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Potentially inadvertent immunomodulation: norepinephrine use in sepsis

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Abstract

Septic shock is a major cause of death worldwide and a considerable healthcare burden in the 21st century. Recently, attention shifted from damaging effects of the pro-inflammatory response to the detrimental role of anti-inflammation, a phenomenon known as sepsis-induced immunoparalysis. Sepsis-induced immunoparalysis may render patients vulnerable to secondary infections and is associated with impaired outcome. The immunoparalysis hypothesis compels us to re-evaluate the current management of septic shock and assess whether we are inadvertently compromising or altering the host immune response. In this perspective, we discuss the potential detrimental role of norepinephrine, the cornerstone treatment for septic shock, in sepsis-induced immunoparalysis. We provide a short overview of the current understanding of the immunologic pathophysiology of sepsis, followed by a detailed description of the immunomodulatory effects of norepinephrine and alternative vasopressors. We conclude that although novel therapies aimed to reverse immunoparalysis are underway, the use of norepinephrine may aggravate the development, extent, and duration of sepsis-induced immunoparalysis. Current *in vitro* and animal data indicate that norepinephrine treatment exerts immunosuppressive and bacterial growth-promoting effects, and may increase susceptibility towards infections. However, evidence in humans is circumstantial, as immunologic effects of norepinephrine have not properly been investigated in experimental or clinical studies. Alternatives such as vasopressin/selepressin, angiotensin II, and phenylephrine could have a fundamental advantage over norepinephrine with respect to their immunologic properties. However, also for these agents, *in vivo* immunologic data in humans are largely lacking. As such, human studies on the immunomodulatory properties of norepinephrine and viable alternatives are highly warranted.

Keywords: norepinephrine, catecholamines, immunoparalysis, septic shock, cytokines

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Introduction

Septic shock continues to be a considerable healthcare burden in the 21st century and a major cause of death on Intensive Care Units (ICUs) worldwide (1). Septic shock is defined as hypotension and compromised organ perfusion with loss of peripheral vascular resistance and vascular leakage secondary to the host response to infection that is refractory to intravenous fluid resuscitation. Treatment necessitates vasopressor therapy, for which norepinephrine has been the primary agent of choice since the birth of modern day critical care medicine in the 1950s. In the past decades, adjunctive strategies have aimed to treat sepsis by targeting pro-inflammatory mediators; however they have failed to improve outcome in numerous clinical trials. Recently, attention shifted from inhibition of the pro-inflammatory response to the detrimental role of the anti-inflammatory phase, termed albeit somewhat simplistically as sepsis-induced 'immunoparalysis'. Immunoparalysis may render patients unable to clear their primary infection and could increase their vulnerability to secondary/opportunistic infections (2, 3). The possible involvement of sepsis-induced immunoparalysis as a contributing factor to poor outcome of sepsis patients has promoted interest in novel therapeutic targets and strategies designed to reverse or prevent immunoparalysis, including programmed death-ligand 1 (PD-L1) , cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), interferon-gamma (IFN-y), granulocyte-macrophage colony stimulating factor (GM-CSF), and interleukin-7 (IL-7) therapy (4-8). However, the

immunoparalysis hypothesis also compels us to re-evaluate the current management of septic shock and assess whether we are inadvertently compromising or altering the host immune response. As the catecholamine and sympathetic neurotransmitter norepinephrine is the cornerstone treatment for septic shock in ICUs worldwide and exerts profound immunomodulatory effects (9), the current treatment of patients in shock might aggravate the development, extent, and duration of sepsis-induced immunoparalysis.

In this perspective, we provide a short overview of the current understanding of the immunologic pathophysiology of sepsis, followed by a detailed description of the immunomodulatory and bacterial growth-promoting effects of catecholamines, thereby focusing on norepinephrine. We include both preclinical and clinical studies that contribute to our understanding of how norepinephrine may contribute to sepsis-induced immunoparalysis. Finally, we will discuss alternative vasopressors that may not contribute to sepsis-induced immunoparalysis.

