EDITORIAL
BASIC SCIENCE

Salvaging the endothelium in acute respiratory distress syndrome: a druggable intersection between TLR4 and NAD$^+$ signalling

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eNAMPT neutralisation may be effective treatment in acute respiratory distress syndrome
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The acute respiratory distress syndrome (ARDS) remains an intractable problem for intensivists, even with advancements in critical care and mechanical ventilation. Mortality rates in ARDS patients are around 35–45% [1]. However, these mortality numbers represent a conservative estimate of the ARDS healthcare impact, because survivors exhibit staggering rates of morbidity and mortality in the aftermath of their intensive care unit (ICU) stay, including neurocognitive dysfunction that can prohibit the return to activities of daily living [2, 3]. Development of medical therapies that improve both the short- and long-term outcomes of these critically ill patients remain an urgent unmet need.

Medical therapies have generally failed to improve outcomes in ARDS patients, which reflects the complexity of the inflammatory milieu in this setting. Both innate and adaptive immunity is activated during ARDS, especially when pneumonia and sepsis represent the initiating insult. While an immune response is essential for host defence, over-exuberant and sustained production of cytokines [4], damage- and pathogen-associated molecular patterns [5, 6], glycosaminoglycans [7] and cytotoxic amyloids [8] elicit secondary tissue injury that negatively impact recovery. Recognising this delicate balance, clinical investigators have made valiant efforts to inhibit the maladjusted cytokine storm "at the right time", commonly, but not exclusively, with corticosteroids. Yet, after many clinical trials, there is no clear answer as to whether, or which, clinical outcomes improve with corticosteroid treatment [9]. SARS-CoV-2 infections have shed some light on the issue, at least in a population of patients with this form of viral pneumonia, where the RECOVERY trial reported that dexamethasone improved 28-day mortality among patients with hypoxaemic respiratory failure [10]. Nonetheless, there is need for a more effective and targeted intervention of the unremitting cytokine storm, at a time when the bacterial and viral loads have been effectively controlled. In this issue of the *European Respiratory Journal*, Quijada *et al.* [11] suggest

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that endothelial extracellular nicotinamide phosphoribosyltransferase, or eNAMPT, represents just such a druggable target.

eNAMPT was first discovered in 1994 using an unbiased screen for genes that drive B cell differentiation [12]. The protein was found outside of the cell, where it exerted a paracrine signalling function. In this context, it was named pre-B-cell colony enhancing factor. Soon thereafter, mechanical stretch, cytokines, lipopolysaccharide and infection were all shown to promote release of pre-B-cell colony enhancing factor from various cell types [13–15], suggesting it plays a central role in the response to inflammation. eNAMPT (also called visfatin) is highly expressed in visceral fat too, and increased circulating concentrations of eNAMPT parallel visceral fat accumulation [16], consistent with a pro-inflammatory state in metabolic syndrome.

Inflammation is rampant in ARDS, and J.G. Garcia and co-workers found high expression of NAMPT in an unbiased search for genes that are prominently expressed in animal and human subjects with ARDS; increased eNAMPT is associated with ARDS severity [17]. Moreover, they identified genetic variants in the *NAMPT* promoter that may be relevant to ARDS outcomes [17, 18]. The eNAMPT amplified the pro-inflammatory environment in ARDS (figure 1). It inhibits apoptosis in neutrophils, prolonging their survival in sepsis [19], and it activates nuclear factor-kB signalling in effector cells, fuelling cytokine synthesis [20, 21]. Indeed, eNAMPT interacts with TLR4, initiating the downstream activation of the NLR3 inflammasome [22, 23]. Infection combined with mechanical ventilation in ARDS strongly promotes eNAMPT release into the airway and the circulation, where it fuels a pro-inflammatory environment within the lung and elsewhere.

However, NAMPT lacks a known secretion sequence, and not all of the NAMPT is found outside of the cell; in fact, how NAMPT is released from the cell remains an open question [21]. Intracellular splice variants of NAMPT (iNAMPT) are found in the cytosol, the mitochondria and the nucleus, where they are rate-limiting enzymes in the nicotinamide adenine dinucleotide (NAD^+) salvage pathway (figure 2). NAD^+ is necessary for metabolic progression through glycolysis and oxidative phosphorylation in the cytosol and mitochondria, respectively [24, 25]. NAD^+ is converted to nicotinamide deacetylases, *i.e.* sirtuins, and ADP-ribosyl transferases, *i.e.* PARPs, which are enzymes that regulate protein function, gene transcription and DNA damage repair [24, 25]. The intracellular pool of iNAMPT is especially important, since the sirtuins and PARPs rely on the salvage pathway to replenish NAD^+ and drive cell survival. Thus, iNAMPT contributes to metabolic requirements and cell survival signalling in a pro-inflammatory environment.

