhem, The Netherlands, [4]Department of Laboratory Medicine, Radboud University Medical Center, Nijmegen, The Netherlands and [5]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands

*Corresponding author. E-mail: matthijs.kox@radboudumc.nl

Abstract

Background: Immunosuppression after surgery is associated with postoperative complications, mediated in part by catecholamines that exert anti-inflammatory effects via the β-adrenergic receptor. Phenylephrine, generally regarded as a selective α-adrenergic agonist, is frequently used to treat perioperative hypotension. However, phenylephrine may impair host defence through β-adrenergic affinity.

Methods: Human leukocytes were stimulated with lipopolysaccharide (LPS) in the presence or absence of phenylephrine and α- and β-adrenergic antagonists. C57BL/6J male mice received continuous infusion of phenylephrine (30–50 μg kg^{-1} min^{-1} i.v.) or saline via micro-osmotic pumps, before LPS administration (5 mg kg_{-1} i.v.) or caecal ligation and puncture (CLP). Twenty healthy males were randomised to a 5 h infusion of phenylephrine (0.5 μg kg^{-1} min^{-1}) or saline before receiving LPS (2 ng kg^{-1} i.v.).

Results: *In vitro*, phenylephrine enhanced LPS-induced production of the anti-inflammatory cytokine interleukin (IL)-10 (maximum augmentation of 93%) while attenuating the release of pro-inflammatory mediators. These effects were reversed by pre-incubation with β-antagonists, but not α-antagonists. Plasma IL-10 levels were higher in LPS-challenged mice infused with phenylephrine, whereas pro-inflammatory mediators were reduced. Phenylephrine infusion increased bacterial counts after CLP in peritoneal fluid (+42%, P=0.0069), spleen (+59%, P=0.04), and liver (+35%, P=0.09). In healthy volunteers, phenylephrine enhanced the LPS-induced IL-10 response (+76%, P=0.0008) while attenuating plasma concentrations of pro-inflammatory mediators including IL-8 (−15%, P=0.03).

Conclusions: Phenylephrine exerts potent anti-inflammatory effects, possibly involving the β-adrenoreceptor. Phenylephrine promotes bacterial outgrowth after surgical peritonitis. Phenylephrine may therefore compromise host defence in surgical patients and increase susceptibility towards infection.

Clinical trial registration: NCT02675868 (Clinicaltrials.gov).

Keywords: endotoxaemia; host defense; immunosuppression; LPS; phenylephrine; surgical peritonitis, cytokines; β-adrenergic receptor

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Editor's key points

- Immunosuppression after surgery, which is associated with infection, is promoted by elevated levels of catecholamines acting via the β-adrenergic receptor.
- In human whole blood and monocytes, b-antagonists reversed the increasing effect of phenylephrine on lipopolysaccharide (LPS)-induced production of the anti-inflammatory cytokine interleukin (IL)-10.
- Phenylephrine infusion in healthy human volunteers also enhanced the LPS-induced IL-10 response, while reducing circulating pro-inflammatory mediators.
- Phenylephrine infusion in mice increased bacterial load after caecal ligation and puncture.
- Phenylephrine exerts potent anti-inflammatory effects, possibly mediated via the β-adrenergic receptor, which may compromise host defence in surgical patients.

Immunosuppression after major surgery is a well-recognised phenomenon with detrimental clinical consequences,[1] for which there is currently no targeted treatment strategy. Surgery is accompanied by the release of both danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), the latter through translocation of bacterial (components) from the gut. In the perioperative setting, both DAMPs and PAMPs can bind to pattern recognition receptors and thereby elicit an inflammatory response, which is followed by suppression of host immunity.[2,3] In addition to increasing the risk for postoperative infections, this immune suppression can have protracted effects, such as increased risk of metastases in cancer patients.[4,5]

There are several indications that catecholamines may play a role in these detrimental sequelae.[6,7] In accordance, studies in other settings have shown that catecholamines exert anti-inflammatory effects, such as decreased production of pro-inflammatory cytokines and increased release of the archetypical anti-inflammatory cytokine interleukin (IL)-10, via stimulation of the β-adrenergic receptor.[8–11] Phenylephrine, a synthetic sympathomimetic, is frequently used to treat hypotension in the perioperative setting. Phenylephrine is widely regarded as a selective α-adrenergic agonist[12]; however, there are indications that it may exert some β-adrenergic affinity as well.[13] For instance, β-adrenergic effects were unmasked in experiments where phenylephrine induced vasodilatation in the human forearm under selective α-adrenergic blockade with phentolamine.[14] Furthermore, this effect was counteracted by pre-treatment with the β-blocker propranolol.[14] Through these putative β-adrenergic effects, perioperative use of phenylephrine could contribute to surgery-induced immunosuppression.

However, immunological effects of phenylephrine have only been sparsely studied. *In vitro*, phenylephrine modestly enhanced IL-10 production upon stimulation with bacterial endotoxin (lipopolysaccharide [LPS])[9] but exerted no effects on LPS-induced production of tumour necrosis factor (TNF)-α or IL-6 in human whole blood stimulation experiments.[8] *In vivo* immunological evidence is limited to studies in rats focused on cardiac inflammation, suggesting anti-inflammatory effects.[15,16]

In the present study, we evaluated the immunological effects of phenylephrine *in vitro* in animal models of inflammation and surgical peritonitis, and *in vivo* in humans.

Methods

Detailed information on study procedures, assays used, and analysis methods are described in the Supplementary data.

Subjects

In vivo human endotoxaemia experiments and blood withdrawal for *in vitro* studies were carried out in accordance with the declaration of Helsinki after approval of the local ethics committee of the Radboud University Medical Centre (CMO identifiers 2015–2079 and 2010–10, respectively). The human endotoxaemia experiments were registered at Clinicaltrials.gov (identifier NCT02675868). All subjects provided written informed consent. All animal procedures were approved by the local animal ethics committee in Nijmegen and by the Dutch Council for Animal Care (identifier AVD103002016447). The Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) checklist for animal experimentation is included in the online data supplement. The murine surgical peritonitis and human endotoxaemia experiments were part of a broader project investigating (immunologic) effects of vasopressors.[11,17,18] Therefore, data of control groups have been published previously as detailed below.

Whole blood and monocyte stimulation experiments

Lithium heparin-anticoagulated venous blood was diluted five times in culture medium (RPMI 1640 Dutch modification [Invitrogen] supplemented with 10 μg ml^{-1} gentamicin, 10 mM Glutamax, and 10 mM pyruvate). This culture medium was used for all *in vitro* experiments (referred to as RPMI). Monocytes were isolated from the peripheral blood mononuclear cell (PBMC) fraction after Ficoll density gradient centrifugation using negative selection magnetic separation (Miltenyi Biotec, Bergisch Gladbach, Germany). All whole blood and monocyte stimulation experiments were performed in duplicate.

