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Sepsis: the need for tolerance not complacency

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Summary

Sepsis is a life-threatening condition that arises as a systemic inflammatory response syndrome to an infection. Its uncontrolled progression can in frequent cases lead to multiple organ failure, which is still associated with high mortality rates. Modern antibiotics made clear that the infection is only an initiating, and not always necessary, event of this syndrome as many patients with sepsis die despite effective eradication of the inciting pathogen. This observation critically contributed to a paradigm shift that focused the pathogenesis of sepsis on the host and not on the pathogen. However, therapeutic strategies based on the inhibition of proinflammatory critical mediators of sepsis or immunostimulation have so far failed to improve sepsis outcome and, therefore, this condition urgently needs transformative therapeutic ideas and strategies. Here we argue that the induction of tolerance, a defence strategy that minimises the impact of an infection on organ function without directly affecting the pathogen burden, is perhaps the missing but essential element to add to the current components of sepsis care and treatment.

Key words: *sepsis; tolerance; DNA damage response; anthracyclines*

Introduction

Based on the pressing need to better define sepsis, to enable clinical trials and to test novel interventions for this devastating condition, an international consensus panel defined sepsis in 1992 as a systemic inflammatory response to infection. The panel also proposed the term “severe sepsis” to describe cases in which sepsis is complicated by acute organ dysfunction, and “septic shock” for sepsis complicated by hypotension that is refractory to fluid resuscitation. The definition and characterisation of sepsis have since evolved, but the key concepts of the original 1992 consensus panel have been for the most part upheld by more recent international consensus panels. “Severe sepsis” and “sepsis” are sometimes now used interchangeably to describe the syndrome of infection complicated by acute organ dysfunction [1, 2].

The pathophysiology and molecular bases of sepsis and multiple organ failure remain poorly understood. As a result, the central elements of treatment (early recognition, sepsis stratification, organ support, namely optimisation of oxygen transport, early adequate antibiotic therapy, and source control if necessary) have not changed substantially in recent decades, and attempts to translate basic research results into effective new interventions have been met with limited or no success [3]. Therefore, perhaps not surprisingly, there are currently no approved drugs that specifically target sepsis. After decades without significant progress in the treatment of sepsis, there is an urgent need for transformative therapeutic alternatives in addition to the thoroughly tested but unsuccessful block of proinflammatory mediators [4] or the more recently proposed, but so far disappointing, stimulation of the immune response [5–8].

Innate immunity is evolutionarily ancient, crucial to fighting infections by invading pathogens or other noxious challenges such as tissue injury, and relies on multiple components, from physical barriers to effector molecules [9]. Innate immune receptors detect signatures of pathogens (pathogen-associated molecular patterns) and also molecules from diseased or dying host cells (damage-associated molecular patterns [10]). The activation of pattern recognition receptors by pathogen- or damage-associated molecular patterns initiates intracellular signalling events and cascades leading to the expression and secretion of inflammatory mediators such as cytokines and chemokines. Ultimately, inflammation needs to be effectively terminated after removal of the original trigger to limit tissue damage and restore homeostasis [11]. Sepsis results from a deranged and excessive systemic inflammatory response to an infection, which can progress to multiple organ dysfunction and has the potential to evolve to multiple organ failure that is often fatal.

Tolerance as an emerging defence strategy

In addition to pathogen *avoidance*, there are two evolutionarily conserved defence strategies against infection that can limit host disease severity. One relies on reducing pathogen load, i.e. *resistance* to infection that is characterised by

and requires inflammation, while the other provides host tissue damage control, limiting disease severity irrespectively of pathogen load, i.e. *tolerance* to infection [12, 13]. Tissue destruction and physiological damage are a consequence of mounting an immune response to fight an infection. To minimise the impact of this damage on health, a host relies on preventive and repair pathways that will affect tolerance but not necessarily resistance defences [14]. This concept of tolerance should not be confused with the classical immunological definition of tolerance that refers to unresponsiveness to self-antigens.

As demonstrated originally for plants and thereafter in *Drosophila*, tolerance to infection also operates in mammals, as revealed for *Plasmodium* [15, 16] and polymicrobial infections in severe sepsis [17, 18]. In disease, immunopathology and excessive inflammation can be the main causes of morbidity and mortality, as immune effectors produced during an excessive immune response and the decrease of available energy for vital functions can cause severe and sustained collateral damage [14]. This is particularly relevant in the case of sepsis, where the effective elimination of the original causative infection does not guarantee a favourable prognosis even with the current best standard of care.

