● PERSPECTIVE

Do neuronal microvascular activation and resultant dysfunction in rheumatoid arthritis contribute to chronic pain?

Microvascular dysfunction and rheumatoid arthritis (RA): Across the UK severe osteoarthritis affects ~5–7% and RA affects ~1% of the population. These are the most common causes of disabling chronic pain and are major burdens on individuals and society. Consequently, the annual financial cost to the UK economy for arthritis is in the billions of pounds. People living with painful inflammatory conditions and in particular RA are at an increased risk of mortality caused by cardiovascular disease (Avina-Zubieta et al., 2008) which is of a magnitude similar to that caused by diabetes (Soubrier et al., 2014). The endothelial dysfunction underlying cardiovascular disease in RA also affects endothelia throughout the body causing a diverse range of diseases including those that are metabolic and renal in nature, and the pathogenesis and worsening of these diseases are intricately linked to dysfunctional endothelia. Endothelial dysfunction and cardiovascular risk are also linked to cognitive deficits in RA (Cutolo et al., 2014). Depression is another co-morbidity of RA and has been linked to increased levels of interleukin (IL)-6, IL-1 and tumour necrosis factor-α (TNFα). All three of these cytokines are associated with endothelial activation and endothelial dysfunction. For example TNFα, which is a potent endothelial activator, also causes endothelial cell apoptosis and this can be inhibited by endothelial cytoprotectants such as brain derived neurotrophic factor (BDNF) (Takeda et al., 2013) and vascular endothelial growth factor-a (VEGF-A) (Liu et al., 2002). Targeting endothelial activation is able to prevent the spread of pain in rodent models of inflammatory arthritis (Beazley-Long et al., 2018) indicating that the spread of neuronal sensitization is dependent on the action of the microvasculature. This has led to the hypothesis that neuronal microvascular activation and ensuing dysfunction could drive the chronic pain experienced by people living with RA. This invited perspective discusses the main research that has led to the formation of this hypothesis and the caveats of targeting endothelial activation *via* vascular endothelial growth factor receptor-2 (VEGFR2). In doing so, I hope to shine the spotlight on the potential role of neurovasculature as a driver of chronic pain in inflammatory conditions.

Endothelial dysfunction in RA: The term endothelial dysfunction refers to a number of conditions affecting endothelia, defective anticoagulant and anti-inflammatory properties, impaired angiogenesis and remodelling including rarefaction, mitochondriopathy and lysosomal dysfunction and impaired endothelium-dependent vasorelaxation. The most common indicator of endothelial dysfunction and defective vasorelaxation is the downregulation of endothelial nitric oxide synthase (eNOS) expression or activity. eNOS, leading to nitric oxide (NO) production is an effector of mechanotransduction. The defective vasorelaxation results in a vasculature unable to respond adequately to the metabolic demands placed upon it and local hypoxia can develop. Decreased endothelial NO bioactivity is described in people living with RA (Steyers and Miller, 2014) and in pre-clinical models of RA (Haruna et al., 2006). The term endothelial activation specifically refers to a pro-inflammatory and procoagulant endothelial state associated with atherosclerosis, diapedesis (trans-endothelial migration) and changes in barrier properties, all of which occur in dysfunctional endothelia.