Concentrations of the different compounds are provided in the figure legends. Diluted blood or monocytes (1×10^5) were pre-incubated in 96-well round bottom plates with phenylephrine (Sigma-Aldrich P6126; Sigma-Aldrich, St. Louis, MO, USA) or RPMI (control) for 60 min, after which 10 ng ml^{-1} *Escherichia coli* LPS (serotype 055:B5) or RPMI was added. After 24 h of incubation at 37°C and 5% CO_2, plates were centrifuged and supernatants were collected and stored at −80°C until analysis. Additional monocyte and whole blood experiments were performed using adrenergic receptor (AR) antagonists (prazosin hydrochloride [α1 antagonist, Sigma-Aldrich P791]; yohimbine hydrochloride [α2 antagonist, Sigma-Aldrich Y3125], propranolol hydrochloride [non-selective β-antagonist, Sigma-Aldrich P0884], and ICI-118,551 hydrochloride [β2 antagonist, Sigma-Aldrich I127]), and using the protein kinase A (PKA) inhibitor H89 (Tocris 2910; Tocris Bioscience, Bristol, UK). Blood or monocytes were pre-incubated with AR antagonists for 30 min before the addition of phenylephrine or RPMI.

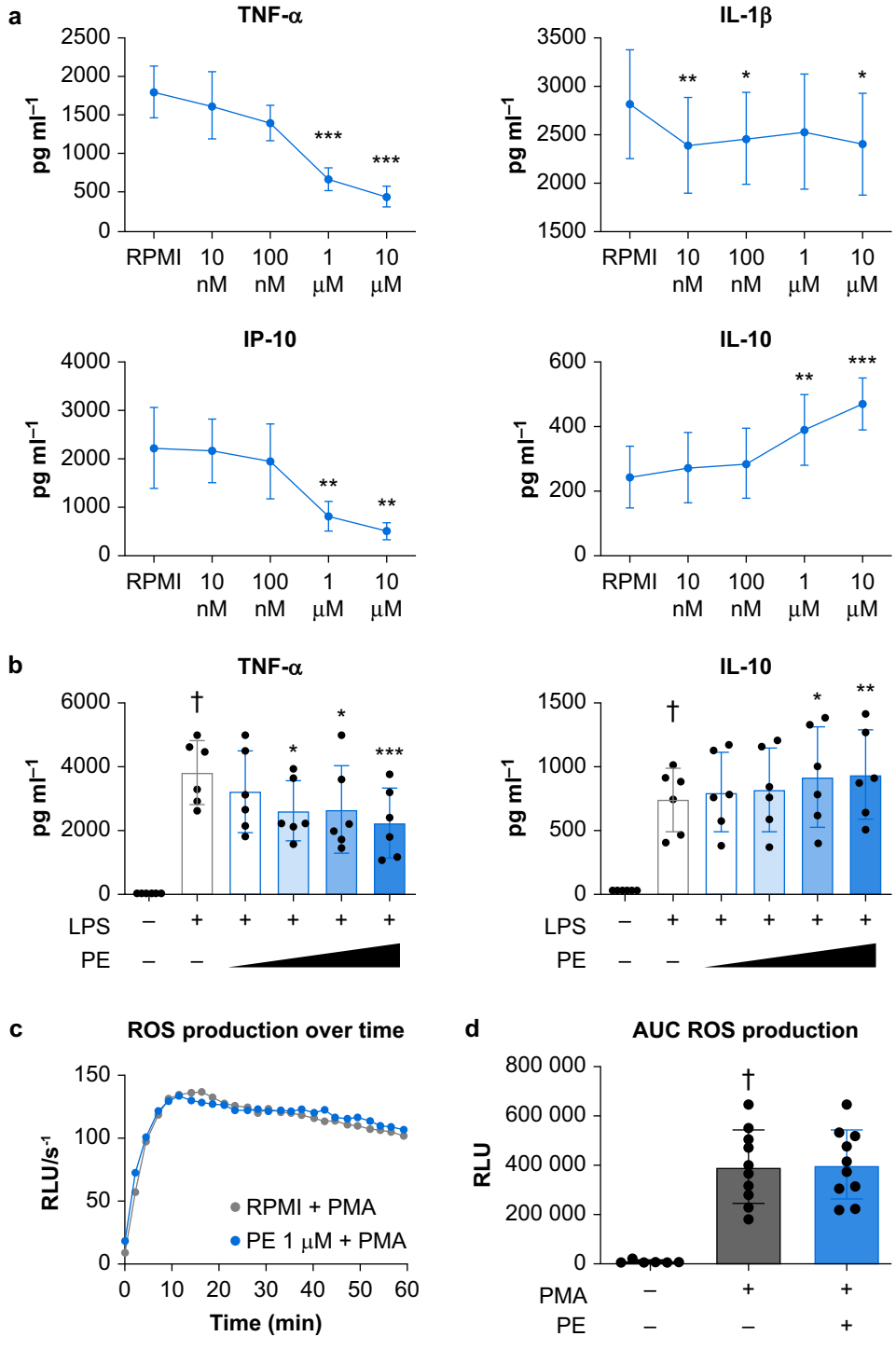

Fig 1. Phenylephrine modulates lipopolysaccharide (LPS)-induced cytokine production by human whole blood and primary human monocytes, whereas phorbol 12-myristate 13-acetate (PMA)-induced reactive oxygen species production is unaffected. (a) Concentrations of tumour necrosis factor (TNF)-α, induced protein (IP)-10, interleukin (IL)-1β, and IL-10 in supernatant of human whole blood pre-incubated with either RPMI (medium control), phenylephrine (PE) in escalating concentrations for 1 h and subsequently stimulated with LPS (10 ng ml^{-1}) for 24 h. (b) Concentrations of TNF-α and IL-10 in supernatant of primary human monocytes pre-incubated with either RPMI or phenylephrine in escalating concentrations (10 nM, 100 nM, 1 μM, 10 μM) for 1 h and subsequently stimulated with LPS (10 ng ml^{-1}) or RPMI for 24 h. (c and d) Primary human monocytes were incubated with RPMI (medium control) or PE (1 μM) for 1 h and subsequently stimulated with PMA (50 ng ml^{-1}) or RPMI in the presence of luminol, and luminescence, reflecting reactive oxygen species (ROS) production, was measured for 1 h. (c) Relative light units (RLU) per second; (d) area under the curve (AUC) of the data presented in panel (c). Data are expressed as mean (standard deviation, SD) (a), individual data points and mean (SD) (b and d), or means (c) of 6–10 individual donors. †$P<0.05$ compared with RPMI; *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with LPS calculated using one-way analysis of variance with Dunnett's *post hoc* tests (a and b) or a t-test (d).

Reactive oxygen species production

A luminol-based luminescence assay was used to determine the production of reactive oxygen species (ROS) by monocytes, with each sample was measured in quadruplicate (intra-assay CV%, 5.2). Monocytes (1×10^5 well^{-1}) were incubated (37°C, 5% CO_2) with phenylephrine or RPMI for 60 min in flat-bottom 96-well plates (Eppendorf; Sigma-Aldrich). After addition of 100 μM luminol and 50 ng ml^{-1} phorbol 12-myristate 13-acetate (PMA), chemiluminescence was determined every 142 s at 37°C during 1 h. Cell death was quantified by was used to measure levels of lactate dehydrogenase (LDH) in stimulated whole blood culture supernatants (Cytotox96 colorimetric assay; Promega, Madison, WI, USA).

Murine *in vivo* studies

Male C57Bl/6J mice (Charles River, Germany) aged 6–9 weeks, used for all experiments, were housed in a light- and temperature-controlled room under specific pathogen-free conditions.

