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Regular Article

The impact of *ADRB2* polymorphisms on immune responses and norepinephrine-induced immunosuppression

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Abstract

Rationale: To evaluate whether common nonsynonymous variants [single-nucleotide polymorphisms (SNPs) or SNP haplotypes] in the β2-adrenergic receptor render subjects more susceptible to norepinephrine-induced immunosuppression and whether they are associated with dysregulated ex vivo and in vivo inflammatory responses.

Methods: Peripheral blood mononuclear cells from healthy volunteers (main cohort: $n = 106$, secondary cohort: $n = 408$) were ex vivo stimulated with various stimuli and production of cytokines was assessed. Additionally, ex vivo modulation of cytokine production by norepinephrine was evaluated in the main cohort. Volunteers from the main cohort also underwent experimental endotoxemia (administration of 1 ng/kg lipopolysaccharide), during which in vivo plasma cytokine concentrations and clinical inflammatory parameters were measured. Subjects were genotyped, common SNPs in the *ADRB2* gene were extracted (*rs1042711*, *rs1042713*, and *rs1042714*), and the presence of haplotypes was identified (CysGlyGln, CysArgGln, and ArgGlyGlu).

Results: In both cohorts, presence of *ADRB2* SNPs or haplotypes was not associated with altered ex vivo cytokine responses. Norepinephrine attenuated production of the proinflammatory cytokines TNF and IL-6 [−26% (−22% to −30%) and −14% (−9% to −18%), respectively, both $P < 0.0001$] and enhanced release of the anti-inflammatory IL-10 [+9% (+3% to +15%), $P = 0.003$]. These effects were not modulated by the presence of *ADRB2* SNPs or haplotypes (all P values >0.37). In addition, no influence of SNPs or haplotypes on in vivo cytokine concentrations or clinical inflammatory parameters was observed (P values >0.14).

Conclusions: Common nonsynonymous variants in the *ADRB2* gene influence neither ex vivo cytokine production or norepinephrine-mediated immunosuppression nor the systemic in vivo inflammatory response induced by lipopolysaccharide administration in healthy volunteers.

Keywords: ADRB2 SNPs, endotoxemia, norepinephrine, cytokines

Abbreviations: ICU, intensive care unit; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; SNP, single-nucleotide polymorphism..

1 Introduction

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.[1] Despite substantial improvements in health care over the past decades, sepsis mortality in the intensive care unit (ICU) remains high.[2,3] It has become apparent that the nature of the dysregulated host response differs greatly between patients with sepsis, varying from hyperinflammation to profound immunosuppression (also termed *sepsis-induced immunoparalysis*). In addition to sepsis, patients admitted to the ICU following surgery also frequently have a dysregulated host response, often displaying an immunosuppressed phenotype similar to sepsis-induced immunoparalysis.[4] Gaining more insight into the large heterogeneity in host response phenotypes observed among sepsis and postoperative patients is of fundamental importance to advance the use of targeted treatments. Genetic variation (in the form of single-nucleotide polymorphisms, SNPs) has been shown to be an important determinant of the host response in healthy subjects,[5] as well as in patients with sepsis or surgery.[6,7] Furthermore, SNPs in several genes were shown to be associated with outcome.[7,8]

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Recently, we discovered that the catecholamine and vasopressor noradrenaline profoundly attenuates the innate immune response and thereby compromises host defense.[9] Noradrenaline is the primary supportive treatment for patients with septic shock and is also frequently administered to counter hypotension in the perioperative period. Therefore, this agent is applied to millions of patients worldwide. Because it may contribute to immune suppression in sepsis and in postoperative patients, further exploration of its immunologic effects is warranted. Norepinephrine's immunosuppressive effects were shown to be primarily mediated via the β2-adrenergic receptor, which is encoded by the *ADRB2* gene located on chromosome 5q31/32. *ADRB2* is a small intronless gene, in which several nonsynonymous common SNPs have been identified, namely, *Arg19Cys* (*rs1042711*), which is located at the 5′ untranslated region as well as *Gly16Arg* (*rs1042713*) and *Glu27Gln* (*rs1042714*), located in the extracellular N-terminus of the receptor. Reports on the functional consequences of these SNPs are conflicting. *In vitro* studies have suggested that the *Arg19Cys* SNP reduces β2-adrenergic receptor expression compared to wild type,[10] *Gly16Arg* is associated with enhanced agonist-induced desensitization,[11] and *Glu27Gln* is associated with resistance to desensitization.[12] A haplotype of the aforementioned SNPs (the CysGlyGln haplotype) was shown to be associated with a slight reduction in isoproterenol (a β-agonist)–induced inhibition of ex vivo CD3/CD28-stimulated lymphocytic IL-5 production.[13] Conversely, in isoproterenol-stimulated lymphocytes[14] and peripheral blood mononuclear cells (PBMCs),[15] *ADRB2* polymorphisms *Gly16Arg* and *Glu27Gln* did not affect production of cAMP, the intracellular messenger molecule downstream of the β2-adrenergic receptor, which was shown to be critical for its anti-inflammatory effects.[9] *In vivo* data on these SNPs are also equivocal and not congruent with the functional consequences observed in the *in vitro* studies. For instance, reduced vascular desensitization upon repeated isoproterenol infusion was observed in carriers of *Gly16Arg*,[16] whereas subjects homozygous for *Glu27Gln* exhibited reduced maximal venodilation.[17] In septic shock, a condition characterized by profound adrenergic stimulation through high endogenous catecholamine and exogenous norepinephrine concentrations,[18] the presence of the CysGlyGln haplotype in *ADRB2* was associated with a higher 28 day mortality in two independent cohorts.[19] Possibly, modulation of immunologic effects exerted by endogenous or exogenous catecholamines could play a role in this association, because partial reversal of the norepinephrine-induced reduction of IL-6 production by a lymphoblastoid cell line upon mixed inflammatory stimulation was observed in carriers of this haplotype.[19] Immunomodulatory effects of the aforementioned individual SNPs or other common haplotypes in *ADRB2* (CysArgGln and ArgGlyGlu) have not been investigated. Elucidating the possible immunologic influence of common *ADRB2* variants is important, as this knowledge could be used to identify patients more vulnerable to the anti-inflammatory effects of catecholamines and facilitate precision medicine.