Immunologic pathophysiology of sepsis

The immune response to infectious agents in sepsis is mediated via pattern recognition receptors (PRRs), present on immune cells such as monocytes, dendritic cells and macrophages, of which the Toll-like Receptor (TLR) family is the most studied. PRRs

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recognize specific components of pathogens, so-called pathogen-associated molecular patterns (PAMPs). Activation of PRRs results in production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β and IL-6, as well as recruitment of other immune cells such as granulocytes and lymphocytes to the infected tissue. This cascade is aimed at elimination of pathogens by inducing cytotoxic and phagocytic properties of neutrophils, T-helper 1 (Th1) cells, and macrophages as well as activation of the complement and coagulation systems. Strong activation of the pro-inflammatory response (a so called "cytokine storm") may result in an excessive and injurious reaction, ultimately resulting in septic shock. Anti-inflammatory mechanisms (for example production of the key anti-inflammatory cytokine IL-10, maturation of T-regulatory cells, and lymphocyte apoptosis) are also initiated to limit collateral tissue damage. However, a too pronounced and/or sustained anti-inflammatory phenotype may result in severe impairment of the immune response, known as sepsis-induced immunoparalysis (3). Important hallmarks of sepsis-induced immunoparalysis are deactivation of monocytes and macrophages, exemplified by decreased HLA-DR expression, and impaired *ex vivo*-stimulated pro-inflammatory cytokine production, upregulation of negative regulatory molecules on immune cells, apoptosis of various immune cell populations, and dysregulated cytokine production, favoring the production of anti-inflammatory over pro-inflammatory cytokines

(3). It has become clear that these immunosuppressive mechanisms are present from the onset of sepsis (3).

Several observational studies indicate that immunoparalysis may contribute to sepsis mortality. This is exemplified by the fact that markers of immunoparalysis such as reduced expression of human leukocyte antigen (HLA)-DR on monocytes and impaired *ex vivo*-stimulated cytokine production, correlate with outcome of septic patients (10, 11). Furthermore, in patients that died from sepsis, a continuous septic focus in 89% of patients who received antibiotic treatment for more than 7 days was observed (12), and multiple signs of immunosuppression, including reduced cytokine production by *ex vivo*-stimulated splenocytes, increased expression of inhibitory receptors such as PD-1, and expansion of suppressive T-cells were demonstrated (2). In addition, frequent infections with e.g. C*andida* species, *Aspergillus* species, and *Acetinobacter* or reactivations of low-virulent micro-organisms (dormant viruses such as cytomegalovirus, Epstein-Barr Virus, Herpes simplex virus-1 and Human Herpes virus-6) (13, 14) are encountered in sepsis patients, to an extent comparable with that observed in transplant patients on high dose immunosuppressive medication (13). Collectively, these findings indicate that the immune suppression associated with sepsis may contribute to the increased susceptibility of these patients for infections caused by pathogens that are not virulent in otherwise healthy hosts. A detailed overview of the pathogenesis of sepsis and sepsis-induced immunoparalysis is beyond the

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scope of this perspective and reviewed elsewhere (3, 15). Next to sepsis itself, pharmacological compounds routinely used in the treatment of sepsis patients might also importantly contribute to immunoparalysis, although relatively little attention has been paid to this.

Immunologic and bacterial growth-promoting effects of the sympathetic nervous system and catecholamines

Sympathetic autonomic pathways are widespread in the human body, with the *'fight or flight'* reaction being the classic example of sympathetic activation. Norepinephrine is the main neurotransmitter of most autonomic sympathetic post-ganglionic fibers, whereas the related catecholamine epinephrine is predominantly produced in the adrenal chromaffin cells and secreted into the blood. Interestingly, next to the adrenal chromaffin cells, leukocytes are an ample source of catecholamines, signaling in an autocrine/paracrine manner (16). Norepinephrine and epinephrine exert their effects through α and β adrenoceptors (α -and β-ARs), each divided in several subtypes. These 7- transmembrane G-protein-coupled receptors are present on virtually all human tissues, including the lymphoid organs (bone marrow, thymus, spleen, and lymph nodes) and most immune cells. Norepinephrine has predominant α-AR affinity, with increasing β-AR affinity in higher concentrations, whereas epinephrine has predominant β-AR affinity. Norepinephrine has

been associated with pro-, but mainly with anti-inflammatory effects, dependent on the specific receptor activated as detailed below.

In vitro studies

Activation of α-ARs by norepinephrine has been shown to augment TNF-α production by macrophages *in vitro* (17). These effects are mediated by activation of NF-κB through protein kinase C-induced IκB phosphorylation, in turn resulting in transcription of pro-inflammatory cytokine genes such as *TNFα, IL1β and IL6* (18). However, another *in vitro* study demonstrated anti-inflammatory effects of α-AR stimulation by norepinephrine (19), marking considerable ambiguity in immunologic effects of α-AR activation. Moreover, already at very low concentrations norepinephrine's immunologic effects appear mainly β-AR-dependent (9, 20), and activation of β-ARs exerts potent anti-inflammatory effects. Ligation of β-ARs enhances intracellular cAMP levels, which induce activation of protein kinase A (PKA), an inhibitor of NF-κB, ultimately resulting in reduced expression of pro-inflammatory cytokine genes (18). Furthermore, synthesis of the anti-inflammatory cytokine IL-10 is enhanced through cAMP- and PKA-dependent pathways (21). In accordance with these β-AR-dependent effects, norepinephrine has been shown to attenuate TNF-α and IL-6 and increase IL-10 production in human whole blood, effects that were diminished by β-blockers metoprolol and propranolol (9, 20). A schematic overview of the intracellular