Micro-osmotic pumps

Isoflurane 5% for 2 min was used to induce anaesthesia, after which mice were anaesthetised with isoflurane 2–2.5% to maintain spontaneous respiration. Depth of anaesthesia was ascertained using the pedal withdrawal reflex.[19] Micro-osmotic pumps (model 2001; Alzet Corporation, Cupertino, CA, USA) with connected jugular vein catheters (0007701; Alzet Corporation) were used in all murine experiments for continuous intravenous phenylephrine or phosphate-buffered saline (PBS) delivery.

Lipopolysaccharide challenge model

Micro-osmotic pumps filled with phenylephrine (50 μg kg^{-1} min^{-1}) or PBS were implanted 3 h before i. v. LPS/saline challenge in 24 mice. Mice were killed by exsanguination under deep isoflurane anaesthesia 90 min after LPS or saline.

Caecal ligation and puncture

Caecal ligation and puncture (CLP; 60% ligation, 21G needle) or a sham operation was performed in 23 mice followed by placement of micro-osmotic pumps, filled with phenylephrine (30 μg kg^{-1} min^{-1}) or PBS. Mice were killed 48 h later. Plasma cytokine and bacterial count data of the sham and PBS + saline groups were published previously.[11]

Human *in vivo* studies of systemic inflammation

Twenty healthy male volunteers participated in a randomised double-blind placebo-controlled experimental endotoxaemia study. Subjects were randomised to receive either phenylephrine (0.5 μg kg^{-1} min^{-1}; Beacon Pharmaceuticals Ltd) for 5 h or sterile saline. To elicit a systemic inflammatory response, 2 ng kg^{-1} U.S. Reference *E. coli* LPS (serotype O:113; Clinical Center Reference Endotoxin, National Institute of Health, Bethesda, MD, USA) was administered i. v. 1 h after the start of phenylephrine/placebo infusion. Data from placebo infusions were published previously.[11]

Randomisation and masking

An online random number generator (https://www.sealedenvelope.com) was used to randomise mice. Human participants were randomised by research nurses not otherwise involved in the study using the sealed envelope method. All biochemical assays were performed in a blinded fashion.

Statistical analysis

Data are presented as mean (standard deviation, SD) or median and inter-quartile range (IQR) or (min–max), depending on distribution (determined using Shapiro–Wilk tests). Data were analysed using one-way analysis of variance (ANOVA) followed by Dunnet's post-hoc tests, *t*-tests, Mann–Whitney *U* tests, or Wilcoxon matched pairs tests. For the experimental human endotoxaemia data, differences between phenylephrine and placebo groups over time were analysed using repeated measures two-way ANOVA (interaction term: time × treatment). A P value <0.05 was considered statistically significant. Statistical tests used are also specified in the figure legends. Note that for the caecal ligation and puncture experiments, it was not always possible to obtain sufficient blood to perform all measurements because of severity of illness, resulting in inadequate blood flow, leading to lower numbers of replicates for some of the presented data. Analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA, USA).

Sample size estimation

For *in vitro* monocyte and whole blood stimulation experiments, sample size was based on previous *in vitro* experiments with vasopressors.[11] For the human and murine endotoxaemia experiments, sample size was calculated a *priori* based on earlier TNF-α results from previous experiments.[20] For the murine CLP experiments, spleen CFU data from pilot experiments were used to calculate the appropriate sample size. Sample size was increased by 20% accommodate for loss of animals during the experiment.

Results

Effect of phenylephrine on responses of primary human leukocytes and monocytes to LPS and on ROS production

In LPS-stimulated whole blood, phenylephrine dose-dependently attenuated production of pro-inflammatory mediators TNF-α, IL-1β, and interferon gamma-induced protein (IP)-10 (maximum inhibition of 75%, 15%, and 77%, respectively; Fig. 1a). In contrast, release of the anti-inflammatory cytokine IL-10 was enhanced by phenylephrine in a dose-dependent fashion (maximum augmentation of 93%; Fig. 1a). Phenylephrine did not affect cell survival (Supplementary Fig. S1). In keeping with the fact that monocytes are the main cytokine producers in short-term LPS stimulation experiments,[21] we confirmed that phenylephrine dose-dependently attenuated the production of TNF-α and enhanced the release of IL-10 in isolated primary human monocytes (Fig. 1b). In addition to cytokine production, we assessed phenylephrine's effect on radical oxygen species (ROS) production, another important innate anti-bacterial defence mechanism. Phenylephrine did not affect ROS production by monocytes stimulated with the