A therapeutic strategy solely based on the targeting of inflammatory pathways has been shown in multiple clinical trials to be essentially unsuccessful [4]. The outcome of these trials is not surprising because by the time sepsis can be clinically diagnosed, most downstream signalling pathways and effectors have already been activated and inflammation has already caused substantial damage. Therefore, targeting mechanisms of tissue tolerance to damage is a viable, and perhaps the critical, therapeutic option in addition to the other current pillars of sepsis management [19], which are critical and not expected to change in the foreseeable future, but are also clearly insufficient.

The main problem for the clinical application of this concept lies in our current general ignorance of the molecular mechanisms of tolerance to tissue damage [14], as very few have so far been identified [20]. One possible strategy to increase tolerance might be through *hormesis*, which refers to the capacity of mild, sublethal stresses to protect against larger subsequent insults [21] and can be defined as any adaptive response exhibiting a biphasic dose response [21]. The elicited responses can either be directly induced or result from compensatory biological processes triggered by an initial disruption in homeostasis, which is interesting because inflammation has been considered as the extreme end-result of substantial deviations of homeostasis [22]. While diverse, hormesis responses induce wide-ranging and long-lasting cytoprotective states that often converge in similar mechanisms. Perhaps surprisingly, rather than being harmful, hormesis-induced mechanisms reduce disease susceptibility and might even prolong the lifespan of the organism where they operate [23].

It has long been known that exposure to low levels of bacterial lipopolysaccharide (LPS), or other inflammatory stimuli, can desensitise the host organism and protect from a subsequent larger dose of LPS that would otherwise be lethal. This phenomenon is known as LPS tolerance and is explained by the initial low dose induction of negative regulators of LPS signalling and suppression of downstream LPS-induced tissue damage genes [24]. A role for the oxidative stress-induced heme oxygenase-1 in tolerance to *Plasmodium* infection and other conditions causing tissue damage [16, 25], for the induction of the unfolded protein response (UPR) to promote tolerance to *Pseudomonas aeruginosa* infection in *Caenorhabditis elegans* [26], and for the nuclear factor, erythroid 2-related factor-2 (Nrf2) gene in cytoprotection have been recently shown. Nrf2 is a transcription factor that regulates host defence against oxidative stress and inflammation-related disorders [27]. It has anti-inflammatory effects in a LPS-induced inflammation mouse model and its down-regulation has been associated with higher organ failure burden and mortality in paediatric septic shock [28]. Lactobacilli have recently been shown to exert a hormetic protective response in the gut by inducing enterocyte production of reactive oxygen species catalysed by NADPH oxidase (Nox) 1, which triggers a Nrf2-dependent protective transcriptional response [29]. Heat shock is known to activate another transcription factor HSF-1 (heat shock factor 1) that prevents proteotoxicity by triggering pathways that control refolding or degradation of misfolded proteins [30]. Endoplasmic reticulum stress activates a transcriptional programme, which includes the *ATF6*, *PERK*, and *IRE1* genes that restore protein homeostasis in the endoplasmic reticulum [31].

In spite of these examples, hormesis mechanisms and cytoprotective pathways remain largely unknown and uncharacterised. The systematic identification and characterisation of such mechanisms is likely to open a complete new field of opportunity to understand at a molecular level core surveillance mechanisms of basic cellular processes with a critical role in the regulation of organ function and whose activation can ultimately promote health and expand longevity. The pharmacological targeting of hormetic pathways will be important for both the mechanistic identification of disease tolerance mechanisms and the therapeutic targeting of diseases that are characterised by extensive tissue damage or multiple organ failure.