In the present study, we investigated whether the presence of common SNPs and haplotypes in the *ADRB2* gene influences cytokine production by ex vivo–stimulated PBMCs or modulates the previously identified immunosuppressive effects of norepinephrine in these cells. Second, we assessed whether these variants are associated with an altered in vivo immune, hemodynamic, and fever response during experimental human endotoxemia, a model of systemic inflammation induced by intravenous administration of lipopolysaccharide (LPS). This model captures various hallmarks of early sepsis[20] and leads to profoundly elevated endogenous catecholamine concentrations.[21]

2 Methods

Detailed study procedures and analysis methods are described in the Supplemental Digital Content.

2.1 *Subjects and ethics*

Main cohort: The 100LPS cohort comprised 113 (male: $n = 58$) healthy individuals of Western European ancestry. After filtering and quality control, imputed SNP data were available for 109 individuals. All subjects provided written informed consent to participate in an interventional cohort study. The study was approved by the local ethics committee of the Radboud University Medical Center (registration number NL68166.091.18, 2018/4983).

Secondary cohort: The 500FG cohort (see www.humanfunctionalgenomics.org) comprised 485 (male: $n = 211$) healthy individuals of Western European ancestry. All volunteers provided written informed consent. After filtering and quality control, imputed SNP data were available for 441 individuals and corresponding ex vivo cytokine data for 408 subjects. The study was approved by the local ethics committee of the Radboud University Medical Center (registration number NL42561.091.12, 2012/550).

All study procedures were performed in accordance with the Declaration of Helsinki, including latest revisions. Subjects of both cohorts were genotyped using Illumina SNP chips (see Supplemental Digital Content). Flowcharts with the number of subjects per analysis are depicted in Supplementary Fig. 1.

2.2 Ex vivo stimulation of peripheral blood mononuclear cells

PBMCs were isolated (for the 100LPS cohort: before in vivo LPS administration; see next section) and were stimulated for 24 h with LPS in the presence or absence of norepinephrine (100LPS cohort) or with a panel of 6 stimuli, all in the absence of norepinephrine (500FG cohort). Concentrations of TNF, IL-6, IL-10, and IL-1β were measured using enzyme-linked immunosorbent assays in culture supernatants.

2.3 In vivo experimental human endotoxemia

Human endotoxemia experiments were conducted in subjects of the 100LPS cohort at the research unit of the Department of Intensive Care Medicine of the Radboud University medical center according to our standard protocol.[20] Vital parameters and temperature were continuously monitored. Subjects received an intravenous bolus of *Escherichia coli* LPS (1 ng/kg) to elicit a transient systemic inflammatory response. During the experiment, blood was withdrawn at various time points and plasma was stored for cytokine analysis.

2.4 Statistical analysis

Distribution of data was determined using Shapiro–Wilk tests. Data are presented as geometric mean with 95% confidence intervals (CIs) or median and range for baseline characteristics. Baseline characteristics were analyzed using chi-square tests for categorical data and Kruskal–Wallis tests for continuous data. Associations between genotypes and outcome data were analyzed using linear regression models (for SNPs) or ANCOVA (for haplotypes) adjusted for sex on logarithmically transformed data. Norepinephrine-induced effects on *ex vivo* cytokine production

Table 1 Demographic characteristics.

100LPS cohort								
Individual SNPs	**rs1042711/rs1042714 [Arg19Cys (C > T)/Glu27Gln (G > C)]**				**rs1042713 [Gly16Arg (G > A)]**			
	CC/GG (n = 13)	**TC/CG (n = 60)**	**TT/CC (n = 36)**	**P value**	**GG (n = 40)**	**GA (n = 56)**	**AA (n = 13)**	**P value**
Age, yr	24 (19–29)	22 (18–33)	23 (20–30)	0.23	22 (18–30)	23 (19–33)	24 (19–26)	0.46
Sex, % male	6 (46)	26 (43)	25 (69)	0.04	17 (43)	33 (59)	7 (54)	0.28
BMI, kg/m^2	23.6 (19.3–31.9)	22.8 (17.7–31.5)	23.2 (19.5–31.3)	0.09	23 (19–31.5)	23.5 (17.7–30.3)	24.6 (20–31.9)	0.17

Haplotypes	***CysGlyGln*** **TTGGCC (n = 3)**	***ArgGlyGlu*** **CCGGGG (n = 13)**	***CysArgGln*** **TTAACC (n = 13)**	**P value**
Age, yr	23 (24–29)	24 (21–27)	23 (19–27)	0.28
Sex, % male	1 (33)	7 (54)	6 (46)	0.79
BMI, kg/m^2	23.5 (21.5–27.7)	24.6 (19.35–29.9)	23.2 (17.7–28.7)	0.69

500FG cohort								
Individual SNPs	***rs1042711/rs1042714 [Arg19Cys (C > T)/Glu27Gln (G > C)]***				***rs1042713 [Gly16Arg (G > A)]***			
	CC/GG (n = 83)	**TC/CG (n = 211)**	**TT/CC (n = 114)**	**P value**	**GG (n = 173)**	**GA (n = 192)**	**AA (n = 43)**	**P value**
Age, yr	23 (18–75)	23 (18–70)	23 (18–70)	0.67	23 (18–75)	23 (18–70)	22 (18–50)	0.52
Sex, % male	47 (57)	120 (57)	68 (60)	0.87	94 (54)	112 (58)	29 (67)	0.28
BMI, kg/m^2	22.3 (17.4–32.2)	22.3 (15.0–32.5)	22 (17.3–34.4)	0.88	22.6 (16.6–34.4)	22 (15.1–32.5)	22.1 (17.3–33)	0.34

Haplotypes	***CysGlyGln*** **TTGGCC (n = 14)**	***ArgGlyGlu*** **CCGGGG (n = 88)**	***CysArgGln*** **TTAACC (n = 47)**	**P value**
Age, yr	23 (20–28)	23 (18–75)	22 (18–50)	0.57
Sex, % male	8 (57)	47 (53)	29 (61)	0.50
BMI, kg/m^2	21.7 (19.5–34.4)	22.3 (17.4–32.2)	22.1 (17.3–33)	0.62

Parameters were measured during screening visit. Data are presented as number (%) or median with range. *P* values were calculated using Kruskal–Wallis tests. BMI, body mass index; SNP, single-nucleotide polymorphism.