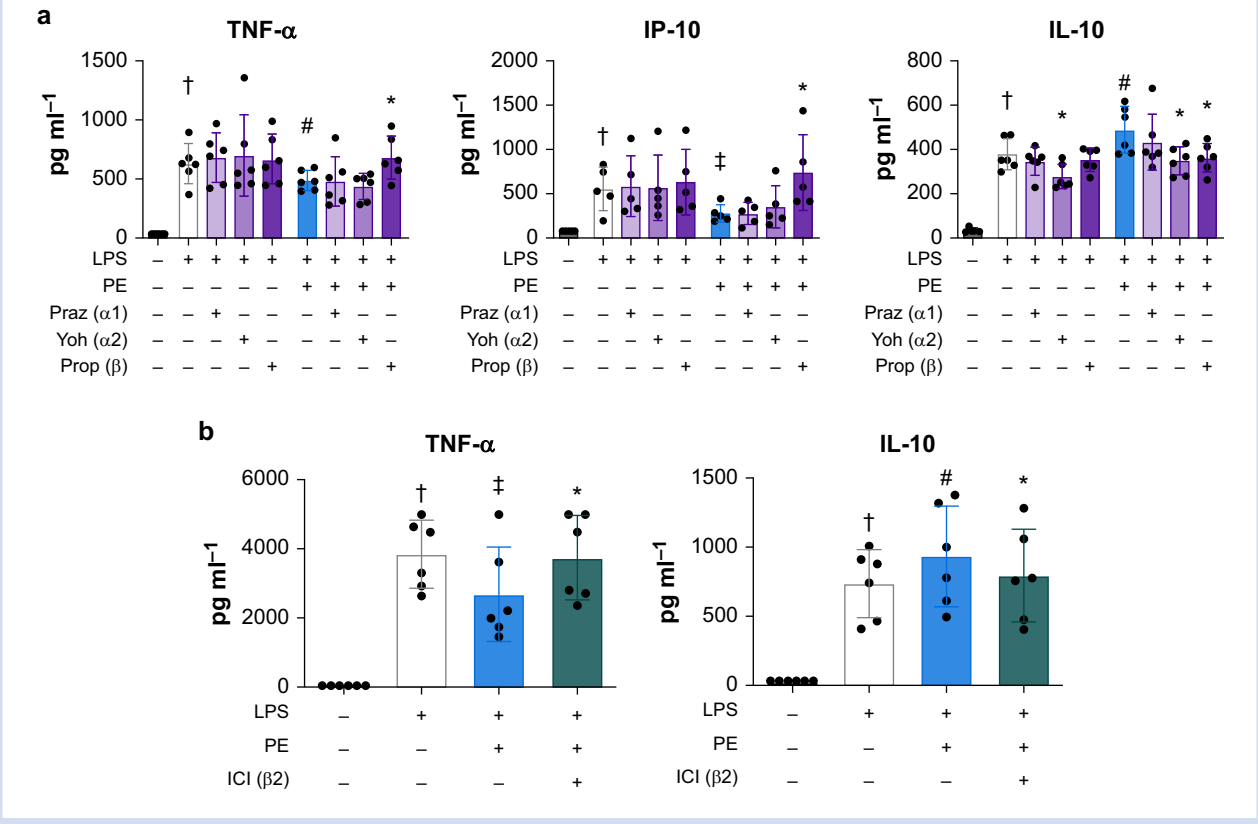

Fig 2. Phenylephrine modulates LPS-induced cytokine production via stimulation of the β2 adrenoreceptor. (a) Concentrations of TNF-α, IP-10, and IL-10 in supernatant of human whole blood pre-incubated with either RPMI (medium control) or adrenoceptor antagonists prazosin (Praz, α1, 1 μM), yohimbine (Yoh, α2, 1 μM), propranolol (Prop, β, 1 μM) for 30 min. This was followed by incubation with phenylephrine (PE, 1 μM) or RPMI for 1 h and subsequent stimulation with LPS (10 ng ml^{-1}) or RPMI for 24 h. (b) Concentrations of TNF-α, and IL-10 in supernatant of primary human monocytes pre-incubated with either RPMI or the β2-antagonist ICI-118,551 (ICI, 0.1 μM) for 30 min, followed by incubation with phenylephrine (PE, 1 μM) or RPMI for 1 h and subsequent stimulation with LPS (10 ng ml^{-1}) or RPMI for 24 h. Data are expressed as individual data points and mean (SD) of six individual donors. †$P<0.05$ compared with RPMI; ‡$P<0.05$, #$P=0.05–0.10$ compared with LPS. *$P<0.05$, ***$P<0.001$ compared with PE + LPS calculated using *t*-tests. LPS, lipopolysaccharide; TNF, tumour necrosis factor; IL, interleukin; IP, induced protein; SD, standard deviation.

nuclear factor kappa B (NF-κB) activator phorbol myristate acetate (Fig. 1c and d).

Adrenoreceptor modulation of anti-inflammatory effects of phenylephrine

The non-selective β-AR antagonist propranolol nullified the effects of phenylephrine on TNF-α, IP-10, and IL-10 production in LPS-stimulated whole blood, whereas the phenylephrine-induced attenuation of TNF-α and IP-10 was not affected by the selective α1-AR antagonist prazosin or the selective α2-AR antagonist yohimbine (Fig. 2a). Nevertheless, yohimbine reversed phenylephrine's IL-10-enhancing effects (Fig. 2a). As previous work demonstrated that selective β2-agonists attenuate pro-inflammatory cytokine production,[22] we investigated the effects of the selective and β2-AR antagonist ICI-1158,551 in isolated primary human monocytes. ICI-118,551 reversed phenylephrine's effects on LPS-induced TNF-α and IL-10 production, indicating a role for the β2-AR in phenylephrine's anti-inflammatory effects (Fig. 2b).

β-ARs are G-protein-coupled receptors that signal via the Gs protein, in turn increasing intracellular cyclic adenosine monophosphate (cAMP) levels. This results in activation of PKA, which prevents NF-κB translocation to the nucleus, ultimately reducing pro-inflammatory cytokine transcription and production while increasing anti-inflammatory IL-10.[23,24] The PKA inhibitor H89 reversed the anti-inflammatory effects of phenylephrine, confirming the involvement of β2-AR signalling in the anti-inflammatory effects of phenylephrine (Supplementary Fig. S2).

Murine experiments

LPS challenge

Under non-inflammatory conditions (saline-injected mice), phenylephrine infusion increased plasma concentrations of IL-6 (+117%) and IP-10 (+91%), whereas other cytokines were not affected (Supplementary Fig. S2). In LPS-challenged mice (Fig 3A), phenylephrine infusion reduced plasma levels of TNF-α (−34%), IL-1β (−31%), IP-10 (−72%), macrophage inflammatory protein (MIP)-1α (−57%), macrophage chemoattractant protein (MCP)-1 (−64%), and keratinocyte-derived chemokine (KC, −32%), whereas concentrations of IL-10 were enhanced (+158%). Similar effects were observed in

