The importance of synovial and subchondral microvessels in maintaining cartilage integrity in inflammatory arthritis.

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Background: Inflammatory arthritis in humans is a group of chronic progressive diseases caused by multiple factors that result in the inflammation of one or more joints. Inflammation and angiogenesis are closely integrated processes in inflammation, and angiogenesis plays an important role in the development and progression of arthritis. The binding of vascular endothelial growth factor A (VEGF-A) to VEGF receptor 2 (VEGFR2) is considered to be the main stimulatory signal of angiogenesis. We have previously shown that tamoxifen inducible knockout of endothelial (Tie2+) VEGFR2 (VEGFR2^{ECKO}) in *vegfr2$^{fl/fl}$* Tie2Cre^{ERT2} mice delayed both the onset and distant spread of pain in a mouse model of inflammatory arthritis. VEGFR2^{ECKO} animals also had more severe tibiotalar cartilage damage in inflammatory arthritis. We therefore hypothesised that this detrimental effect on cartilage integrity only under inflammatory conditions may result from a reduction in synovial angiogenesis and the resulting reduction in synovial vascular perfusion in VEGFR2^{ECKO} mice.
Methods: Transgenic mice underwent unilateral sub-cutaneous, peri-articular (tibiotarsal) injection of Complete Freund's Adjuvant (CFA) [2 x 80 µg CFA in 40 µL oil, on either side of the joint under isoflurane anaesthesia (2–3% in oxygen)] to induce inflammatory tibiotalar (ankle) arthritis two weeks after tamoxifen induction of VEGFR2 knockout (n=5, 5 daily injections, 1 mg/100 µL in sunflower oil, i.p.). Controls included VEGFR2^{ECKO} mice that underwent anaesthesia and injection site preparation without CFA injection (sham, n=4) and uninduced *vegfr2$^{fl/fl}$* Tie2Cre^{ERT2} mice with either CFA (n=5) or vehicle (n=4) peri-articular injections. Inflammation and pain were confirmed with increased ankle joint diameter and nociceptive withdrawal tests for 2 weeks after CFA/vehicle. VEGFR2KO was confirmed in lung and spinal cord by flow cytometry for Tie2+ and VEGFR2 in CD31+ cells (Beazley-Long et al 2018). Ankle joints were fixed, decalcified, sectioned at 10µm, and stained for CD31, and blood vessel density quantified in synovium and subchondral bone.
Results: There was a significant reduction in both synovial p = 0.0043 (VEGFR2^{ECKO} uninflamed: Mean = 5.900, SEM= 0.6117; inflamed: Mean = 1.810, SEM= 0.4265) and subchondral blood vessels density p= 0.0082 (VEGFR2^{ECKO} uninflamed Mean = 1.710, SEM= 0.5128: inflamed (Mean = 0, SEM= 0) in VEGFR2^{ECKO} mice with CFA inflammation as compared with control groups, with an almost complete loss of subchondral vessels in the region closest to the osteochondral junction.
Conclusion: The decrease in vessel density in synovium and almost complete vascular regression in superficial subchondral bone regions suggests that loss of vascular integrity by endothelial VEGFR2KO exacerbates cartilage damage. There is debate about the source of oxygen and nutrition for articular cartilage; our data suggest that subchondral bone vessels have an important role in maintaining cartilage integrity in inflammatory arthritis.

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