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mechanisms behind the effects of α- and β-AR stimulation on cytokine production is provided in Figure 1. In addition to effects on cytokine production, other *in vitro* studies have shown that norepinephrine diminishes NK-cell cytotoxicity and downregulates IL-2 production by Th1 cells in a β2-AR-dependent manner (22, 23). Th2 function was shown to be unaffected, presumably due to the lack of β2-AR expression on these cells (23). Via this mechanism, norepinephrine may skew the immune response from a Th1 towards a Th2 cell phenotype (23), one of the hallmarks of sepsis-induced immunoparalysis. Next to immunologic effects, norepinephrine directly promotes bacterial growth *in vitro*, which has been shown for both Gram-positive and Gram-negative bacteria (24).

In general, the effects of norepinephrine *in vitro* can be characterized as being generally anti-inflammatory, mediated through β-ARs, as well as directly bacterial growth-promoting. This may compromise the ability of the host to combat infection and thus contribute to sepsis-induced immunoparalysis, although this cannot be deduced from these *in vitro* studies.

Animal studies

There are animal studies that confirm and extend the *in vitro* findings by demonstrating that norepinephrine exerts anti-inflammatory effects *in vivo*. For instance, administration of exogenous norepinephrine in the portal vein of rats resulted in increased IL-10 serum levels,

although concentrations of IL-1β were also elevated (25). Along these lines, acute brain injury in rats, associated with high endogenous catecholamine release, triggered systemic release of IL-10 which was completely prevented by propranolol (26), and atenolol increased lipopolysaccharide (LPS)-induced TNF-α production in mice, suggested to be mediated by blocking the effects of endogenous norepinephrine (27). Animal studies have also demonstrated that norepinephrine indeed compromises the host's ability to combat infection. For instance, neutrophils incubated with norepinephrine display an immunosuppressive phenotype, and inoculation of mice with these neutrophils increased susceptibility for sepsis induced by cecal ligation and puncture and resulted in increased mortality (28). Interestingly, in the same study it was demonstrated that the non-infectious inflammatory conditions pancreatitis or burn injury result in generation of neutrophils with the same suppressive phenotype *in vivo*, suggesting a significant role of endogenous release of norepinephrine (28). In line with these observations, destruction of noradrenergic nerve endings renders mice more resistant against *Listeria monocytogenes* infection (29). Furthermore, there is also *in vivo* evidence for norepinephrine's bacterial growth-promoting properties; a murine study revealed that endogenous release of norepinephrine during trauma directly increases the growth of bacteria in the gastro-intestinal system (30). This phenomenon could be an explanation for the high incidence of infectious complications after trauma.

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Taken together, *in vivo* animal data on the immunologic and bacterial growth-promoting effects of norepinephrine corroborates the previously described *in vitro* findings. However, the translation of animal data to humans is naturally hampered by species differences. In addition, norepinephrine's profound effects on organ perfusion and thus tissue ischemia can be a confounding factor, as tissue ischemia may induce and/or propagate inflammation.

Clinical studies

To date, no clinical studies have specifically investigated the immunologic effects of norepinephrine in humans. Circumstantial evidence in support of norepinephrine's immunosuppressive effects can however be deduced from several studies, although clear interpretation is cumbersome, as outlined below. Sepsis and septic shock are associated with high levels of endogenously- and exogenously-derived circulating catecholamines. For instance, in septic patients without shock, endogenous norepinephrine plasma concentrations up to 0.9 ng/ml have been reported, compared with levels up to 2.9 ng/ml in septic shock patients (31). If septic shock persists, endogenous production of catecholamines is exhausted (32) and high circulating levels result from exogenous administration. After 5 days of exogenous norepinephrine infusion in non-surviving critically ill patients concentrations up to 10 ng/ml were found (33). There are observational studies indicating that high arterial norepinephrine levels are associated with mortality in septic shock patients