The spread of pain is dependent on neuronal microvasculature: People living with RA present with painful symptoms of central sensitization. Central sensitization drives the enhancement of neuronal responses in central pain transmission pathways and includes increased sensitivity of second-order neurons in response to afferent inputs, enlargement of receptive fields and increased neuronal excitability. The chronic effects are driven by transcriptional and morphological changes, and heightened pain signalling, due to disturbed descending control mechanisms (decrease in descending inhibition/increase in descending facilitatory signaling). The clinical manifestations of central sensitization include enhanced pain sensitivity to nocuous stimuli (hyperalgesia), the sensation of pain from innocuous stimulus (allodynia), referred pain, unprovoked sensory abnormalities including paraesthesia (pins and needles, and numbness) and dysaesthesia (unusual burning or itching sensation). These clinical manifestations present in people living with RA. The importance of vascular health for maintaining normal neuronal function is becoming increasingly apparent. In the peripheral nervous system (PNS), neural microvascular endothelial cells (endoneurial endothelium), are able to initiate many of the painful changes observed in nerve injury (Moreau et al., 2016). This study indicates that peripheral endothelial stimulation and resultant dysfunction are able to trigger hallmarks of pain, such as mechanical allodynia in the absence of a typical injury (nerve injury, inflammatory insult) and highlights the important role blood vessels in the periphery can play can in the generation and maintenance of pain. The mammalian brain contains many more glia and other non-neuronal cells than neurons with endothelial and other vascular cells making up a large proportion of the non-neuronal cells. In a model of RA, inflammation causes brain endothelial dysfunction and the prevention of this leads to an increase in the levels of the endothelial and neuronal cytoprotectant BDNF (Pedard et al., 2018). Microvessels in the spinal cord dorsal horn (central nervous system, CNS) and dorsal root ganglia (DRG, PNS) are activated in pre-clinical rodent models of painful inflammatory arthritis (Beazley-Long et al., 2018). In the CNS astrocytic end-feet encase the microvasculature, forming the glia limitans, and maintain the tight blood-brain/spinal cord barrier and endothelial health. In the dorsal horn of arthritic animals there is a significant increase in the number of reactive astrocytic end-feet on microvessels. In both the CNS and dorsal root ganglia there is an increase in endothelial expression of intercellular adhesion molecule-1 (ICAM-1) and in the dorsal horn there is an increase in the number of $CD11b^{+}$ cells associated with the microvasculature. These results reveal a novel glio-vascular response to peripheral inflammatory arthritis. Furthermore, targeting a receptor that mediates many vascular effects including endothelial activation - endo-

thelial VEGFR2 - either by pharmacological inhibition or by inducible and endothelial-specific knockout, suppressed the glio-vascular response and endothelial ICAM-1 expression, and importantly prevented the development of secondary (referred) mechanical allodynia. This strongly suggests that the spread of neuronal sensitization in the spinal cord is dependent on the activation of neuronal microvasculature, with endothelial VEGFR2 playing a key role in vascular activation.

What are the possible detrimental and painful consequences of the observed glio-vascular activation? The glio-vascular response is likely to lead to changes in barrier function and permeability as well as the possible trans-endothelial migration of immune cells into the affected areas (diapedesis). Furthermore, perivascular cells such as pericytes are able to migrate into neuronal parenchyma in pathology and this process could contribute to a sensitized state (Ozen et al., 2014). How the astrocytic end-feet, endothelial cells, pericytes and other vascular associated cells and ultimately neurons communicate and interact in central sensitization, in particular in central sensitization driven by peripheral inflammation, are largely unknown. The vasculature in the CNS, and also the PNS, play a significant role in the spread of pain and the development of chronic pain, possibly more so than previously considered. What causes the glio-vascular activation is an additional follow up question. Possible explanations include 1) the barrage of painful nociceptive signalling coming into the cord causes neuronal release of factors such as calcitonin gene related peptide that activate the microvasculature either directly or *via* glia activation (neurogenic neurovascular inflammation). 2) The increased neuronal activity generates a local hypoxic environment which stimulates glio-vascular activation. 3) The glio-vascular activation is a consequence to circulating factors (peripherally driven endothelial activation). 4) A combination of the above depending on the neuronal area of concern (spatial aspect) and the time of disease (temporal aspect). In summary, CNS microvascular activation is associated with the development of secondary pain, suggesting that endothelial activation may drive aberrant neuronal sensitization (central sensitization) that underlies the development and/or maintenance of chronic pain states. **Figure 1** proposes the possible CNS neurovascular events that when triggered by pathological peripheral inflammation could lead to neuronal sensitisation and ultimately the development of chronic pain.