were analyzed using paired *t* tests on logarithmically transformed data. To correct for multiple testing, *P* values obtained from the regression models were adjusted using the false discovery rate (FDR) method according to Benjamini and Hochberg.[22] For the analysis of *in vivo* cytokine responses, area under curve plasma cytokine concentrations were used as dependent variables in the regression models/ANCOVAs, as these represent an integral measure of the *in vivo* cytokine production over time in response to LPS administration. In the 100LPS cohort, 3 subjects were inadvertently administered a lower dose of LPS due to a faulty vial and were therefore excluded for the *in vivo* analyses. These subjects carried the TC/CG, TC/CG, and TT/CC genotype for the *rs1042711/rs1042714* SNPs (which were in complete linkage disequilibrium; see Results section) and the GA, GA, and GG genotype for the *rs1042713* SNP but were not carriers of any of the 3 haplotypes investigated in the present study. A *P* value <0.05 was considered significant. Analyses were performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA), SPSS 25 (IBM Statistics, New York, NY, USA), and R (version 3.6.3, Free Software Foundation, Boston, MA, USA).

3 Results

3.1 Baseline characteristics

A graphical overview of the study is provided in the graphical abstract, and flowcharts of the two cohorts used with the number of subjects per analysis are depicted in Supplementary Fig. 1. Demographic characteristics of the subjects of both cohorts are listed in Table 1. *rs1042711* and *rs1042714* are in almost complete linkage disequilibrium ($R^2 = 1.0$ GBR [british in England and Scotland cohort], $R^2 = 1.0$ TSI [toscani in Italy cohort], $R^2 = 0.96$ CEU [Utah residents with Northern and Western European Ancestry][23,24]) and were in complete linkage disequilibrium in the 100LPS cohort. Given that *rs1042711* was not genotyped in the 500FG cohort, we therefore used *rs1042714* as a proxy SNP, which we henceforth indicate as *rs1042711/14*. In the 100LPS cohort, carriers of the TT/CC genotype of *rs1042711/14* were more likely men (69% vs. 43% $P = 0.04$) compared to the other genotypes, but no other differences were observed between genotypes of *rs1042711/14* or *rs1042713*. In the 500FG cohort, no differences in baseline characteristics were present between genotypes.

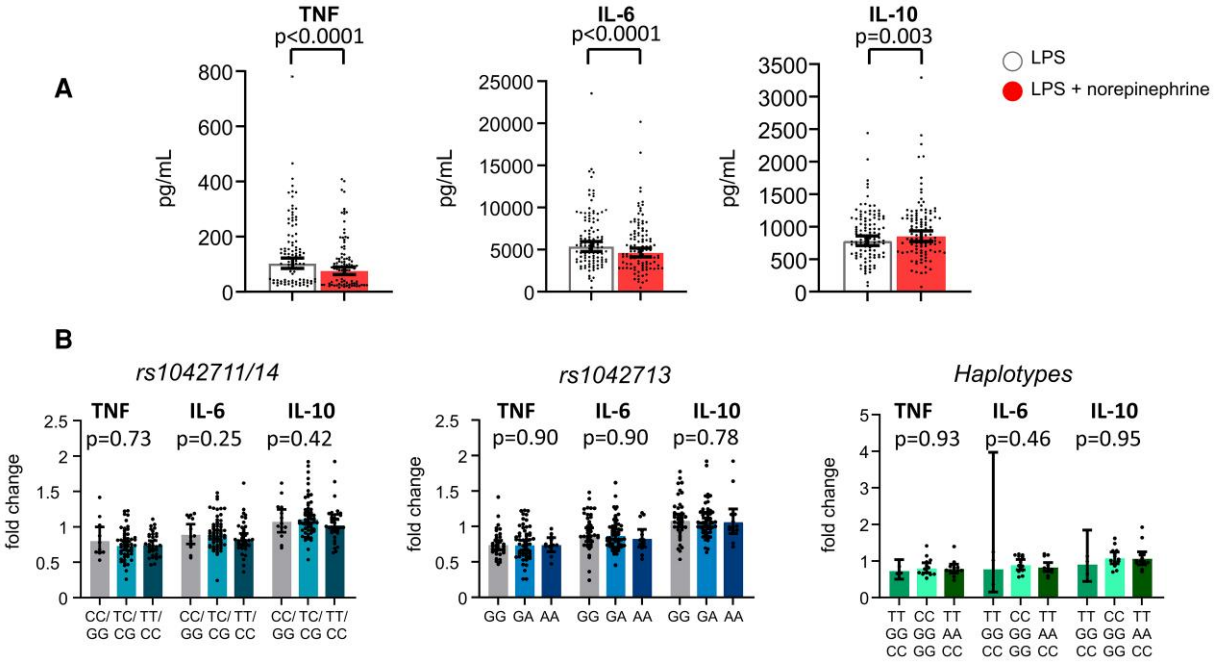

Fig. 1. *ADRB2* polymorphisms do not influence norepinephrine-induced modulation of lipopolysaccharide (LPS)–induced cytokine production by peripheral blood mononuclear cells. (A) Concentrations of TNF, IL-6, and IL-10 in supernatants of peripheral blood mononuclear cells of 109 subjects who were stimulated ex vivo with LPS (10 ng/mL) in the presence or absence of norepinephrine (NE, 1 μM). (B) Norepinephrine-induced fold changes in production of TNF, IL-6, and IL-10 in supernatants of peripheral blood mononuclear cell cultures according to single-nucleotide polymorphism (SNP) genotype or haplotype [rs1042711/rs1042714 CC/GG: $n = 13$, TC/CG: $n = 60$, TT/CC: $n = 36$; rs1042713 GG: $n = 40$, GA: $n = 56$, AA: $n = 13$; CysGlyGln (TTGGCC): $n = 3$, ArgGlyGlu (CCGGGG): $n = 13$, CysArgGln (TTAACC): $n = 13$]. Data are expressed as individual datapoints with geometric mean and 95% confidence interval. *P* values were calculated using (A) paired *t* tests on log-transformed data or (B) linear regression analysis (SNPs) or ANCOVA (haplotypes) on log-transformed data with sex as the covariate. False discovery rate correction was applied.