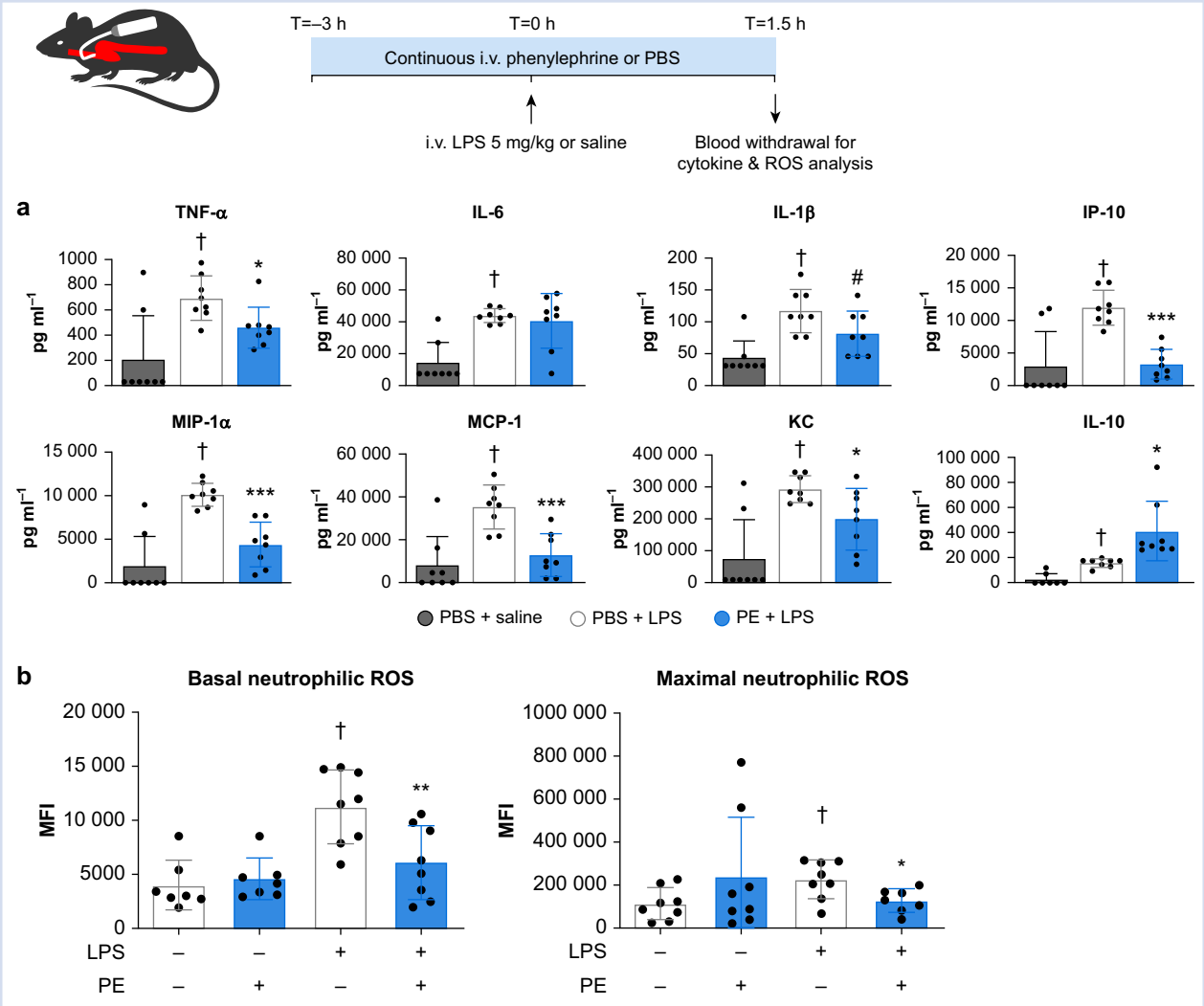

Fig 3. Phenylephrine modulates *in vivo* cytokine responses and *ex vivo* neutrophilic reactive oxygen species production in LPS-challenged mice. (a) Plasma concentrations of TNF-α, IL-6, IL-β, IP-10, MIP-1α, MCP-1, KC, and IL-10 in C57B/6J mice intravenously infused with phenylephrine (PE, 50 μg kg^{-1} min^{-1}) or PBS via a micro-osmotic pump connected to a jugular vein catheter for 4.5 h and challenged intravenously with LPS (5 mg kg^{-1}) or saline 3 h after start of infusion. (b) Reactive oxygen species (ROS) content and PMA-induced maximal respiratory burst of neutrophils isolated at the end of the experiment depicted in panel A. Data are expressed as individual data points and mean (SD) of eight animals per group. †$P<0.05$ compared with PBS + saline; #$P=0.05–0.10$, *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with PBS + LPS calculated using *t*-tests. LPS, lipopolysaccharide; IL, interleukin; IP, induced protein; MCP, macrophage chemoattractant protein; MFI, mean fluorescence intensity; KC, keratinocyte-derived chemokine; SD, standard deviation; TNF, tumour necrosis factor; PBS, phosphate-buffered saline.

spleen and lung tissue of LPS-challenged mice (Supplementary Fig. S3). LPS-induced increase in both basal neutrophilic ROS production and the PMA-induced maximal respiratory burst was reduced after phenylephrine infusion (−54% and −57%, respectively), whereas no such effects were observed under non-inflammatory conditions (Fig. 3b). Phenylephrine infusion in saline-injected mice also attenuated IFN-γ production by splenocytes *ex vivo* stimulated with PMA and ionomycin (−57%), whereas statistical significance was not reached for TNF-α (−43%, $P=0.09$) (Supplementary Fig. S4).

Caecal ligation and puncture

Compared with sham-operated mice, bacterial counts in peritoneal fluid, blood, liver, and spleen were profoundly increased in animals that underwent CLP (Fig. 4). Compared with PBS-infused mice, phenylephrine infusion increased bacterial counts in the peritoneal fluid (+42%) and spleen (+59%), whereas a trend towards increased counts was observed in the liver (+35%, $P=0.09$). No robust augmentation in blood bacterial counts were observed (+51%, $P=0.47$; Fig. 4). Phenylephrine treatment did not influence plasma cytokine

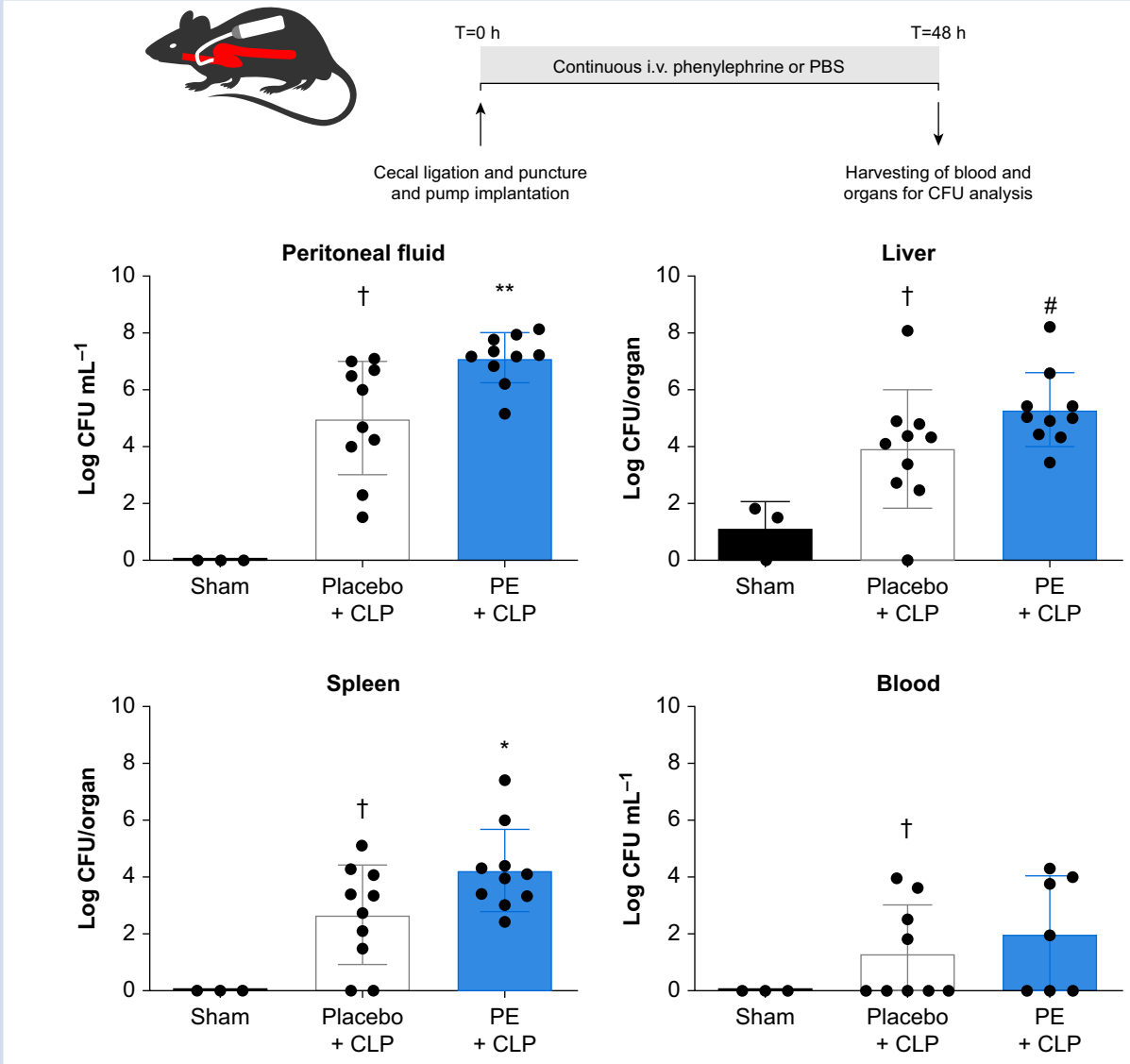

Fig 4. Phenylephrine treatment increases bacterial dissemination during surgical peritonitis. Bacterial load (expressed as colony forming units [CFU]) in peritoneal fluid, blood, liver, and spleen of C57B/6J mice intravenously infused with phenylephrine (PE, 30 μg kg^{-1} min^{-1}) or PBS for 2 days via a micro-osmotic pump connected to a jugular vein catheter and subjected to caecal ligation and puncture (CLP) to induce peritonitis or a sham operation. Data are expressed as individual data points and mean (SD) of 10 animals per group.; †P<0.05 compared with sham; #P=0.05–0.10, *P<0.05, **P<0.01 compared with PBS + CLP, calculated using t-tests. PBS, phosphate-buffered saline; SD, standard deviation.

levels determined 4 h after induction of CLP induction (Supplementary Fig. S5).

