Commentary

Attack of the NETs! NETosis primes IL-1β-mediated inflammation in diabetic foot ulcers

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In volume 133 issue 4 of Clinical Science, Liu et al. showed that neutrophils release extracellular traps (NETs) in the setting of diabetes which acts as a stimulus for NLRP3 inflammasome activation in macrophages to promote IL1β-dependent exacerbation of inflammation. They also provide evidence to show that degrading NETs improves the wound healing process. These findings provide an insight into how NETs communicate with other cells in the vicinity (e.g. macrophages) to exacerbate the inflammatory response. Most importantly, they provide novel avenues to improve wound healing process such as diabetic foot ulcers (DFUs) by targeting NETs.

Diabetes is a chronic disease that occurs when the body cannot maintain normal blood glucose levels due to the failure of the pancreas to produce sufficient levels of insulin in order for the body to take up glucose for energy. One common complication in diabetes is an impaired ability of the body to heal wounds such as diabetic foot ulcers (DFUs) [1]. DFUs are open sores generally located at the bottom of the foot where the skin tissue breaks down exposing layers underneath. In patients with poorly controlled diabetes without well-managed foot care, serious bacterial infections can occur. These non-resolving infected wounds can, in some instances, may lead to amputation. In a previous issue of Clinical Science, Liu et al. uncover a new mechanistic pathway to improve wound healing process in diabetes [2]. They provide evidence to show how neutrophils release extracellular traps (NETs) to prime macrophages towards the release of inflammatory cytokines such as interleukin 1β (IL-1β). They also demonstrate that eliminating NETs from activated neutrophils, significantly improve the wound healing process in rats with diabetes via lowering the release of IL-1β thus, uncovering a novel approach to treat DFUs (Figure 1).

DFUs undergo inflammatory immune cell responses involving both the innate and adaptive arms of the immune system. Neutrophils are the first cells to arrive at the sites of inflammation playing a crucial role in healing via phagocytosis, generation of reactive oxygen species (ROS), degranulation of cytoplasmic granules containing toxic enzymes and alarmins, and the generation of neutrophil extracellular traps by a process known as NETosis [3]. In the present study, the authors took skin samples from healthy and diabetic patients with DFU and found that citrullinated histone 3 (Cit-H3) was significantly up-regulated, the gold-standard marker for NETosis. During the process of NETosis, histones lose the charged state needed for compact packaging of DNA resulting in decondensation of DNA in nuclei or mitochondria. Once free of its histone tether, DNA is unfurled into the extracellular space resulting in the extrusion of NETs to the extracellular space. NETosis is construed as a form of cell death but distinct from either apoptosis or necrosis. NETosis is independent of caspases and is not affected by the caspase inhibitor zVAD-fmk [4,5], or by inhibition of RIP1 kinases by necrostatin-1. An additional feature of NETosis is fragmentation of both nuclear and granular membrane allowing for mixing of nuclear, granular, and cytoplasmic components, followed by cell membrane rupture and release of NETs into the extracellular milieu [6].
When wounds develop on the feet of people with the diabetes the hyperglycemic conditions (along with other unidentified factors) activate neutrophils causing them to undergo NETosis. These NETs interact with macrophages to promote the production of IL-1β which sustains a chronic inflammatory state and prevents wound resolution. Targeting pathways including NETs (DNase I), Macrophage activation (antioxidants, NLRP3) or preventing IL-1β signaling (IL-1R, canakinumab) may inhibit inflammation and promote wound closure.

