Diabetes Primes Neutrophils to Undergo NETosis, Which Impairs Wound Healing

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INTRODUCTION

Background

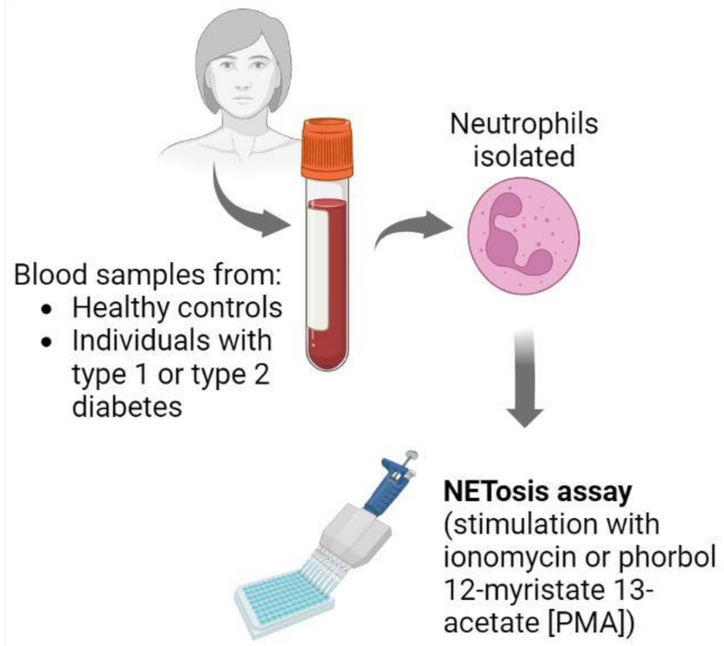
- Wound healing is impaired in diabetes, resulting in significant morbidity and mortality.
- Neutrophils are the main leukocytes involved in the early phase of healing. **Neutrophil extracellular traps (NETs)**, decondensed chromatin lined with cytotoxic proteins, are released in anti-microbial defense. NETs, however, can also induce tissue damage.
- Under diabetic conditions, there is increased neutrophil production of superoxide and cytokines, as well as elevated tumor necrosis factor-α (which primes neutrophils for NETosis). It has thus been proposed that **the diabetic microenvironment may facilitate NETosis**.

Objectives

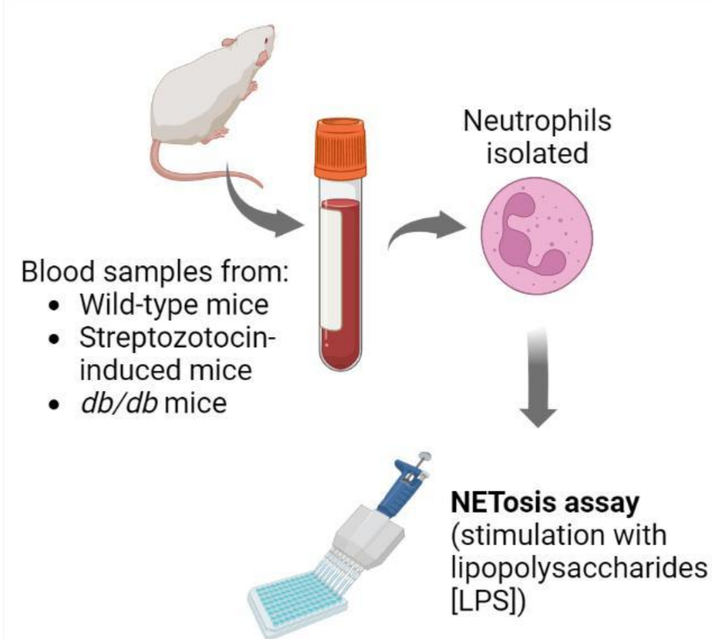
- To investigate whether type 1 and type 2 diabetes predisposes neutrophils to NETosis.
- To examine the role of peptidylarginine deiminase 4 (PAD4, critical enzyme for NETosis) and the impact of NETs on diabetic wound healing.
- To investigate the role of DNase 1 treatment in wound healing.