In this issue of the *European Respiratory Journal*, both lipopolysaccharide ("one-hit" model) and lipopolysaccharide combined with ventilator-induced lung injury ("two-hit" model) increased lung NAMPT that amplified the inflammatory response [11], raising the possibility that eNAMPT represents a tractable target in ARDS, potentially discriminating between its extracellular and intracellular functions. To test this idea, an eNAMPT-neutralising goat polyclonal antibody and a first-in-kind humanised monoclonal antibody were delivered intravenously following the onset of ARDS. Both antibodies

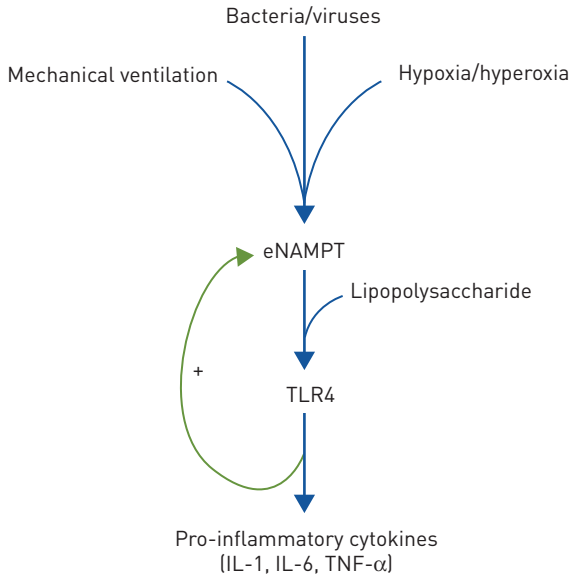

FIGURE 1 eNAMPT amplifies the inflammatory response in acute respiratory distress syndrome. An inflammatory milieu increases eNAMPT in the airway and the circulation, including bacterial and viral infection, airway stretch, as occurs with mechanical ventilation, and hypoxia. eNAMPT further promotes TLR4 signalling, which amplifies the production of eNAMPT. IL: interleukin; TNF: tumour necrosis factor.