The authors also discover an increase in NLRP3 protein in human DFU samples, which is mainly expressed in innate immune cells. During inflammation, after the recruitment of neutrophils, monocytes from the circulation are also recruited where they differentiate into macrophages and augment the inflammatory response, after which they facilitate the wound healing process [7]. To study the immune cell mechanisms of DFU in the skin, the authors used a model of DFUs in diabetic rats. In addition to showing increased levels of NLRP3, elevated levels of pro-IL-1β was also observed, which could be responsible for impaired wound healing in patients with DFU. The activation of the NLRP3 inflammasome triggers a complex signaling pathway, involving the maturation and release of inflammatory caspases and cytokines such as IL-1β and IL-18, leading to lytic cell death. We have shown that inflammasome activation in adipose tissues macrophages in diabetes/obesity by neutrophil-borne S100 calcium binding proteins (S100A8/A9) promotes IL-1β-dependent myelopoiesis in the bone marrow. Interaction of IL-1β with its receptor, IL-1R1 in hematopoietic stem and myeloid progenitor cells results in enhanced production of inflammatory monocytes and neutrophils, thus, exacerbating the inflammatory cycle in chronic diseases [8]. Of note, NETosis is essential for the release of S100A8/A9 from neutrophils [9] and S100A8/A9 constitute the bulk of major NET-bound cytosolic proteins [4] suggesting the NETosis in intimately linked to inflammasome activation. The Papayannopoulos group has also demonstrated an interplay between NETs and macrophage-released IL-1β in the context of non-diabetic atherosclerosis [10]. These findings suggest that “neutrophil secretome” may act as a potential link between NETosis and inflammasome activation in macrophages.

To study mechanistically how NETs and IL-1β are associated with impaired wound healing, the authors isolated primary neutrophils and macrophages from the intraperitoneal cavity of rats. They first stimulated neutrophils with PMA to induce NETosis followed by incubation with DNase I (which degrades NETs). DNase I is an endonuclease that cleaves or digests single- or double-stranded DNA [11]. Next, they co-cultured neutrophils with macrophages and found that NETs indeed activated the NLRP3 inflammasome and increased IL-1β, this was not seen in the samples treated with DNase I. Release of IL-1β via the NLRP3 inflammasome complex requires two steps, priming and activation. Priming will up-regulate the mRNA and protein expression of NLRP3 and the immature forms of IL-1β (pro-IL-1β) while the activation step will promote the formation of the NLRP3-inflammasome, activating caspase-1 trigger the cleavage of pro-IL-1β to its mature form, along with forming gasdermin D pores to punch holes in the cell membrane to release IL-1β [12]. To understand whether NETs act as a priming signaling for NLRP3, the authors...
used common inhibitors of TLR4 and TLR9 to show that the TLR/NF-κB signaling pathway which is involved in the priming step, leads to up-regulation of NLRP3 and pro-IL-1β. The authors then go on to demonstrate that ROS are required as a second stimuli by using a ROS scavenger such as N-acetylcysteine (NAC), in which they demonstrated that NAC reduced NET-induced IL-1β expression in macrophages.

Finally, when the authors administered DNase I to diabetic rats, they found that NET degradation improved wound healing, which coincided with a significant reduction in swelling and infiltrating immune cells compared with controls. Even though the authors have studied the effect of DNase treatment on wound healing, it would be interesting to look at the effects of inhibiting the IL-1β pathway, either by targeting NLPR3 (i.e. MCC950) or IL-1 receptor (IL-1R) blockade with IL-1R antagonist (anakinra) on wound healing in diabetes. In addition, demonstration of gASPERA M cleavage in macrophages in response to NET treatment would further reinforce the findings that IL-1β is important. It would also be interesting to speculate on the exact component of NETs responsible for inflammation some activation in macrophages. However, as with many studies implicating IL-1β, it would be important to find out where (i.e. tissue/cells) IL-1β is acting and what the direct functional consequences are, rather than simply speculating "inflammation."

In conclusion the authors study lends further support to the idea that NETs are the drivers of DFUs. By demonstrating accelerated wound healing via degradation of NETs (by DNase), and downstream changes to IL-1β, the present study provide important clues to develop novel therapeutic approaches for diabetic patients. Importantly, DNase I is commonly used in patients with cystic fibrosis (CF) to reduce the viscosity of sputum to help clear secretions from the lung since 1994 [13]. Therefore, the topical use of DNase I may be repurposed for the treatment of DFUs. Alternative approaches could be to target macrophage activation, via antioxidants or NLRP3 inhibitors, or IL-1β signaling (i.e. IL-1R antagonists or neutralizing antibodies) (Figure 1). Furthermore, an important question remains as to how high glucose is sensed by neutrophils and how this activates NETosis in the setting in diabetes. Understanding this in future studies may give us more insights on how to treat those with DFUs.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations
DFU, diabetic foot ulcer; IL-1β, interleukin 1β; NET, neutrophils release extracellular trap; ROS, reactive oxygen species.

References