independent of disease severity score and hemodynamic parameters (34, 35); however, these findings are at high risk of confounding by indication and no immunological parameters were assessed. Recent findings indicate that blocking β-adrenergic effects may exert beneficial effects in septic shock patients (36). A retrospective study showed that septic patients treated with β-blockers before ICU admission displayed lower mortality, although causes of mortality were not specified (37). Furthermore, in a randomized controlled trial, esmolol treatment decreased mortality by 30% in patients with high norepinephrine load, although the mortality in the control group was very high (36). Naturally, cardiac effects of esmolol, such as a mildly reduced volume load and increased stroke volume were observed, but its beneficial effects might be beyond modulation of these hemodynamic parameters (36). Again, unfortunately no immunologic markers were assessed. Currently, the putative beneficial effects of esmolol are further evaluated in a larger clinical trial (NCT02068287), in which immunologic markers are assessed as well. Along these lines, blocking the actions of endogenous catecholamines with propranolol in pediatric burn patients, another condition accompanied by high catecholamine release, was associated with a lower rate of secondary infections (38). It could be contended that these beneficial effects are due to intrinsic immune-stimulatory effects of β-blockers, however, these have not been reported *in vitro* (9) and there are no indications that β-blockade exerts advantageous immune effects in diseases not associated with increased endogenous

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catecholamine levels. Finally, in traumatic brain injury patients, it was shown that profound sympathetic activation induces systemic release of IL-10 (26). This IL-10 release was shown to be associated with an immunosuppressive monocyte phenotype (decreased cell-surface HLA-DR expression), and an increased infection rate, although the relative contribution of norepinephrine and epinephrine was not assessed (26).

Collectively, these clinical studies suggest that high norepinephrine levels encountered in septic patients may contribute to sepsis-induced immunoparalysis, although to date, studies into sepsis-induced immunoparalysis have not assessed the potential role of norepinephrine. Furthermore, clear assessment of these effects in studies in septic shock patients will always be difficult for several reasons. First, until alternative vasopressors become standard of practice there is an absolute requirement of norepinephrine administration in patients with septic shock to achieve hemodynamic stabilization (31), as such, it would be unethical to withhold this treatment. Second, similar to animal studies, differences in hemodynamic effects of norepinephrine compared with a fluid heavy approach or alternative vasopressors could induce differences in organ perfusion and/or tissue ischemia, which, as alluded to before, hampers interpretation of immunologic effects as well. Therefore, the most promising approach to increase our understanding of intrinsic immunologic effects of norepinephrine is the use of experimental human *in vivo* models of inflammation or clinical conditions which are not associated with compromised organ perfusion and/or tissue

ischemia. As of yet, there is only such data on the highly related catecholamine epinephrine. Exogenous administration of this cathecholamine was shown to potently attenuate plasma TNF-α levels, whereas it increased circulating anti-inflammatory IL-10 concentrations during experimental human endotoxemia (20). Epinephrine infusion also reduced the capacity of blood leukocytes to release IL-1β upon stimulation with LPS *ex vivo* (39). These findings of epinephrine-induced immunosuppression were recently extended by another human endotoxemia study which showed that increasing endogenous epinephrine levels through a behavioral intervention also resulted in markedly reduced plasma concentrations of pro-inflammatory cytokines and increased IL-10 levels (40). Interestingly, using the same human endotoxemia model, it was demonstrated that the specific β1-AR agonist dobutamine does not influence cytokine release, suggesting that the anti-inflammatory effects of epinephrine and other β-AR agonists in humans *in vivo* are mediated via β2-ARs (41). There are strong indications that immunologic effects of norepinephrine resemble the anti-inflammatory effects demonstrated for epinephrine, as norepinephrine and epinephrine exert equally potent immunosuppressive effects in LPS-stimulated human whole blood or isolated monocytes *in vitro* (9, 20, 42).

Studying adrenoceptor gene polymorphisms could represent an alternative approach to shed light on the contribution of norepinephrine to sepsis-induced immunoparalysis. Interestingly, in human lymphoblastoid cell lines carrying the CysGlyGln haplotype variant of

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the β2-AR, norepinephrine suppressed IL-6 production to a lesser degree compared with other haplotypes (43). Furthermore, in two large cohorts of septic shock patients, this haplotype was associated with decreased norepinephrine sensitivity, higher lactate levels, more organ dysfunction, and increased mortality (43). It appears plausible that the increased mortality was probably due to hemodynamic rather than immunologic effects, however no immunologic parameters in patients or incidence of secondary infections were reported (43).

Taken together, evidence obtained from *in vitro* and animal studies indicate that norepinephrine exerts anti-inflammatory and bacterial growth-promoting effects, which lead to increased susceptibility towards infections. This is supported by experimental human studies that show potent immunosuppressive effects of the highly related catecholamine epinephrine. As such, although clinical evidence is as of yet circumstantial, norepinephrine could importantly contribute to sepsis-induced immunoparalysis and use of alternative vasopressors may be superior in this respect.

Figure 2 provides an overview of receptor- and cell-specific immunologic and bacterial-growth promoting effects of norepinephrine derived from *in vitro* and animal studies and how these might contribute to sepsis-induced immunoparalysis.