METHODS

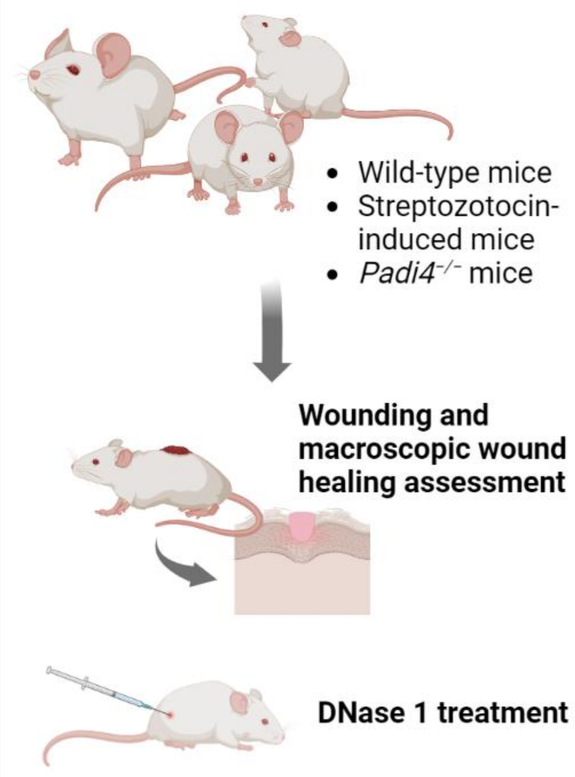
Human studies – *in vitro*



Animal (mouse) studies – *in vitro*



Animal (mouse) studies – *in vivo*



RESULTS

1. Neutrophils in diabetic humans (Fig. 1a) and mice (Fig. 1b, 1c) produced more NETs than those in normoglycemic humans and mice.
2. Citrullinated histone H3 (H3Cit) is a marker of NET formation. NETs are found in wounds of wild-type (WT) but not *Padi4*$^{-/-}$ mice. (Fig. 2)
3. PAD4 is an enzyme which allows for chromatin decondensation required for NETosis. Diabetic mice without PAD4 and hence impaired NETosis had better wound healing compared to mice with PAD4. (Fig. 3)
4. DNase 1, an extracellular enzyme which breaks down NETs, improved wound healing in diabetic mice. (Fig. 4)

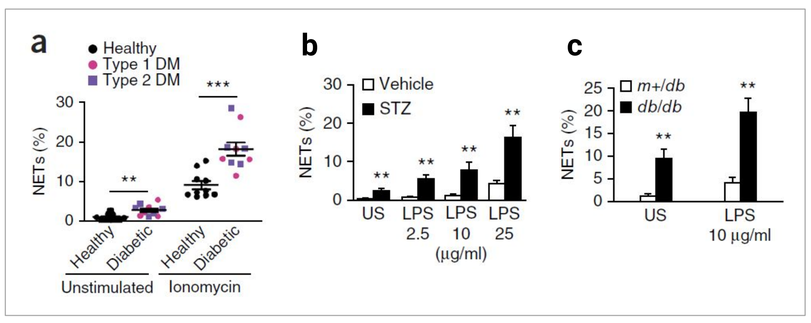


Figure 1. (a) In both diabetics and healthy individuals, there was a higher percentage of NETs produced in both stimulated and unstimulated neutrophils. Healthy individuals (n=10), type 1 diabetes mellitus (n=5), type 2 diabetes mellitus (n=5). **(b)** There was a significant (P<0.01, Student's t-test) increase in percentage of NETs produced by T1DM mice model (n = 10) compared to normoglycemic (n = 12) mice. **(c)** There was a significant (P<0.01, Mann-Whitney test) increase in percentage of NETs produced by T2DM mice model (n = 8) compared to normoglycemic (n = 7) mice.

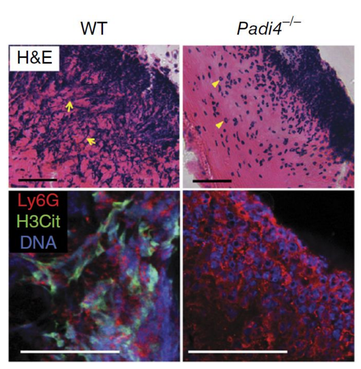


Figure 2. H3Cit (suggesting NETs formation) was present in wounds of WT mice but not in *Padi4*$^{-/-}$ mice, on H&E stain (top) and confocal microscopy (bottom)

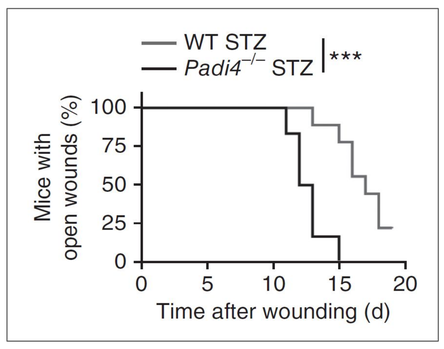


Figure 3. Percentage of mice with open wounds remaining against time. *Padi4*$^{-/-}$ diabetic mice (n = 6) showed significantly (P<0.001, log-rank test) faster wound healing than WT diabetic mice (n = 9).

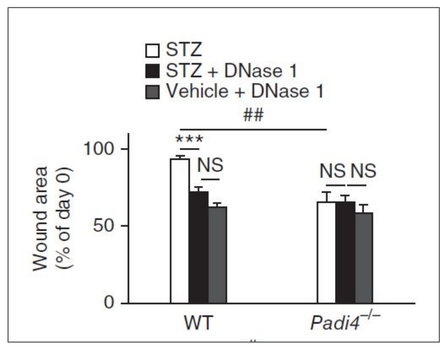


Figure 4. Wound area (as a percentage of initial wound) with diabetic mice (n = 6), diabetic mice with DNase 1 (n = 8) and normoglycemic mice with DNase 1 (n = 9). There was a significant (P<0.001, Kruskall Wallis test followed by Dunn's post-test) reduction in wound area for diabetic mice with DNase 1 compared to diabetic mice, but no significant reduction in wound area for diabetic mice with DNase 1 and normoglycemic mice with DNase 1.

DISCUSSION & CONCLUSION

The results demonstrate that diabetes increases production of NETs in neutrophils, causing impaired wound healing.

Implications for Clinical Practice and Future Research

- Further experiments are needed to determine DNase 1 effect on wound healing in humans.
- Beside wound healing, other complications of diabetes have been hypothesised to share similar pathological pathways of NETosis dysregulation. PAD4 inhibitors therefore present an exciting potential therapeutic agent in the treatment of other complications of diabetes beyond wound healing.

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