3.2 ADRB2 polymorphisms do not influence cytokine production or norepinephrine-induced modulation of cytokine responses in peripheral blood mononuclear cells

The influence of *ADRB2* SNPs and haplotypes on the ex vivo inflammatory response was first explored by stimulating PBMCs obtained from subjects of the 100LPS cohort with LPS for 24 h. No associations between any of the SNPs or haplotypes and LPS-induced production of the proinflammatory cytokines TNF and IL-6 and the anti-inflammatory cytokine IL-10 were observed (all *P* values >0.1, Supplementary Tables 1 and 2). This finding was validated in the larger 500FG cohort, in which no associations between cytokine production (TNF, IL-6, and IL-1β) by PBMCs and any of the SNPs or haplotypes were observed (all *P* values >0.1, Supplementary Tables 3 and 4).

To investigate whether presence of the *ADRB2* SNPs affected norepinephrine-induced modulation of cytokine production, PBMCs obtained from subjects of the 100LPS cohort were stimulated with LPS in the presence and absence of norepinephrine for 24 h, after which the production of TNF, IL-6, and IL-10 was assessed. In accordance with our previous study,[9] norepinephrine attenuated LPS-induced TNF and IL-6 production [−26% (−22% to −30%), and −14% (−9% to −18%), respectively, both $P < 0.0001$], while release of IL-10 was enhanced [+9% (+3% to +15%), $P = 0.003$, Fig. 1A]. However, neither individual SNPs nor haplotypes in the *ADRB2* gene influenced the immunomodulatory effects of norepinephrine (Fig. 1B). Furthermore, no influence of individual SNPs or haplotypes on the norepinephrine-induced decrease of the TNF/IL-10 or IL-6/IL-10 ratios was observed (data not shown).

3.3 ADRB2 polymorphisms do not affect the in vivo cytokine response induced by intravenous LPS administration

During endotoxemia, endogenous catecholamine concentrations increase and subsequently can influence the immune response,[21] an effect that may be modulated by the presence of *ADRB2* SNPs/haplotypes. Therefore, we investigated the influence of the presence of these SNPs and haplotypes on the systemic inflammatory response induced by intravenous LPS administration in the 100LPS cohort, quantified by plasma cytokine concentrations. LPS administration induced a marked increase in plasma concentrations of TNF, IL-6, IL-8, IP-10, IL-10, IL-1RA, macrophage inflammatory protein 1α, monocyte chemoattractant protein 1, and granulocyte colony stimulating factor in all subjects (Fig. 2A–E and Supplementary Fig. 2A–E). However, individual *ADRB2* SNPs or haplotypes did not affect any of these cytokine responses (Fig. 2F–J, Supplementary Figs. 2F–J, 3, Supplementary Tables 1 and 2).

3.4 ADRB2 polymorphisms are not associated with alterations in the hemodynamic or temperature response induced by intravenous LPS administration

Heart rate and mean arterial blood pressure showed a typical pattern for human endotoxemia: a gradual decrease in mean arterial pressure [maximum decrease of 19% (18% to 20%); Fig. 3A] and a compensatory increase in heart rate [maximum increase of 37% (37% to 38%); Fig. 3B]. Furthermore, LPS administration resulted in an increase in body temperature [maximum increase of 1.0 °C (0.7 °C to

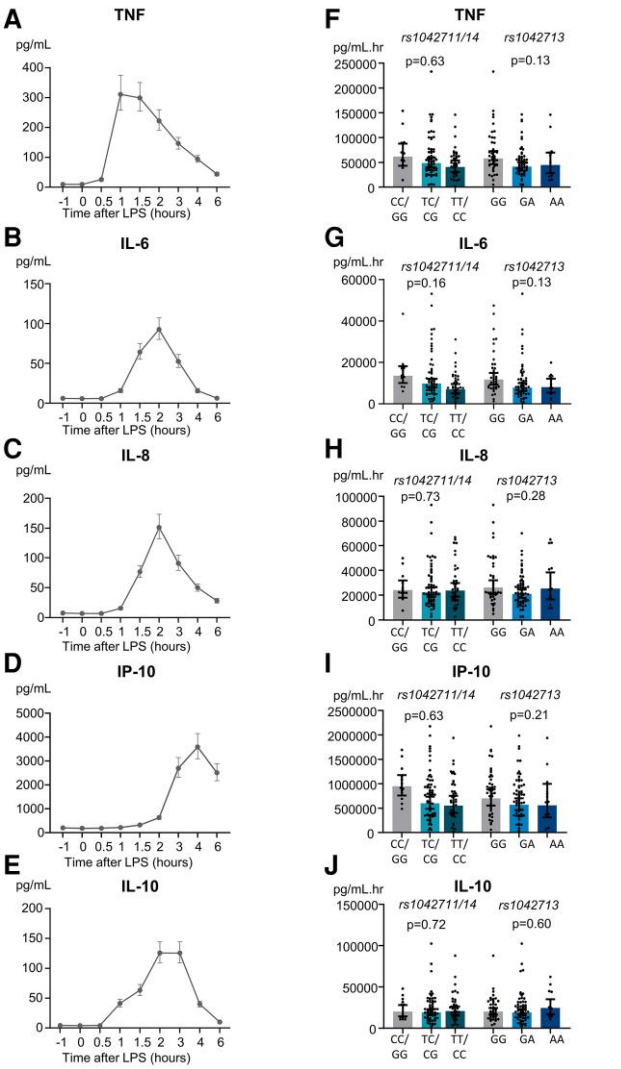

Fig. 2. ADRB2 polymorphisms do not affect the in vivo cytokine response induced by intravenous lipopolysaccharide administration. Plasma concentrations of TNF, IL-6, IL-8, IP-10, and IL-10 during experimental human endotoxemia in 106 subjects (A–E) and area under curve cytokine responses (F–J) according to single-nucleotide polymorphism genotype (rs1042711/rs1042714 CC/GG: $n = 13$, TC/CG: $n = 58$, TT/CC: $n = 35$; rs1042713 GG: $n = 39$, GA: $n = 54$, AA: $n = 13$). Data are expressed as geometric mean and 95% confidence interval (A–E) or individual datapoints with geometric mean and 95% confidence interval (F–J). *P* values were calculated using linear regression analysis on log-transformed data with sex as covariate and false discovery rate correction was applied. Data of other cytokines (IL-1RA, monocyte chemoattractant protein 1, macrophage inflammatory protein 1α, and granulocyte colony stimulating factor) are provided in Supplementary Fig. 2. All cytokine responses according to haplotype are provided in Supplementary Fig. 3.