Alternative vasopressors

Three alternative vasopressors for the treatment of septic shock that may not, or to a lesser extent contribute to immunoparalysis are vasopressin/ selepressin, angiotensin II (ATII), and phenylephrine. In this perspective, we focus on direct vasopressors and not blockers of endogenous vasodilators. Therefore we will not discuss the nitric oxide inhibitors methylene blue and L-NMMA. Furthermore, it might be argued that dopamine is a viable alternative. Nevertheless, a large RCT in shock patients slanted in favor of norepinephrine, although a significantly decreased mortality was only found in a subgroup of cardiogenic shock patients (44). This advantage was mainly explained by a lower number of adverse events (44). In addition, dopamine exerts potent anti-inflammatory effects, both through stimulation of dopaminergic receptors and β-ARs (45), so it appears plausible that it confers no immunologic advantages. In accordance, the incidence of new infectious episodes in the aforementioned RCT was similar between groups (44). Similar to dopamine, epinephrine exerts immunosuppressive effects, as described earlier, and did not show benefit over norepinephrine plus dobutamine in a large RCT (46).

Vasopressin is 8-arginine-vasopressin, a synthetic analogue of the endogenous anti-diuretic hormone (ADH). To date, vasopressin is mostly used as an adjunctive treatment to norepinephrine in severe septic shock, and reduction of the norepinephrine infusion rate by addition of vasopressin reduced mortality in a subgroup of patients with relatively mild

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septic shock (47). However, in this trial vasopressin was added as an adjunctive treatment to existing vasopressor treatment to allow tapering of vasopressor dosages, and was started after several hours of this existing treatment, which consisted of norepinephrine in the great majority of patients. Therefore, most patients in the vasopressin group initially received norepinephrine and many of them continued to receive it, albeit in lower dosages. This hampers clear interpretation and assessment of (immunological) effects, as norepinephrine already exerts immunosuppressive effects at low concentrations (9). Small studies did however show that vasopressin is a viable alternative for norepinephrine as first-line treatment in septic shock (48), which is evaluated in a currently ongoing larger clinical trial (ISRCTN 20769191). As of yet, little is known about the immunomodulatory properties of vasopressin in septic shock. In a post-hoc assessment of the previously mentioned trial (47), vasopressin was associated with a more pronounced decrease of plasma cytokine levels in the first 24 hours of treatment (49). Unfortunately, as mentioned before, sustained norepinephrine administration in the patients in the vasopressin group in this study impedes clear interpretation of these results. Furthermore, the difference reported failed to retain statistical significance following correction for baseline characteristics such as corticosteroids, APACHE II score, shock severity, age, and sex (49). Therefore, from this study no clear conclusions can be drawn on immunologic properties of vasopressin. Small animal studies, which are more easily interpretable, may suggest anti-inflammatory properties.

Vasopressin treatment resulted in slightly decreased intestinal IL-6 levels compared with norepinephrine in a porcine sepsis model, while levels of circulating TNF-α, IL-6, and IL-10 were similar (50). In mice, vasopressin attenuated LPS-induced pulmonary but not systemic IL-6 levels, which was abolished by a V2-receptor antagonist (51). Likewise, recent data concerning early administration of a selective V1 receptor agonist (selepressin) in an ovine sepsis model showed reduced systemic IL-6 levels compared with norepinephrine (52). However, organ perfusion in general was better with selepressin compared to norepinephrine, which may indirectly affect systemic cytokine production due to attenuation of tissue ischemia and resulting inflammation (52). At present, selepressin is being evaluated in a multi-centre trial for clinical efficacy and safety (NCT02508649). One might argue that terlipressin, another vasopressinergic agent with V1-receptor selectivity, represents a suitable alternative vasopressor as well. This agent is frequently used as rescue therapy in addition to norepinephrine, however its use as a first-line vasopressor for septic shock is hampered by its long half life, which compromises exact titration (53).

Angiotensin II has no reported immunosuppressive effects. In a pilot study in patients with high-output shock, angiotensin II was well-tolerated and markedly reduced norepinephrine requirement, albeit with considerable interindividual variation (54). Angiotensin II is currently further investigated in an ongoing phase 3 trial in sepsis patients (NCT02338843).