Human volunteer experiment

Haemodynamic changes after LPS infusion in presence or absence of phenylephrine

Subjects randomised to receive a 5 h infusion of either low-dose phenylephrine (0.5 μg kg^{-1} min^{-1}; n=10) or saline (n=10) had similar characteristics (Supplementary data). After 1 h of infusion, phenylephrine increased MAP by 13 (3) mm Hg; Fig. 5a) and reduced heart rate by 10 (1) beats min^{-1} (Fig. 5b). One hour after start of phenylephrine/placebo infusion, 2 ng kg^{-1} LPS administration resulted in flu-like symptoms, lower MAP, and higher heart rate in both groups (Fig. 5a and b). LPS-induced increase in body temperature was also similar between the two groups (Fig. 5c). Endogenous circulating norepinephrine levels were lower after phenylephrine infusion (reduction of 24% in area under the time–concentration curve [AUC], P=0.053; Fig. 5d); no effect on the LPS-induced increase in plasma epinephrine concentrations occurred (Fig. 5e).

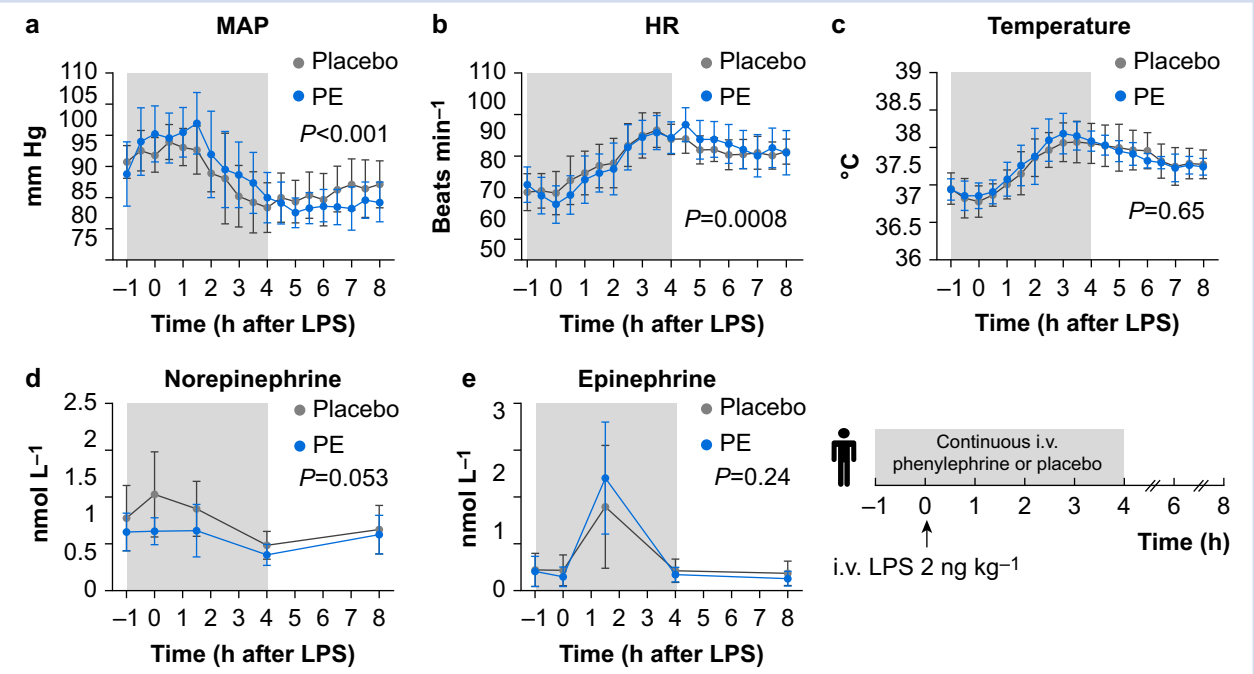

Fig 5. Haemodynamics, body temperature, and plasma (nor)epinephrine levels during experimental human endotoxaemia. (a) Mean arterial pressure, (b) heart rate, (c) body temperature, (d) plasma norepinephrine levels, and (e) plasma epinephrine levels in healthy volunteers randomised to a 5 h intravenous infusion with either saline (placebo) or low-dose phenylephrine (PE, 0.5 μg kg^{-1} min^{-1}) and challenged intravenously with 2 ng kg^{-1} LPS 1 h after start of infusion to elicit a systemic inflammatory response. Data are expressed as mean (SD) of 10 subjects per group. The grey area represents the period during which phenylephrine, or saline was infused. *P*-values were calculated using repeated measures two-way analysis of variance (interaction term: time × treatment) *vs* the placebo group. SD, standard deviation.

Circulating leukocytes and cytokine levels after LPS infusion in presence or absence of phenylephrine

LPS-induced changes in circulating numbers of monocytes, neutrophils, or lymphocytes were not affected by phenylephrine infusion (Fig. 6a). LPS administration caused a transient increase in plasma concentrations of TNF-α, IL-6, IL-8, IP-10, MCP-1, granulocyte colony-stimulating factor (G-CSF), and IL-10 in all subjects (Fig. 6b). Phenylephrine treatment significantly attenuated plasma levels of IL-8, IP-10, and MCP-1 (reduction in AUC of 15%, 41%, and 27%, respectively) while enhancing the IL-10 response (+76%; Fig. 6b).

Discussion

We provide the first comprehensive translational study into the immunologic effects of phenylephrine. We demonstrate that phenylephrine attenuates LPS-induced pro-inflammatory cytokine production *in vitro*, whereas it enhances the release of the anti-inflammatory cytokine IL-10. These effects are not mediated through the α-adrenergic receptor, but instead appear to involve the β2 receptor. Furthermore, we show that, in both mice and humans, phenylephrine infusion shifts the LPS-induced cytokine profile towards a distinct anti-inflammatory phenotype. The functional relevance of these immunosuppressive effects of phenylephrine is illustrated by increased bacterial dissemination during surgical peritonitis in mice, indicative of impaired host defence.