The pros and cons of anti-VEGFR2 dosing to target microvascular activation in RA: A selection of agents that target activated blood vessels are approved for clinical use throughout the world including anti-VEGF-A agents. Anti-VEGF-A agents are used to treat a number of angiogenesis-dependent disease conditions such as age-related macular degeneration and various cancers. VEGF-A levels are elevated in both the serum and joints of people living with RA. Our research indicates that targeting neurovascular activation through the inhibition of endothelial VEGFR2 may be able to limit endothelial activation and the likely painful consequences during the inflammatory episode. However, VEGF-A working through VEGFR2 also stimulates endothelial and neuronal cytoprotection, and further research from our collaborators has shown that VEGFR2 is an endogenous endothelial cytoprotectant in the spinal cord - specifically knocking out endothelial VEGFR2 in otherwise normal mice is sufficient to cause endothelial dysfunction and thermal stimulus sensitivity (Ved et al., 2018). This research was performed in the same mice in which inducible endothelial VEGFR2 knock out prevents the spread of neuronal sensitization caused by peripheral inflammation. These results are further evidence of the pleiotropic and complex nature of VEGFR2 and the importance of vasculature health and function in neuronal homeostasis. The complex biology of VEGFR2 suggests that long-term anti-VEGFR2 dosing in RA is likely to cause additional and unwanted neuronal microvasculature complications and result in unwanted detrimental effects however acute anti-VEGFR2 dosing may limit the painful consequences of neurovascular activation during an inflammatory episode.

Future perspective: Neurovasculature plays a significant role in the spread of neuronal sensitization and therefore potentially plays a role in the development of chronic pain. If the inflammatory flairs that afflict people living with RA cause endothelial activation not only at the affected inflamed joints but also at other sites throughout the body including the CNS, then repeated inflammatory episodes are likely to lead to the observed CNS endothelial dysfunction in RA. In support of this hypothesis TNFα which is heavily implicated in the inflammatory progression in RA causes both endothelial activation (adhesion molecule expression and increased permeability), and endothelial apoptosis and dysfunction. Therefore, repeated inflammatory flairs with increased circulating inflammatory factors, such as TNFα and VEGF-A, and possible neurogenic gliovascular activation may eventually lead to a chronic endothelial dysfunctional state. In the CNS and PNS, defective eNOS/NO and hypoxia resulting from endothelial dysfunction may in turn drive aberrant neuronal sensitization and chronic pain. However, the relationship between neurovascular activation and dysfunction, and neuronal function in the context of chronic pain is currently severely under researched. Continued investigation may reveal that targeting neurovascular activation during inflammatory flairs and promoting long-term neurovascular health are beneficial in the fight against chronic pain in inflammatory conditions such as RA.

The author would like to thank Alexandra Durrant for commenting on this article.

Work contained within this article was funded by Arthritis Research UK [grant number 21338].

Nicholas Beazley-Long*
Arthritis Research UK Pain Centre & School of Life Sciences, Medical School, University of Nottingham, Nottingham, UK
***Correspondence to:** *Nicholas Beazley-Long, BSc, PhD, nicholas.beazley-long@nottingham.ac.uk.*
***orcid:** 0000-0001-7342-2771 (Nicholas Beazley-Long)*
***Received:** June 12, 2018*
***Accepted:** August 6, 2018*

***doi:** 10.4103/1673-5374.243710*
***Copyright license agreement:** The Copyright License Agreement has been signed by the author before publication.*
***Plagiarism check:** Checked twice by iThenticate.*
***Peer review:** Externally peer reviewed.*
***Open access statement:** This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non-Commercial-ShareAlike 4.0 License, which allows others to remix, tweak,*