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Phenylephrine is a pure α-adrenergic agonist. Its use in septic shock patients has thus far been limited due to concerns for splanchnic blood flow. However, no difference was found in any regional hemodynamic parameters when compared with norepinephrine in septic shock (55). In addition, a recent meta-analysis failed to show a survival benefit for norepinephrine over phenylephrine (56). As described before, *in vitro* studies have shown that α-AR stimulation is mainly associated with pro-inflammatory effects. In accordance, LPS-stimulated monocytes exhibited increased IL-1β production after incubation with phenylephrine (57), however these results are not supported by work showing that phenylephrine does not affect TNF-α or IL-6 production in LPS-stimulated whole blood (9). *In vivo* data is limited to a canine endotoxemia model revealing that phenylephrine treatment resulted in a very modest increase in circulating TNF-α levels (58). Given the limited and pre-clinical nature of the current evidence, no definitive conclusions can be drawn on the immunological properties of vasopressin/selepressin, angiotensin II, and phenylephrine in humans *in vivo*, and studies into these effects are highly warranted as these compounds might represent viable less- or no immunosuppressive alternatives to norepinephrine. Lastly, administration of non immunosuppressive alternative vasopressors would be most advantageous in the later phases of sepsis, when immunoparalysis is the overriding immune dysfunction (59) and endogenous catecholamine release is exhausted (32). Nevertheless, it is increasingly recognized that anti-inflammatory mechanisms are activated simultaneous with

the pro-inflammatory response early on in sepsis (3). Furthermore immunologic changes induced by early norepinephrine treatment, for instance a skewed Th1/Th2 balance (23), can be long-lasting and thus have a detrimental influence on the entire course of the disease. In this respect, restriction of norepinephrine use should be pursued already early on in sepsis.

An overview of the different classes of vasopressors described in this perspective and their immunologic effects is provided in Table 1.

Conclusion

Sepsis-induced immunoparalysis is a common phenomenon in septic shock patients and may contribute to the development of secondary infections and late mortality. Next to sepsis itself, our current treatment approach may substantially contribute to the development of immunoparalysis. Furthermore, as many of these therapies are also administered in non-sepsis critically ill patients, such as those suffering from major trauma or traumatic brain injury, it might also be an explanation for the high risk of nosocomial infections in these populations. While novel therapies aimed to reverse immunoparalysis are underway, we believe that a reassessment of our old therapies is indicated. Current *in vitro* and animal data indicate that norepinephrine treatment exerts immunosuppressive effects and may increase infection rates. Furthermore, restricted use of norepinephrine is propagated due to

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its multitude of adverse effects (60). However, to date, immunologic effects of norepinephrine and markers of immunoparalysis have not properly been investigated in experimental or clinical studies in humans. Alternatives such as vasopressin/selepressin, angiotensin II, and phenylephrine could have a fundamental advantage over norepinephrine with respect to these immunologic properties. However, also for these agents, *in vivo* immunologic data in humans are largely lacking. As such, human studies on the immunomodulatory properties of norepinephrine and viable alternatives are highly warranted.

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Figure legends

Figure 1. Intracellular mechanisms behind the effects of α- and β-AR stimulation on cytokine production. Panel A: Stimulation of the α-AR results in activation of PKC, which induces IκB phosphorylation, in turn resulting in translocation of NF-κB to the nucleus. NF-κB facilitates pro-inflammatory cytokine transcription and this ultimately leads to enhanced pro-inflammatory cytokine production. Panel B: Stimulation of the β-AR increases intracellular cAMP levels, which activate PKA. PKA prevents NF-κB from entering the nucleus, resulting in reduced pro-inflammatory cytokine transcription and production and increased production of anti-inflammatory IL-10.

AR= adrenoceptor, cAMP=cyclic adenosine monophospate, IκB= Inhibitor nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB= nuclear factor kappa-light-chain-enhancer of activated B cells, PKA=Protein Kinase A, PKC= Protein Kinase C, IL= interleukin, TNF=Tumor necrosis factor.

Figure 2. Several mechanisms by which norepinephrine may contribute to sepsis-induced immunoparalysis. Activation of the α-AR has been associated with both pro-and anti-inflammatory effects. Activation of the β-AR exerts anti-inflammatory effects, including decreased production of pro-inflammatory cytokines, increased production of anti-inflammatory cytokines, skewing of the immune response from a Th1 towards a Th2 cell phenotype, and decreased NK cell cytotoxicity. Furthermore, norepinephrine induces the generation of immunosuppressive neutrophils and directly promotes bacterial growth. AR= adrenoceptor, IL= interleukin, TNF=Tumor necrosis factor, Th= T-helper, NK= natural killer.

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Table 1. Overview of different vasopressors, their classes, their receptors and immunologic effects.