1.3 °C); Fig. 3C]. SNPs or haplotypes in the *ADRB2* gene did not influence any of these hemodynamic and temperature responses (Fig. 3D–F, Supplementary Fig. 4, Supplementary Tables 1 and 2).

Discussion

In the present study, we investigated whether nonsynonymous polymorphisms in the β2-adrenergic receptor gene *ADRB2* influence cytokine production by *ex vivo*–stimulated PBMCs of healthy volunteers of Western European origin or the immunomodulatory effects of norepinephrine in these cells. Furthermore, we explored whether these polymorphisms were associated with altered cytokine, hemodynamic, and temperature responses following LPS administration in vivo. We demonstrate that none of the above parameters were influenced by *ADRB2* polymorphisms.

There have been conflicting results from *in vitro* and *ex vivo* studies on the functionality of *ADRB2* polymorphisms. For example, it was reported that Gly16 and Glu27 variants in human lung mast cells are resistant to isoproterenol desensitization compared to the Arg16 and Gln27 variants, when examining histamine production after IgE stimulation (inhibition of histamine was demonstrated to be a β-adrenergic-driven effect),[25] suggesting these variants are associated with reduced sensitivity for adrenergic stimulation. However, an analysis in 96 individuals from two European cohorts revealed no influence of any of these SNPs on receptor expression or affinity in circulating PBMCs.[15] In addition, when examining intracellular production of cAMP, the intracellular messenger molecule induced by β-adrenergic receptor stimulation, in lymphocytes or PBMCs of patients with asthma upon ex vivo isoproterenol stimulation, no influence of the presence of these polymorphisms was found as well.[14,15] The results from our ex vivo stimulation experiments in PBMCs also reveal no effects of the presence of individual *ADRB2* SNPs on the previously established β2-adrenergic receptor-mediated immunomodulatory effects of norepinephrine (i.e., attenuation of proinflammatory cytokine production and enhanced production of the anti-inflammatory cytokine IL-10), which were shown to be cAMP dependent.[9] Other *in vitro* and *ex vivo* investigations have focused on the effects of the presence of several haplotypes of *ADRB2*, with equivocal results as well. An examination of the three haplotypes that were also assessed in the present study in lymphocytes of nonasthmatic, nonallergic subjects showed no association with β2-adrenergic receptor expression but found increased desensitization upon repeated isoproterenol-induced cAMP production for the CysGlyGln haplotype compared to the CysArgGln and ArgGlyGlu haplotypes, suggestive of enhanced signaling.[13] However, isoproterenol-mediated inhibition of IL-5 production after CD3/CD28 stimulation was attenuated in individuals carrying the CysGlyGln haplotype,[13] as was suppression of IL-6 release by norepinephrine in a lymphoblastoid cell line after mixed inflammatory stimulation,[19] suggesting an impaired adrenergic signaling in subjects bearing the CysGlyGln haplotype. A similar examination in PBMCs derived from several asthmatic patient cohorts demonstrated no influence of several haplotypes, including CysGlyGln, on β2-adrenergic receptor expression and cAMP production after isoprenaline stimulation.[15] In our ex vivo stimulation experiments, no influence on cytokine production capacity was found for the ArgGlyGlu, CysArgGln, and CysGlyGln haplotypes. As only three subjects of the 100LPS cohort carried the CysGlyGln haplotype, we cannot draw meaningful conclusions on the influence of this haplotype on noradrenaline-induced dysregulation of ex vivo cytokine production. We used the 500FG cohort for validation of our initial findings in a much larger cohort. Furthermore, we extended our initial analyses in this cohort by evaluating cytokine production induced by multiple stimuli in addition to LPS, again revealing no influence of any of the *ADRB2* SNPs or haplotypes.

We also observed no influence of the presence of individual *ADRB2* SNPs or haplotypes of these SNPs on the *in vivo* inflammatory response in subjects challenged with LPS. The experimental human endotoxemia model used in the present study is a reproducible standardized and controlled model of systemic inflammation and shares many hallmarks of early sepsis, among which is an increase in circulating catecholamine concentrations.[20]