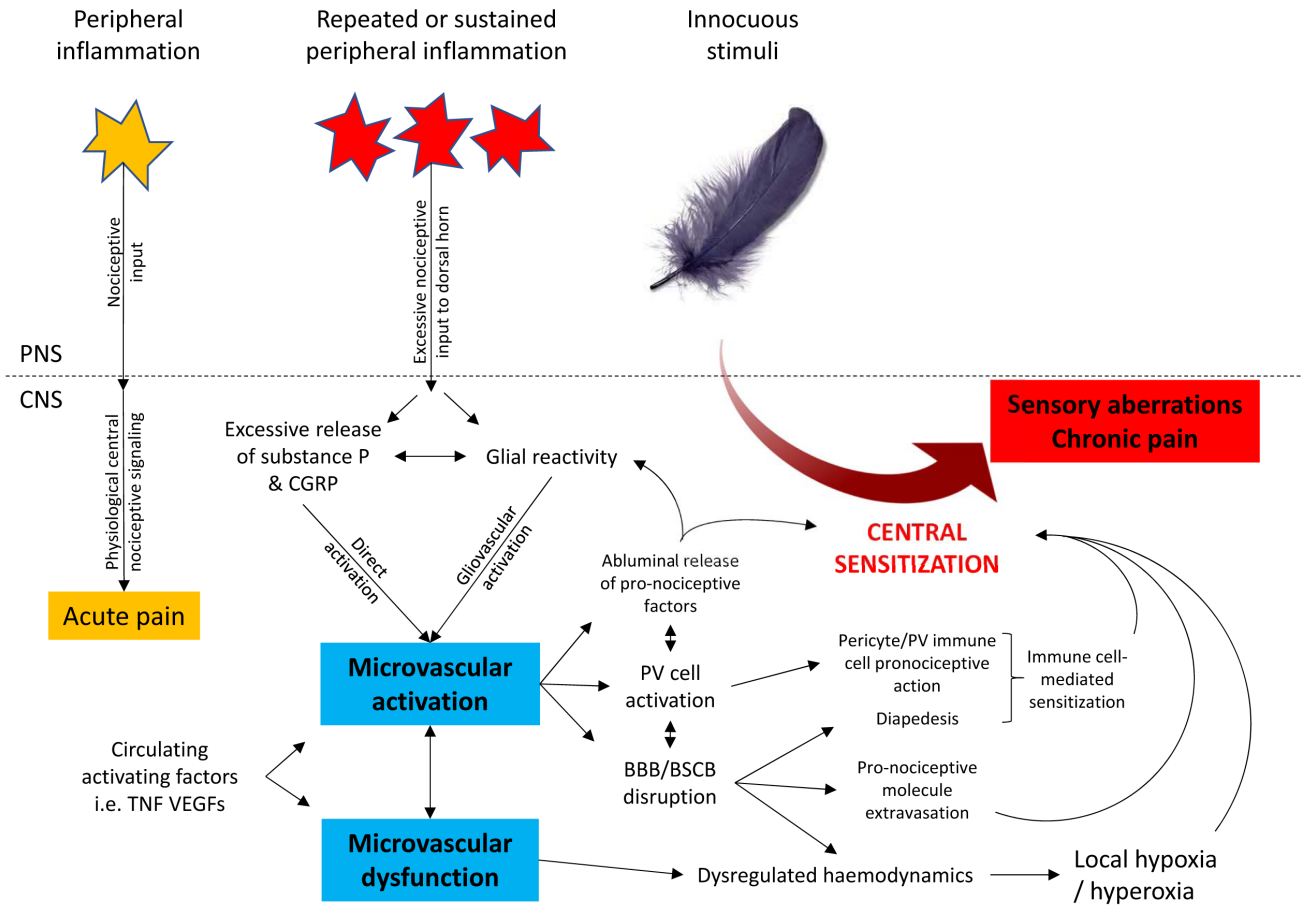

Figure 1 Flow diagram proposing possible CNS neurovascular inflammatory events within that may contribute to the development of chronic pain in peripheral inflammatory conditions.
BBB: Blood spinal cord barrier; BSCB: blood spinal cord barrier; PV: perivascular; TNF: tumour necrosis factor; VEGF-A: vascular endothelial growth factor-a; CNS: central nervous system; PNS: peripheral nervous system; CGRP: calcitonin gene related peptide.