Drug	Class	Receptor affinity	Immunologic effects
Epinephrine	Catecholamines	$\alpha_1, \alpha_2, \beta_1, \beta_2$	*In vitro* TNF-α↓ , IL-6↓, IL-8↓, IL-10↑ (20, 42) *In vivo* Human endotoxemia: circulating TNF-α↓, IL-10↑ (20)
Norepinephrine	Catecholamines	$\alpha_1, \alpha_2, \beta_1 > \beta_2$	*In vitro* TNF-α↓, IL-6↓, IL-8↓, IL-10↑, Th1/Th2 ratio↓, NK cell cytotoxicity↓, bacterial growth↑ (9, 20, 22-24, 42) *In vivo* Rats (norepinephrine injection in portal vein): circulating IL-1β↑, IL-10↑ (25)
Phenylephrine	Phentylamines	α_1	*In vitro* IL-1β↑, TNF-α–, IL-6– (9, 57) *In vivo* Canine endotoxemia: circulating TNF-α↑(58)
Vasopressin	Synthetic vasopressin-analogue	V_1, V_2	*In vivo* Pig sepsis (fecal peritonitis): circulating TNF-α–, IL-6–, IL-10– , intestinal TNF-α–, IL-6↓, IL-10–, compared with norepinephrine (50) Murine endotoxemia: circulating IL-6–, pulmonary IL-6↓(51)
Selepressin	Synthetic vasopressin-analogue	V_1	*In vivo* Sheep sepsis (fecal peritonitis): IL-6↓ compared with norepinephrine (52)
Angiotensin II	Angiotensin	AT_1, AT_2	Not investigated

TNF= Tumor necrosis factor, IL= interleukin, Th= T-helper, NK= natural killer, ↑= increased, ↓= decreased, –= not affected.