Phenylephrine is widely regarded as a selective α-adrenergic agonist, although some cardiovascular β-adrenergic effects have been reported.[13,14] Remarkably, we reveal that its immunomodulatory effects in terms of cytokine production are highly similar to those of specific β-AR agonists[22,25] and to the β-AR-dependent effects of (nor)epinephrine.[8–11] In accordance, our data show that the nonspecific β-AR antagonist propranolol and the specific β2-AR antagonist ICI-118-551 block the effects of phenylephrine on LPS-induced cytokine production. Therefore, phenylephrine cannot be considered as a specific α-agonist, as it exerts functional immunological β-adrenergic effects as well. This concurs with previous data showing that vascular effects of phenylephrine were counteracted by co-administration of a β-AR antagonist.[14] β-AR affinity was also implied by a study demonstrating uterine contraction by phenylephrine mediated by transient cAMP increases,[26] which are also observed after β-AR stimulation with norepinephrine.[23] Our data also show that phenylephrine-induced immunomodulation is mediated by the cAMP/PKA pathway, which concurs with our earlier results on norepinephrine[11] and those on other β-adrenergic agonists.[27] Interestingly, investigations into the inotropic properties of phenylephrine revealed that its overriding effects are dependent on the relative distribution of α- and β-adrenergic receptors in the target tissue.[28] As immune cells mainly

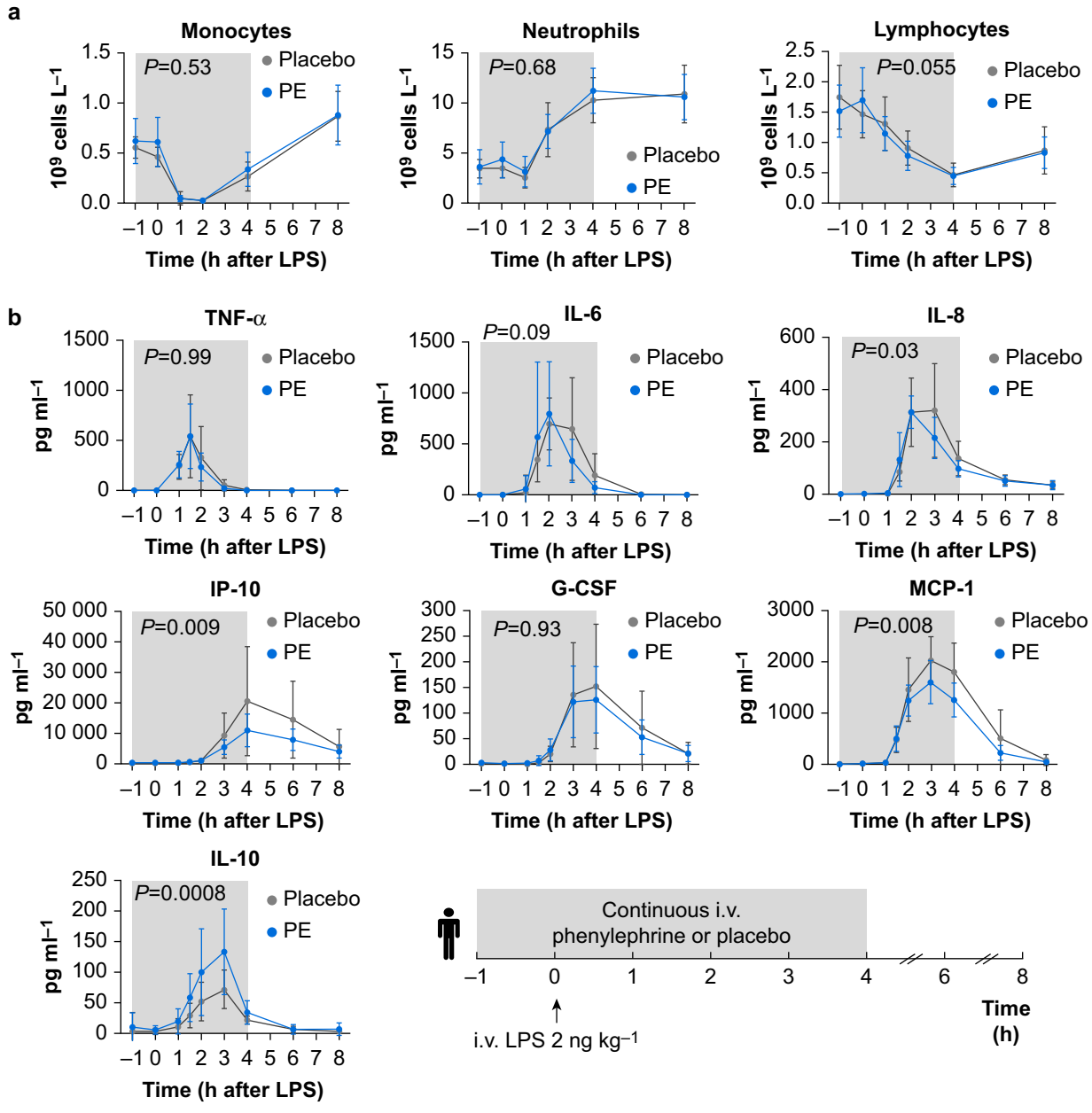

Fig 6. Low-dose phenylephrine infusion attenuates pro-inflammatory cytokine levels, while enhancing the anti-inflammatory IL-10 response during experimental human endotoxaemia. (a) Circulating monocyte, neutrophil, and lymphocyte numbers; (b) plasma concentrations of TNF-α, IL-6, IL-8, IP-10, G-CSF, MCP-1, and IL-10, in healthy volunteers randomised to a 5 h intravenous infusion with saline (placebo) or low-dose phenylephrine (PE, 0.5 μg kg^{-1} min^{-1}) and challenged intravenously with 2 ng kg^{-1} LPS 1 h after start of infusion to elicit a systemic inflammatory response. Data are expressed as mean (SD) of 10 subjects per group. The grey area represents the period during which phenylephrine or saline was infused. *P*-values were calculated using repeated measures two-way analysis of variance (interaction term: time × treatment) *vs* the placebo group. G-CSF, granulocyte colony stimulating factor; LPS, lipopolysaccharide; IL, interleukin; IP, induced protein; MCP, macrophage chemoattractant protein; SD, standard deviation; TNF, tumour necrosis factor.

express β-ARs,[29] this may provide an explanation for the anti-inflammatory effects of phenylephrine observed in the present study. Our data are, to some extent, in contrast with previous reports showing no effects of phenylephrine on LPS-induced production of TNF-α by human whole blood,[8] although the enhanced IL-10 production we observed was also demonstrated before.[9] This discrepancy might be explained by the differences in the duration of stimulation. IL-10 is a potent negative regulator of pro-inflammatory cytokine production, but its production is delayed relative to that of pro-

inflammatory cytokines such as TNF-α, with maximal expression observed only after 8 h.[30] Therefore, in the previous study using a 4 h stimulation period,[8] IL-10-mediated negative feedback on pro-inflammatory cytokine production was likely limited compared with the 24 h stimulation period we used, which may more accurately capture the complex interplay between the release of different inflammatory mediators.