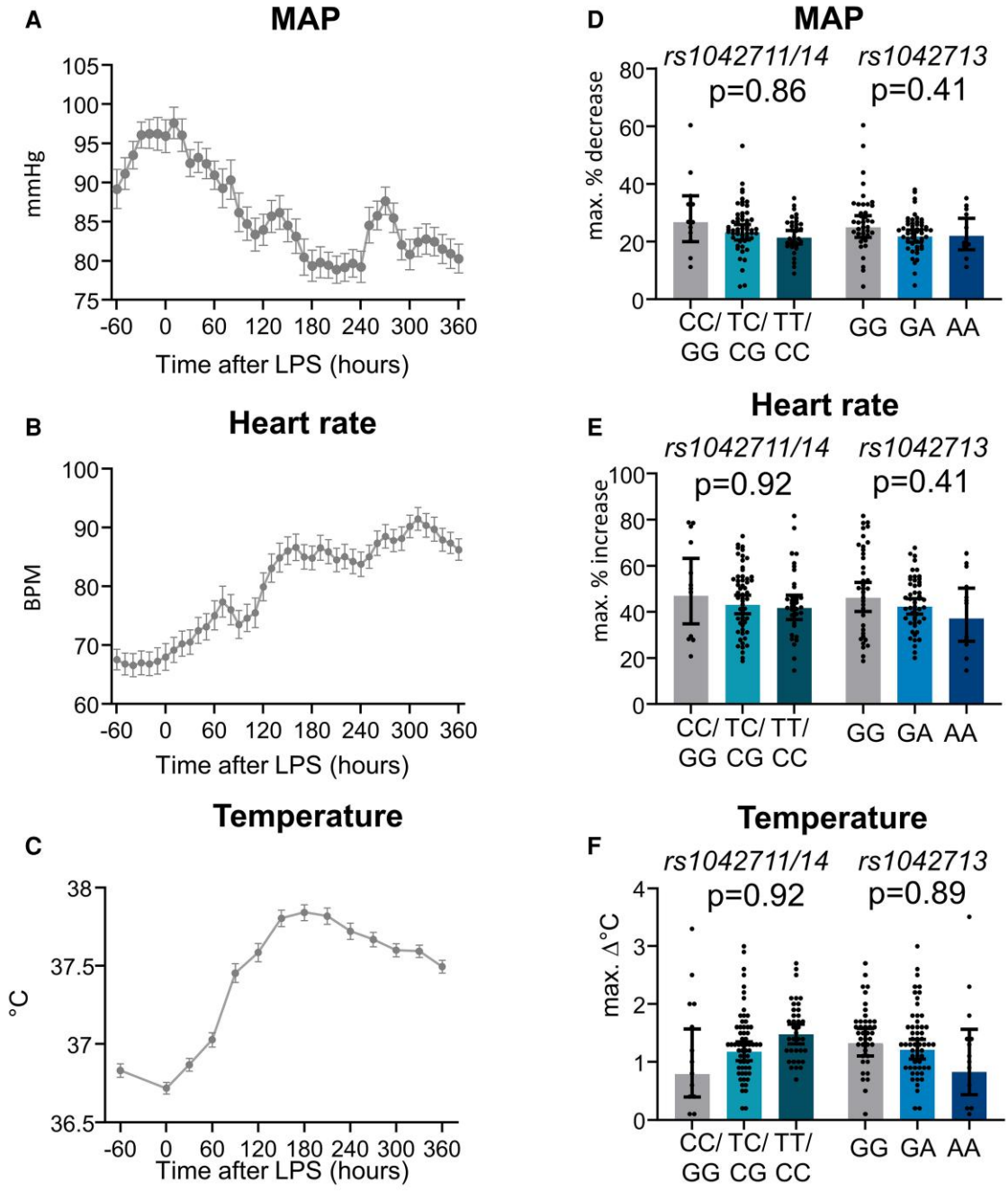

Fig. 3. *ADRB2* polymorphisms are not associated with alterations in the hemodynamic or temperature response induced by intravenous lipopolysaccharide administration. Mean arterial pressure (MAP) (A), heart rate (B), and body temperature (C) during experimental human endotoxemia in 106 subjects and percentage changes in mean arterial pressure (D) and heart rate (E) and changes in body temperature (F) according to single-nucleotide polymorphism genotype (rs1042711/rs1042714 CC/GG: $n = 13$, TC/CG: $n = 58$, TT/CC: $n = 35$; rs1042713 GG: $n = 39$, GA: $n = 54$, AA: $n = 13$). Data are expressed as geometric mean and 95% confidence interval (A–C) or individual datapoints with geometric mean and 95% confidence interval (D–F). *P* values were calculated using linear regression analysis on log-transformed data with sex as covariate and false discovery rate correction was applied. Hemodynamic and temperature responses according to haplotype are provided in Supplementary Fig. 4.

Strikingly, genetic variants in *hTLR4*, the gene encoding for the LPS receptor TLR4, were also shown not to influence the inflammatory response during experimental human endotoxemia,[26] illustrating the possibility that compensatory mechanisms are in play even if apparently essential pathways are affected. Marked anti-inflammatory effects by catecholamines such as epinephrine, norepinephrine, and the synthetic sympaticomimetic agent phenylephrine have been demonstrated previously by our group and others using this model.[9,21,27,28] Furthermore, norepinephrine and phenylephrine were shown to facilitate bacterial dissemination in experimental sepsis, and norepinephrine use was related to dysregulated cytokine profiles in patients with septic shock.[9] These immunosuppressive effects of norepinephrine could be reversed by selective β2-adrenergic receptor blockers in *in vitro* experiments and were attenuated in patients with sepsis who were treated with a β-adrenergic receptor blocker for cardiovascular reasons prior to ICU admission,[9] highlighting a critical role for the β2-adrenergic receptor. As adrenergic agents, especially

norepinephrine, are routinely used in daily practice on the intensive care for hemodynamic support of patients in shock, their unfavorable immunologic effects could be of major clinical relevance.[29,30] Based on our findings however, variations in the *ADRB2* gene do not render subjects more or less susceptible to catecholamine-induced immunologic dysregulation. Interestingly, we previously demonstrated that vasopressin, a noncatecholaminergic vasopressor, is "immunologically inert" and therefore does not compromise host defense.[9] In the context of personalized medicine, a tailored vasopressor approach, in which patients characterized by hyperinflammation are treated with norepinephrine and immunoparalyzed patients with vasopressin, could therefore be envisioned. The present study provides no indication that the presence of SNPs in *ADRB2* should be considered in such an approach.

The presence of the CysGlyGln haplotype has been associated with increased mortality in 2 separate cohorts of patients with septic shock.[19] Furthermore, this haplotype was associated with an increased heart rate and higher norepinephrine requirements, which the authors attribute to increased systemic inflammation because of attenuation of catecholamine-induced anti-inflammatory effects.[19] We did not find signals of increased systemic inflammation during experimental endotoxemia in carriers of this haplotype. However, as mentioned before, CysGlyGln was present in only few subjects of the 100LPS cohort, so definitive conclusions cannot be drawn.

Most research into in vivo effects of *ADRB2* polymorphisms concerns vascular studies. For instance, it was demonstrated that subjects homozygous for Gly16 exhibited a more pronounced isoproterenol (a nonselective β-adrenergic receptor agonist)–induced increase in forearm blood flow (i.e., mediated by resistance arteries) compared to homozygotes for Arg16, whereas subjects with Gln27 demonstrated an attenuated increase in forearm blood flow compared with Glu27 homozygotes.[17] Furthermore, homozygous carriers of Gly16 exhibited reduced desensitization for hand vein dilation upon repeated isoproterenol infusion compared to homozygous Arg16 carriers, irrespective of their Glu27Gln status.[16] Finally, increased systolic and diastolic blood pressure upon terbutaline infusion for Gly16-bearing subjects compared to Arg16 carriers was shown under noninflammatory conditions.[31] However, again, other work reported no hemodynamic effects associated with the presence of *ADRB2* polymorphisms.[32] We did not observe functional hemodynamic effects of the polymorphisms during experimental endotoxemia. Of course, while this model is characterized by substantial increases in endogenous catecholamines,[20,21] no exogenous adrenergic stimulation was applied. Therefore, we conclude that possible functional effects of individual SNPs and haplotypes are too small to affect macrocirculatory changes induced by increased endogenous catecholamine concentrations.