We have recently screened approximately 2 300 compounds (including most that are approved for clinical use) for their ability to inhibit inflammatory cytokine production and have identified members of the anthracycline family of chemotherapeutic agents (epirubicin, doxorubicin and daunorubicin) as potent antagonists [18]. In an experimental model of severe sepsis [32], caecal ligation and puncture (CLP), where at least 80% of C57BL/6 mice die within 48 hours after the initial procedure, administration of a low dose of epirubicin at the time of CLP and again 24 hours later at a total dose of 1.2 μg/g body weight reproducibly increases by 80% the survival of C57BL/6 mice subjected to CLP without the use of antibiotics [18]. Protection against severe sepsis is not due to an antibiotic effect, as epirubicin also protects C57BL/6 mice from lethal septic shock subsequent to LPS administration [18]. Moreover, epirubicin-treated mice subjected to CLP show similar numbers of bacteria circulating in blood and in target organs of sepsis (e.g., spleen, liver and kidney) at 24 hours post-CLP as compared with untreated controls [18]. This suggests that epirubicin confers disease tolerance to polymicrobial infection, a host defence strategy against infec-

tion that acts irrespectively of pathogen burden [20]. In fact, serum concentration of lactate dehydrogenase (lung and general cellular damage), creatine kinase (muscle), alanine aminotransferase (liver) and urea (kidney) are reduced to almost basal levels in mice treated with epirubicin compared with untreated mice [18], suggesting that anthracyclines provide tissue damage control and sustain organ function. Ataxia telangiectasia mutated (ATM) is a master regulator of the DNA damage response [33] and is known to be activated by anthracyclines [34]. ATM-deficient ($Atm^{-/-}$) mice are not protected against CLP by epirubicin and die with similar kinetics to those of wild-type ($Atm^{+/+}$) animals that are treated with phosphate-buffered saline alone. Therefore, ATM expression is necessary to mediate the protective effect of epirubicin [18]. Accordingly, epirubicin does not normalise the serological markers of organ lesion in $Atm^{-/-}$ mice or decrease the levels of inflammatory mediators.

The lung is not only the most common site of infection leading to sepsis but also respiratory dysfunction/failure (independently of where the infection originated) occurs often, at a very early stage in the pathophysiology of sepsis [35–38] and can possibly initiate a cascade of events leading to multiple organ failure that carries a very high mortality rate [39].

Interestingly, key molecular steps of sepsis protection induced by epirubicin, including the activation of DNA damage responses and autophagy pathways, take place in the lung [18, 40]. Their activation is necessary in the lung for systemic protection, because depletion of ATM and ATG7 (autophagy related 7) specifically in the lung eliminates the protection conferred by epirubicin when given systemically.

Preliminary evidence from our laboratory suggests that lung protection is not only necessary, but also sufficient for systemic protection as direct delivery of epirubicin to the lung confers sepsis protection that is similar to that obtained by systemic administration of the drug (Velho et al., unpublished). If confirmed, these observations have potential fundamental clinical significance because that would mean that if signs of lung dysfunction are detected early enough in patients, and necessary and effective measures can be used, it is then possible to stop the cascade of events leading to the domino effect causing the progressive failure of more organs, including the kidneys and liver, that are likely to culminate in multiple organ failure, a condition with a very poor prognosis once established [1].

Conclusion

Tolerance is an emerging defence strategy that has tremendous potential to be a central element of the treatment of sepsis and multiple organ failure as an adjuvant to the current standard of care. Hormesis might constitute an effective possibility to induce tolerance. Interestingly, low doses of DNA damaging agents, such as anthracyclines, induce strong protection against mouse models of sepsis, even in the absence of broad-spectrum antibiotics, by promoting tolerance without affecting the burden of infection. While this hormetic effect was very strong and reproducible in several mouse models of sepsis, it might not always be applicable to real clinical settings in terms of both therapeutic window and organs primarily affected, because tissues differ in tolerance capacity and resilience of physiological processes. In fact, tolerance capacity varies in different organs and tissues as a function of their intrinsic damage susceptibility, repair capacity, functional autonomy and the consequences of a certain level of damage for a particular tissue. Therefore, studying how anthracyclines protect from sepsis downstream of the activation of DNA damage responses might constitute an unprecedented window into the identification of novel tolerance, cytoprotective mechanisms and tissue damage repair mechanisms, which are attractive molecular targets for the treatment of sepsis and multiple organ failure. They would have the advantage of specificity, fewer potential side effects and improved efficacy in a wide-range of clinical cases, because they can predictably be activated at any point and severity of organ dysfunction. Finally, uncovering the role of DNA damage responses in sepsis also opens the possibility to understand better its pathophysiology at the molecular level and how organisms deal with stress, age and set limits to their lifespan.

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