In our murine studies, phenylephrine was administered via micro-osmotic pumps connected to a jugular vein catheter. This allowed for continuous i.v. administration in conscious animals, thereby excluding well-known confounding effects of sedatives.[31] The phenylephrine dosages used were based on previous studies using similar dosages, which were shown to induce an increase in blood pressure but were nevertheless tolerated for more than 14 days.[32] Previous animal studies investigating the immunomodulatory properties of phenylephrine are scarce. In a murine endotoxaemia study, either one or two subcutaneous bolus injections of phenylephrine did not reduce plasma TNF-α levels[33]; this might be related to the mode of administration used (bolus *vs* continuous infusion) in conjunction with phenylephrine's short half-life, the timing relative to LPS injection (0–45 min after LPS administration instead of 1 h before LPS challenge in our experiments), or both. Another study demonstrated protective effects of a single subcutaneous bolus administration of phenylephrine on myocardial dysfunction in rats subjected to CLP, exemplified by preservation of cardiac function and reduced myocardial TNF-α and IL-6 content, although plasma levels of TNF-α and IL-6 were not significantly reduced,[16] which may again be related to timing and mode of administration. Furthermore, in our CLP experiments, we also did not observe clear effects of phenylephrine infusion on cytokine levels. Like in other studies, this is plausibly related to timing, as we measured cytokines only on a single, relatively early time point, on which concentrations were still relatively low. No other time points were assessed owing to the limited amount of blood that can be obtained from mice. This represents a limitation of our work.

Although the anti-inflammatory effects of phenylephrine might be beneficial locally in the heart, our results indicate that these come at a cost of impaired host defence, and a subsequent increased susceptibility for infections. The most plausible underlying mechanism behind the increased bacterial dissemination observed in our CLP experiments is enhanced IL-10 release and an impaired pro-inflammatory cytokine response. This is illustrated by murine experiments showing that a more pronounced IL-10 response is associated with impaired bacterial clearance.[34] The impaired IFN-γ production by splenocytes of phenylephrine-treated mice observed in the present work is also of interest, as this cytokine was also shown to be pivotal in the defence against bacterial infections in mice[34,35] and its production is significantly attenuated in patients suffering from sepsis-induced immunoparalysis.[36] Furthermore, exogenous IFN-γ administration has successfully been used to restore immunocompetence in human models of immunosuppression[37] and in several small studies in immunosuppressed patients.[38,39] Although neutrophilic ROS generation was impaired in phenylephrine-infused mice, this may not represent a direct effect of phenylephrine for several reasons. First, phenylephrine did not decrease ROS production in our *in vitro* experiments. Second, the effect was only apparent in LPS-challenged mice and not in saline-challenged mice. As the inflammatory response elicited by LPS potentiated both basal and maximal neutrophilic ROS production, the attenuated ROS generation in phenylephrine-infused mice that were challenged with LPS is likely a consequence of the overall decreased inflammatory response in these animals.

To the best of our knowledge, this is the first study to investigate immunomodulatory effects of phenylephrine in humans. Recently, we published a comprehensive investigation of immunomodulatory properties of noradrenaline, a catecholamine with both α- and β-adrenergic affinity.[11] Noradrenaline is the cornerstone treatment for patients with septic shock, but is also frequently used in the operating theatre to counteract hypotension.[40] We demonstrated that, similar to phenylephrine, noradrenaline enhances the IL-10 response and attenuates pro-inflammatory mediators *in vitro* and *in vivo* through β-adrenergic signalling.[11] Unlike phenylephrine, noradrenaline also impaired monocytic ROS production.[11] Possibly as a result of these effects on both cytokines and ROS, noradrenaline increased bacterial dissemination in CLP mice more profoundly than phenylephrine did in the present study.[11] These dissimilarities might be caused by the different affinities of noradrenaline and phenylephrine for β-adrenergic receptors.

Clinically, phenylephrine is predominantly used in the surgical setting to treat hypotension and is either administered as a bolus or by continuous infusion. In this setting, higher dosages than those we used in our human endotoxaemia study (0.5 μg kg^{-1} min^{-1}) are commonly used. For instance, a median infusion rate of 0.95 μg kg^{-1} min^{-1} was used in obstetric surgery patients to treat anaesthesia-associated hypotension.[40] Nevertheless, we demonstrate that continuous infusion of this relatively low dose of phenylephrine enhances the IL-10 response and attenuates concentrations of several pro-inflammatory cytokines during experimental human endotoxaemia (i.e. IL-8, IP-10, and MCP-1). Given the dose-dependent effects of phenylephrine in our *in vitro* experiments, stronger anti-inflammatory effects are anticipated in the clinical setting, which are likely of relevance. For example, augmented IL-10 levels have been associated with an increased incidence of sepsis in patients undergoing surgery and in trauma patients.[41,42] Furthermore, a 74% increase in the IL-10/TNF-α ratio (reflecting the anti-/pro-inflammatory balance) has been linked to increased disease severity and mortality in sepsis patients.[43] For comparison, the relatively low phenylephrine dose used in our human endotoxaemia experiments increased the IL-10/TNF-α ratio by 93%. The effects of phenylephrine are neither mediated via enhanced release of norepinephrine, nor by increased circulating concentrations of epinephrine, another catecholamine which was previously shown to increase IL-10 and impair pro-inflammatory responses during experimental human endotoxaemia.[9] As IL-8 plays a pivotal role in neutrophil chemotaxis,[44] it is of interest to note that impaired neutrophil chemotaxis has been linked to increased susceptibility towards postoperative sepsis in patients.[3] In addition to influencing such short-term postoperative outcomes, it has been suggested that perioperative immune suppression affects long-term outcomes as well, and there are several indications of β-adrenergic involvement. For example, use and release of

catecholamines has been linked to suppressed anti-metastatic immunity and thereby increased risk of metastatic recurrence after cancer surgery.[5] Furthermore, in a breast cancer model in rats, metastases dose-dependently increased after treatment with the β-agonist metaproterenol, an effect which could be mitigated by the β-antagonist nadolol.[45] Likewise, in a rat model of surgical stress, it was demonstrated that pretreatment with nadolol prevented the surgery-induced suppression of T- and B-cell function.[46]

Finally, in a clinical study, the use of β-blockers was associated with a significantly reduced incidence of sepsis and mortality after emergency colonic surgery, although this was a retrospective study with risk for selection bias and confounding.[47] In a recent systematic review, this effect was not confirmed for noncardiac surgery patients.[48] Based on our experimental results, we argue that a clinical study aimed to investigate possible detrimental effects of phenylephrine-induced immunomodulation is warranted, and that use of alternative vasopressors should be further explored.

A limitation of our study is that we only included male subjects. There are considerable differences in the cytokine response to LPS between males and females.[49] This is likely influenced by menstrual cycle-related hormonal variations that can affect the immune response. As human endotoxaemia experiments are very labour-intensive and costly studies, and for ethical reasons (we want to expose as few volunteers as possible to endotoxaemia), we therefore only include male subjects in virtually all of our endotoxaemia studies. However, as no sex differences were demonstrated for anti-inflammatory effects of other adrenergic agonists,[50] it is unlikely that phenylephrine will have different effects in females.

In conclusion, phenylephrine, a sympathomimetic drug frequently used during surgery, exerts anti-inflammatory effects *in vitro* and *in vivo*. These effects are not mediated through α-adrenergic receptor agonism, but via the β2-adrenoceptor, and functionally result in impaired bacterial clearance. Phenylephrine could therefore compromise host defence in surgical patients, with potential detrimental effects on both the short- and long-term outcomes.

Authors' contributions

Study design: RFS, PP, MK
Conduct of experiments: RFS, FN, EvdP, JS, SB, AEvH, JG, RS, MR
Statistical analyses: RFS
Drafting of the article: RFS
Critical revision of the article and project supervision: JGvdH, HvL, PP, MK
All authors read and approved the final article.

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Declarations of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2020.11.040.

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