Our study is limited by the fact that we did not genotype and select subjects before study inclusion, leading to low numbers of subjects carrying the relatively rare CysGlyGln haplotype in the 100LPS cohort. Nevertheless, the results obtained in the 500FG cohort, with a higher number of CyGlyGln carriers, also revealed no indications for an altered *ex vivo* immune response. Furthermore, we were able to properly assess possible functional effects of individual *ADRB2* SNPs and of two other common haplotypes—namely, ArgGlyGlu and CysArgGln. Considering the common linkage disequilibrium encountered in *ADRB2* polymorphisms, it is essential to include the functionality of haplotypes in addition to the functionality of individual SNPs in an analysis.[33] Another limitation is the use of young participants in the 100LPS cohort, whereas patients, especially those with sepsis, are usually older. There are several reasons why only young subjects were included in this cohort, the first of which is safety. For example, injection of LPS in older volunteers was associated with a more pronounced reduction in blood pressure compared with younger subjects,[34] which may present risks. Likewise, elder subjects are likely to have (not yet diagnosed) comorbidities, which may also compromise safety. Second, because endotoxemia experiments are very labor intensive and expensive, very large group sizes are not feasible. Therefore, interindividual variability should be kept relatively low to draw meaningful conclusions. Nevertheless, no influence of *ADRB2* SNPs or haplotypes on *ex vivo* cytokine production induced by various stimuli was observed in the 500FG cohort as well, even though this cohort also included older subjects.

Conclusions

We report no consequences of common nonsynonymous variants in the *ADRB2* gene on cytokine production by *ex vivo*–stimulated PBMCs or norepinephrine-mediated immunosuppression in these cells. Furthermore, no influence of *ADRB2* polymorphisms on the systemic *in vivo* inflammatory and hemodynamic response induced by LPS administration was observed. Consequently, these genetic variants do neither influence the immune response per se nor the susceptibility toward catecholamine-induced dysregulation of the host response.

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Authorship

R.F.S. and M.K. conceived and designed the study. N.B., A.J., and J.G. performed the experiments. R.F.S., N.B., R.T.H., and I.R.P. performed statistical analyses. R.F.S. drafted the manuscript. H.v.d.H., V.K., M.N., P.P., and M.K. critically revised the article and supervised the project. All authors read and approved the final article.

Supplementary material

Supplementary material is available at *Journal of Leukocyte Biology* online.

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Disclosures

None declared.

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche J-D, Coopersmith CM, *et al.* The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016:315(8):801–810. https://doi.org/10.1001/jama.2016.0287
2. Angus DC, van der Poll T. Severe sepsis and septic shock. *N Engl J Med*. 2013:369(21):2063. https://doi.org/10.1056/NEJMra1208623