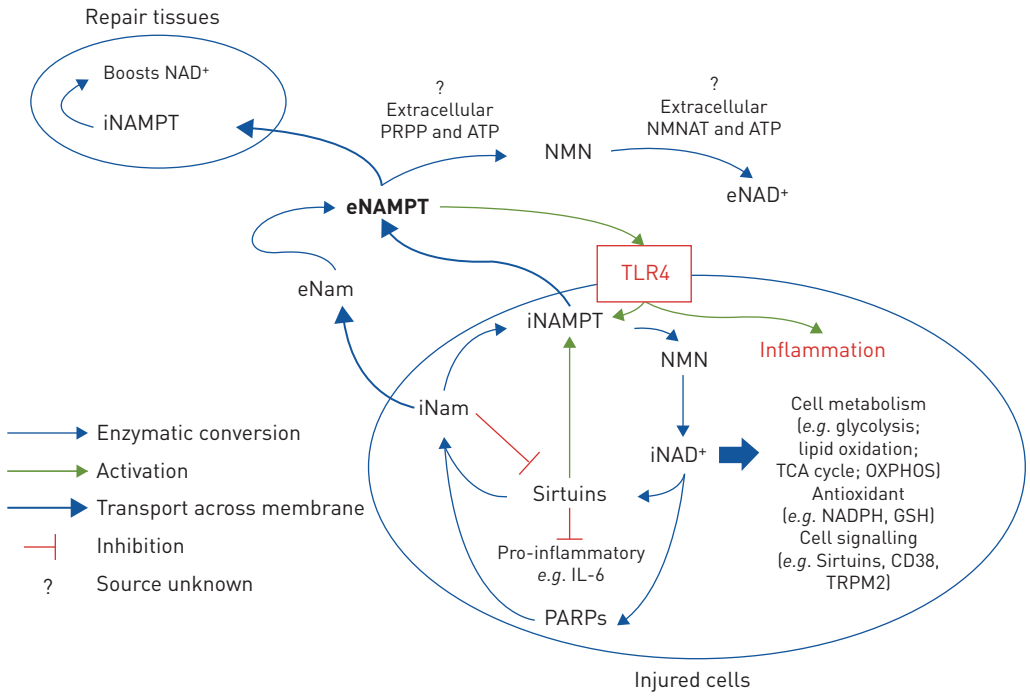

FIGURE 2 NAMPT is a rate limiting enzyme in the NAD^+ salvage pathway, necessary in control of cellular bioenergetics and gene transcription that controls cell survival. Infection and inflammatory stimuli increase eNAMPT, which promotes TLR4 signalling. However, whether eNAMPT contributes to NAD^+ synthesis in the fluid-phase of the circulation remains unknown. If its substrates are available at sufficiently high concentrations during inflammation, eNAMPT could contribute to an extracellular NAD^+ pathway in an inflammatory environment. IL: interleukin; PRPP: 5-phosphoribosyl-1-pyrophosphate.

effectively reduced major indices of acute lung injury, including exudative oedema, the number of neutrophils that were recruited to the airways, cytokine production and the acute lung injury severity score. Thus, eNAMPT can be targeted, *i.e.* neutralised, early after the onset of lung injury, resulting in preservation of the gas exchange barrier.

Preservation of the gas exchange barrier requires intimate contact between the alveolar epithelium and the capillary endothelium. The capillary endothelium is a highly specialised cell phenotype [26], and it is subjected to both chemical and mechanical signals that drive lung injury in ARDS. To investigate whether the endothelium contributes to the increase in eNAMPT during acute lung injury, endothelial-specific NAMPT knockout mice were generated. These mice were protected from both "one-hit" and "two-hit" models of lung injury. A majority of the lipopolysaccharide-induced eNAMPT was abolished altogether in the knockout animals, suggesting lung endothelium uniquely responds to airway infection to coordinate the immune response, and in this case, drive exuberant innate immune signalling.

The endothelium fulfils an essential role in coordinating the host's response in sepsis and ARDS. It serves as a permeability barrier, it contributes to haemostatic balance (*i.e.* anti- *versus* pro-coagulant), and it is a gateway to leukocyte recruitment [26]. It is exquisitely mechanosensitive, directly responsive to lipopolysaccharide binding to TLR4 receptors, and therefore, as highlighted by Quijada *et al.* [11], it is capable of amplifying an innate immune response during acute lung injury. The endothelium appears to contribute the majority of the increase in eNAMPT during ARDS.

While eNAMPT neutralisation provided a clear benefit over the short term [11], in future studies it will be interesting to address the impact of this approach on lung repair following injury. NAMPT may not just serve as a ligand driving the innate immune response, it could contribute to a fluid-phase circulating NAD^+ salvage pathway. Mitochondrial dysfunction is well-recognised in sepsis and ARDS [27, 28], and if such a circulating pathway exists, the increase in eNAMPT during this inflammatory insult may be needed to generate NAD^+ that supplements the bioenergetic demands of repairing tissues [5]. Alternatively, eNAMPT itself could be internalised by repairing tissues, and boost NAD^+ (figure 2). Future studies will be able to resolve the contribution of eNAMPT to long-term tissue repair.

eNAMPT does not have to be catalytically competent to activate TLR4 [5]. In this context, assessing the impact of eNAMPT neutralisation on the circulating metabolome could be important [29]. eNAMPT

requires both 5-phosphoribosyl-1-pyrophosphate (PRPP) ribose and nicotinamide to generate nicotinamide mononucleotide in the salvage pathway that leads to NAD^+ (figure 2). PRPP is not usually available at sufficiently high concentrations in the circulation to enable this reaction, but it remains unclear as to whether its bioavailability is increased during sepsis and ARDS. It will be important to confirm that eNAMPT neutralisation does not reduce iNAMPT activity, as iNAMPT supports $iNAD^+$ (200–500 μM) levels, and thus, cell survival. NAD^+ drives neoangiogenesis. NAD^+ in endothelium activates sirtuin 1 (SIRT1), which suppresses the Foxo1 anti-angiogenic transcription factor [30], and endothelial specific SIRT1 knockout mice have reduced capillary densities and limited vascular expansion following exercise and ischaemic injury, indicating SIRT1 is necessary for vascular maintenance. NAMPT activity may be needed for the endothelium to regenerate new blood vessels following an injury.

Neutralising the endothelial-derived eNAMPT with a first-in-kind humanised monoclonal antibody provided clear benefit in these pre-translational models of ARDS [11]. This is great news. The results provide hope for the development of novel medical therapy. However, translating between animal models and ARDS patients has proven to be difficult. Nonetheless, these studies look promising and it will be exciting to see how they translate to the human experience, in ARDS and in other acute or chronic inflammatory states.

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