and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.
Open peer reviewers: *John Mitrofanis, University of Sydney, Australia; Peter Shortland, Western Sydney University, Australia.*
Additional file: *Open peer review reports 1 and 2.*

References

Avina-Zubieta JA, Choi HK, Sadatsafavi M, Etminan M, Esdaile JM, Lacaille D (2008) Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. Arthritis Rheum 59:1690-1697.

Beazley-Long N, Moss CE, Ashby WR, Bestall SM, Almahasneh F, Durrant AM, Benest AV, Blackley Z, Ballmer-Hofer K, Hirashima M, Hulse RP, Bates DO, Donaldson LF (2018) VEGFR2 promotes central endothelial activation and the spread of pain in inflammatory arthritis. Brain Behav Immun doi: 10.1016/j.bbi.2018.03.012.

Cutolo M, Kitas GD, van Riel PL (2014) Burden of disease in treated rheumatoid arthritis patients: going beyond the joint. Semin Arthritis Rheum 43:479-488.

Haruna Y, Morita Y, Komai N, Yada T, Sakuta T, Tomita N, Fox DA, Kashihara N (2006) Endothelial dysfunction in rat adjuvant-induced arthritis: vascular superoxide production by NAD(P)H oxidase and uncoupled endothelial nitric oxide synthase. Arthritis Rheum 54:1847-1855.

Liu WL, Guo X, Chen QQ, Guo ZG (2002) VEGF protects bovine aortic endothelial cells from TNF-alpha- and H_2O_2-induced apoptosis via co-modulatory effects on p38-and p42/p44-CCDPK signaling. Acta Pharmacol Sin 23:45-49.

Moreau N, Mauborgne A, Bourgoin S, Couraud PO, Romero IA, Weksler BB, Villanueva L, Pohl M, Boucher Y (2016) Early alterations of Hedgehog signaling pathway in vascular endothelial cells after peripheral nerve injury elicit blood-nerve barrier disruption, nerve inflammation, and neuropathic pain development. Pain 157:827-839.

Ozen I, Deierborg T, Miharada K, Padel T, Englund E, Genove G, Paul G (2014) Brain pericytes acquire a microglial phenotype after stroke. Acta Neuropathol 128:381-396.

Pedard M, Demougeot C, Prati C, Marie C (2018) Brain-derived neurotrophic factor in adjuvant-induced arthritis in rats. Relationship with inflammation and endothelial dysfunction. Prog Neuropsychopharmacol Biol Psychiatry 82:249-254.

Soubrier M, Barber Chamoux N, Tatar Z, Couderc M, Dubost JJ, Mathieu S (2014) Cardiovascular risk in rheumatoid arthritis. Joint Bone Spine 81:298-302.

Steyers CM 3rd, Miller FJ Jr (2014) Endothelial dysfunction in chronic inflammatory diseases. Int J Mol Sci 15:11324-11349.

Takeda K, Kermani P, Anastasia A, Obinata Y, Hempstead BL, Kurihara H (2013) BDNF protects human vascular endothelial cells from TNFalpha-induced apoptosis. Biochem Cell Biol 91:341-349.

Ved N, Da Vitoria Lobo ME, Bestall SM, C LV, Beazley-Long N, Ballmer-Hofer K, Hirashima M, Bates DO, Donaldson LF, Hulse RP (2018) Diabetes-induced microvascular complications at the level of the spinal cord; a contributing factor in diabetic neuropathic pain. J Physiol doi: 10.1113/JP275067.

P-Reviewers: Mitrofanis J, Shortland P; C-Editors: Zhao M, Yu J; T-Editor: Liu XL