3. Gaieski DF, Edwards JM, Kallan MJ, Carr BG. Benchmarking the incidence and mortality of severe sepsis in the United States. *Critical Care Med*. 2013:41(5):1167–1174. https://doi.org/10.1097/CCM.0b013e31827c09f8
4. Hogan BV, Peter MB, Shenoy HG, Horgan K, Hughes TA. Surgery induced immunosuppression. *Surgeon*. 2011:9(1):38–43. https://doi.org/10.1016/j.surge.2010.07.011
5. Li Y, Oosting M, Smeekens SP, Jaeger M, Aguirre-Gamboa R, Le Kieu TT, Deelen P, Ricaño-Ponce I, Schoffelen T, Jansen AFM, *et al.* A functional genomics approach to understand variation in cytokine production in humans. *Cell*. 2016:167(4):1099–-1110.e1014. https://doi.org/10.1016/j.cell.2016.10.017
6. Davenport EE, Burnham KL, Radhakrishnan J, Humburg P, Hutton P, Mills TC, Rautanen A, Gordon AC, Garrard C, Hill AVS, *et al.* Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study. *Lancet Respir Med*. 2016:4(4):259–271. https://doi.org/10.1016/S2213-2600(16)00046-1
7. Kumpf O, Giamarellos-Bourboulis EJ, Koch A, Hamann L, Mouktaroudi M, Oh D-Y, Latz E, Lorenz E, Schwartz DA, Ferwerda B, *et al.* Influence of genetic variations in TLR4 and TIRAP/Mal on the course of sepsis and pneumonia and cytokine release: an observational study in three cohorts. *Critical Care*. 2010:14(3):R103. https://doi.org/10.1186/cc9047
8. Rautanen A, Mills TC, Gordon AC, Hutton P, Steffens M, Nuamah R, Chiche J-D, Parks T, Chapman SJ, Davenport EE, *et al.* Genome-wide association study of survival from sepsis due to pneumonia: an observational cohort study. *Lancet Respir Med*. 2015:3(1):53–60. https://doi.org/10.1016/S2213-2600(14)70290-5
9. Stolk R, van der Pasch E, Naumann F, Schouwstra J, Bressers S, van Herwaarden AE, Gerretsen J, Schambergen R, Ruth MM, van der Hoeven JG, *et al.* Norepinephrine dysregulates the immune response and compromises host defense during sepsis. *Am J Respir Crit Care Med*. 2020:202(6):830–842. https://doi.org/10.1164/rccm.202002-0339OC
10. McGraw DW, Forbes SL, Kramer LA, Liggett SB. Polymorphisms of the 5' leader cistron of the human beta2-adrenergic receptor regulate receptor expression. *J Clin Invest*. 1998:102(11):1927–1932. https://doi.org/10.1172/JCI4862
11. Green SA, Turki J, Bejarano P, Hall IP, Liggett SB. Influence of beta 2-adrenergic receptor genotypes on signal transduction in human airway smooth muscle cells. *Am J Respir Cell Mol Biol*. 1995:13(1):25–33. https://doi.org/10.1165/ajrcmb.13.1.7598936
12. Moore PE, Laporte JD, Abraham JH, Schwartzman IN, Yandava CN, Silverman ES, Drazen JM, Wand MP, Panettieri RA, Shore SA. Polymorphism of the beta(2)-adrenergic receptor gene and desensitization in human airway smooth muscle. *Am J Respir Crit Care Med*. 2000:162(6):2117–2124. https://doi.org/10.1164/ajrccm.162.6.9909046
13. Oostendorp J, Postma DS, Volders H, Jongepier H, Kauffman HF, Boezen HM, Meyers DA, Bleecker ER, Nelemans SA, Zaagsma J, *et al.* Differential desensitization of homozygous haplotypes of the β_2-adrenergic receptor in lymphocytes. *Am J Respir Crit Care Med*. 2005:172(3):322–328. https://doi.org/10.1164/rccm.200409-1162OC
14. Lipworth BJ, Hall IP, Tan S, Aziz I, Coutie W. Effects of genetic polymorphism on ex vivo and in vivo function of β_2-adrenoceptors in asthmatic patients. *Chest*. 1999:115(2):324–328. https://doi.org/10.1378/chest.115.2.324
15. Lipworth B, Koppelman GH, Wheatley AP, Le Jeune I, Coutie W, Meurs H, Kauffman HF, Postma DS, Hall IP. β_2 adrenoceptor promoter polymorphisms: extended haplotypes and functional effects in peripheral blood mononuclear cells. *Thorax*. 2002:57(1):61–66. https://doi.org/10.1136/thorax.57.1.61
16. Dishy V, Sofowora GG, Xie H-G, Kim RB, Byrne DW, Stein CM, Wood AJJ. The effect of common polymorphisms of the β_2-adrenergic receptor on agonist-mediated vascular desensitization. *N Engl J Med*. 2001:345(14):1030–1035. https://doi.org/10.1056/NEJMoa010819
17. Cockcroft JR, Gazis AG, Cross DJ, Wheatley A, Dewar J, Hall IP, Noon JP. β_2-adrenoceptor polymorphism determines vascular reactivity in humans. *Hypertension (Dallas, Tex: 1979)*. 2000:36(3):371–375. https://doi.org/10.1161/01.HYP.36.3.371
18. Benedict CR, Grahame-Smith DG. Plasma noradrenaline and adrenaline concentrations and dopamine-beta-hydroxylase activity in patients with shock due to septicaemia, trauma and haemorrhage. *Q J Med*. 1978:47(185):1–20.
19. Nakada TA, Russell JA, Boyd JH, Aguirre-Hernandez R, Thain KR, Thair SA, Nakada E, McConechy M, Walley KR. β_2-adrenergic receptor gene polymorphism is associated with mortality in septic shock. *Am J Respir Crit Care Med*. 2010:181(2):143–149. https://doi.org/10.1164/rccm.200903-0332OC
20. van Lier D, Geven C, Leijte GP, Pickkers P. Experimental human endotoxemia as a model of systemic inflammation. *Biochimie*. 2019:159:99–106. https://doi.org/10.1016/j.biochi.2018.06.014
21. Kox M, van Eijk LT, Zwaag J, van den Wildenberg J, Sweep FCGJ, van der Hoeven JG, Pickkers P. Voluntary activation of the sympathetic nervous system and attenuation of the innate immune response in humans. *Proc Natl Acad Sci U S A*. 2014:111(20):7379–7384. https://doi.org/10.1073/pnas.1322174111
22. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B (Methodological)*. 1995:57(1):289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x
23. Cunningham F, Allen JE, Allen J, Alvarez-Jarreta J, Amode MR, Armean IM, Austine-Orimoloye O, Azov AG, Barnes I, Bennett R, *et al.* Ensembl 2022. *Nucleic Acids Res*. 2021:50(D1):D988–D995. https://doi.org/10.1093/nar/gkab1049
24. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics*. 2015:31(21):3555–3557. https://doi.org/10.1093/bioinformatics/btv402
25. Chong LK, Chowdry J, Ghahramani P, Peachell PT. Influence of genetic polymorphisms in the β_2-adrenoceptor on desensitization in human lung mast cells. *Pharmacogenetics*. 2000:10(2):153–162. https://doi.org/10.1097/00008571-200003000-00007
26. Calvano JE, Bowers DJ, Coyle SM, Macor M, Reddell MT, Kumar A, Calvano SE, Lowry SF. Response to systemic endotoxemia among humans bearing polymorphisms of the Toll-like receptor 4 (hTLR4). *Clin Immunol*. 2006:121(2):186–190. https://doi.org/10.1016/j.clim.2006.05.012
27. Stolk RF, Naumann F, van der Pasch E, Schouwstra J, Bressers S, van Herwaarden AE, Gerretsen J, Schambergen R, Ruth M, van der Hoeven HG, *et al.* Phenylephrine impairs host defence mechanisms to infection: a combined laboratory study in mice and translational human study. *Br J Anaesth*. 2021:126(3):652–664. https://doi.org/10.1016/j.bja.2020.11.040
28. van der Poll T, Coyle SM, Barbosa K, Braxton CC, Lowry SF. Epinephrine inhibits tumor necrosis factor-alpha and potentiates interleukin 10 production during human endotoxemia. *J Clin Invest*. 1996:97(3):713–719. https://doi.org/10.1172/JCI118469
29. Stolk RF, van der Poll T, Angus DC, van der Hoeven JG, Pickkers P, Kox M, *et al.* Potentially inadvertent immunomodulation: norepinephrine use in sepsis. *Am J Respir Crit Care Med*. 2016:194(5):550–558. https://doi.org/10.1164/rccm.201604-0862CP

30. Stolk RF, Kox M, Pickkers P. Noradrenaline drives immunosuppression in sepsis: clinical consequences. *Intensive Care Med*. 2020:46(6):1246–1248. https://doi.org/10.1007/s00134-020-06025-2
31. Hoit BD, Suresh DP, Craft L, Walsh RA, Liggett SB. β_2-Adrenergic receptor polymorphisms at amino acid 16 differentially influence agonist-stimulated blood pressure and peripheral blood flow in normal individuals. *Am Heart J*. 2000:139(3):537–542.
32. Bruck H, Leineweber K, Büscher R, Ulrich A, Radke J, Insel P, Brodde O-E. The Gln27Glu β_2-adrenoceptor polymorphism slows the onset of desensitization of cardiac functional responses in vivo. *Pharmacogenetics*. 2003:13(2):59–66. https://doi.org/10.1097/00008571-200302000-00001
33. Leineweber K, Brodde O-E. β_2-adrenoceptor polymorphisms: relation between in vitro and in vivo phenotypes. *Life Sci*. 2004:74(23):2803–2814. https://doi.org/10.1016/j.lfs.2003.10.025
34. Krabbe KS, Bruunsgaard H, Qvist J, Hansen CM, Møller K, Fonsmark L, Madsen PL, Kronborg G, Frandsen U, Andersen HØ, *et al*. Hypotension during endotoxemia in aged humans. *Eur J Anaesthesiol*. 2001:18(9):572–575. https://doi.org/10.1097/00003643-200109000-00003