Role of neutrophil extracellular traps (NETs) for disturbed fracture healing in pre-/diabetics

DFG funded PhD Project

Project Start / Duration: As soon as possible / 3 years

Background: In Orthopedic and Trauma-Surgery pre-/diabetics have an elevated risk to develop post-surgical complications, resulting in prolonged hospital stays - in our clinic on average +2 days for pre-diabetics and +6 days for diabetics. With a current prevalence of 46% pre-diabetics and 16% diabetics in our clinic, this is not only of high medical but also economical relevance.
In 2015, excessive neutrophil extracellular trap (NETs) formation has been found in disturbed wound healing of diabetic mice (Wong et al. 2015 Nature Medicine). NETs represent a special defense mechanisms of neutrophils, which are the first cells to arrive at the wound site. The NETs consist of the neutrophils' DNA covered with proteins like neutrophil elastase, myeloperoxidase and citrullinated histones. Known to catalyze the conversion of arginine to citrulline, protein deiminase 4 (*PADI4* gene) is considered as key regulator to induce NETosis. In the study mentioned above blocking of the *PAD4* expression rescued wound healing in the diabetic mice.
First data from patients show also increased PAD4 expression and NETosis markers in diabetics. In humans, additionally, specific *PADI4* polymorphisms have been described that correlate with the severity of rheumatoid arthritis. First results from our institute suggest that the *PADI4* polymorphisms also correlate with the clinical outcome in our diabetic patients, especially wound healing and tissue infections. However, the underlying mechanisms are so far not known.

Aim of the Study: Aim of this project is to identify underlying mechanisms how NETs may disturb wound/fracture healing in our pre-/diabetic patients. To reach this goal the project is divided in an *ex vivo* (patient samples) and an *in vitro* (mechanistic analyses) part.
The *ex vivo* part includes the correlation of NET markers, and *PADI4* polymorphisms with the clinical outcome in our patients. NETs will be detected in patients tissue. Furthermore, the composition of the isolated NETs (Pre-/Diabetics vs. controls) will be characterized. Ideally, these results may help to identify patients at high risk to develop post-surgical complications and serve as base for the development of therapeutic strategies.
This will be further addressed with the *in vitro* part of the project. There it will be investigated if specific *PADI4* polymorphisms correlate with PAD4 levels and thus sensitize neutrophils for NET formation. The influence of the formed NETs on bone and skin cells will be analyzed in a diabetic context, in order to identify disease relevant triggers that cause the excessive NET formation observed in diabetic wounds. Furthermore, it will be investigated how different anti-diabetic drugs influence this system.

Methods: literature and data base research, isolation and quantification of DNA + ARMS-PCR, neutrophil isolation by density gradients, PAD4 Expression (qRT-PCR, Western blot) and activity, NETosis quantification (SYTOX green assay, Immunofluorescence staining, extracellular DNA), oxidative-/nitrosative stress detection, cell culture models (co-culture + 3D), cell death analyses, immuno-/histological stainings, etc.

Kontakt: PD Dr. Sabrina Ehnert (sabrina.ehnert@med.uni-tuebingen.de)
Siegfried Weller Institut an der BG Unfallklinik Tübingen
Eberhard Karls Universität Tübingen
Tel: +49-7071-606-1065