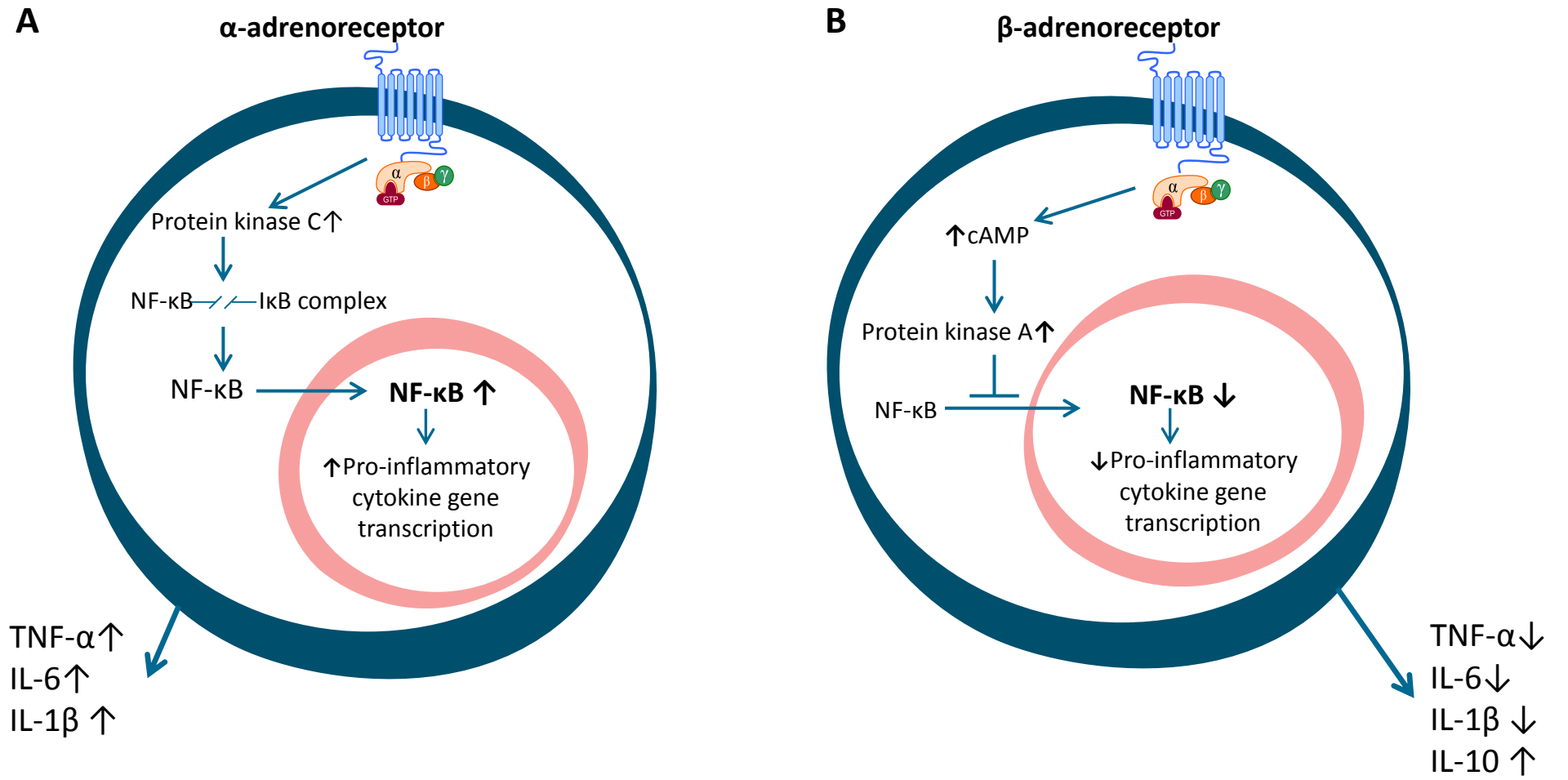

Figure 1.

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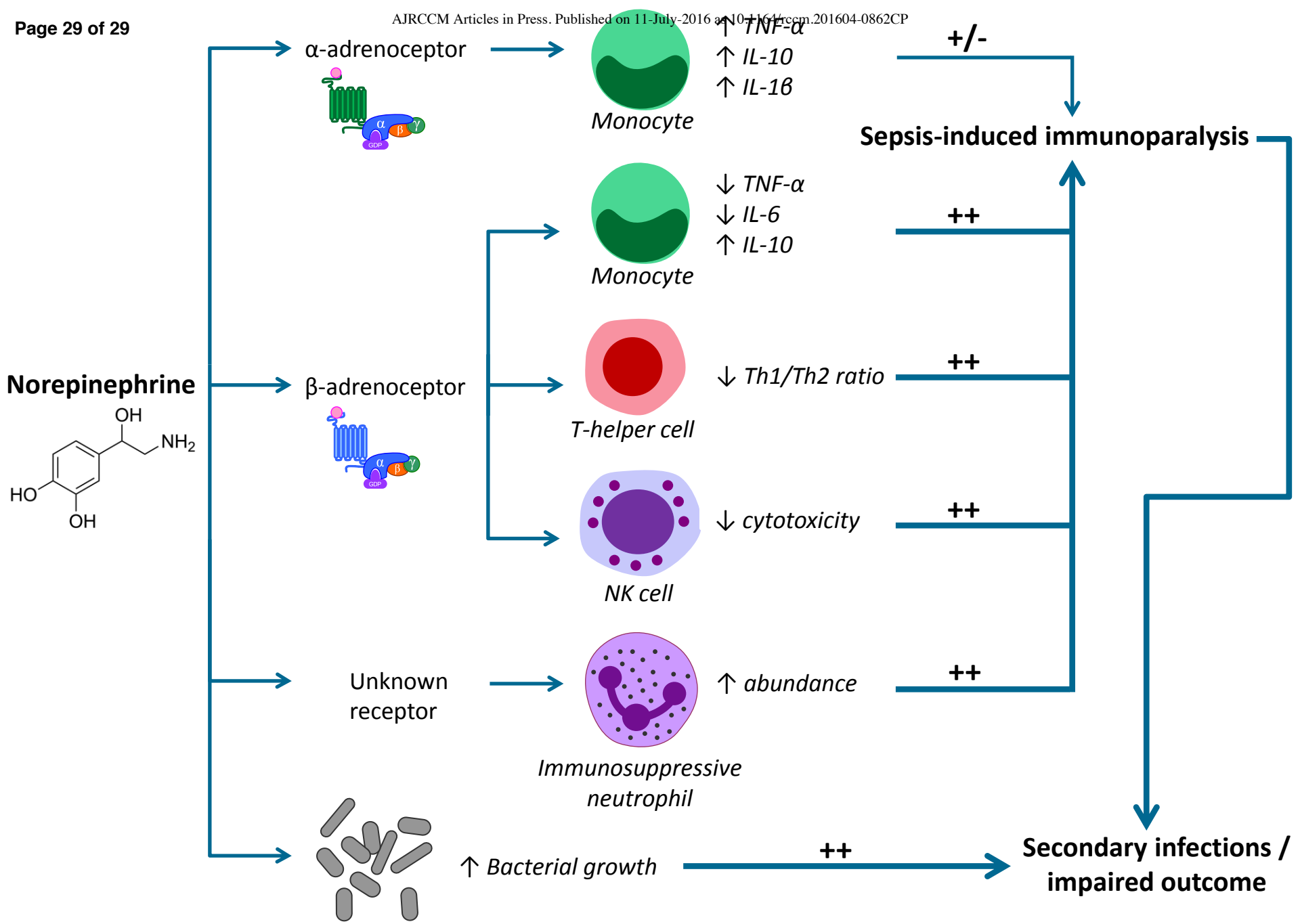

Figure 2.