Visualization of neutrophil extracellular traps in atherosclerosis

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Neutrophil extracellular traps (NETs), a unique type of cell death characterized by active release of nuclear chromatin and granule proteins, are known to capture and kill invading microbes extracellularly (Brinkmann et al., 2004). However, formation of NETs may also be involved in sterile inflammatory environment of atherosclerosis. Here we visualize the presence of NETs in murine and human atherosclerotic plaques as revealed by in vivo two-photon laser scanning microscopy and immunohistochemistry.

ApoE−/− mice expressing green fluorescent protein under control of Lysozyme M (Lysmgf/gfgfp/Apoe−/−) mice were fed high fat diet for 4 weeks, a time point shown to be important in neutrophil-dependent atherogenesis (Drechsler et al., 2010). Following monocyte depletion by single injection of chlodronate-filled liposomes, Lysmgf/gfgfp/Apoe−/− mice carry green fluorescent neutrophils only. The carotid bifurcation was exposed and imaged in vivo using two-photon laser scanning microscopy (LaVision Biotec Trimscope, Bielefeld, Germany; Olympus FV1000MP, Hamburg, Germany). Visualization of NETs was enabled by intravenous injection of the viable cell impermeable DNA-binding dye propidium iodide immediately after surgery and five minutes prior to imaging. Two-photon microscopy revealed presence of luminally adherent neutrophils releasing DNA into extracellular space.

Subsequent to our observation of luminal NETs in experimental atherosclerosis, we aimed to identify presence of NETs in human atherosclerosis. Human plaque material obtained by endarterectomy was instantly fixed and after further processing stained for neutrophil elastase and the neutrophil surface marker CD177. Both intra- and extracellular presence of DNA was marked by DAPI. The latter experiments confirmed the presence of luminal NETs in human atherosclerosis. For the first time NETs have been detected in an atherosclerotic environment, both in mice in vivo and in human plaque. Described results will stimulate further research on the contribution of NETs in atherogenesis.

Literature
