Peripheral nerve coordinates signal transduction from the periphery to the central nervous system for processing and transmission back as required for normal mammalian function. Peripheral nerves and nerve roots are structurally divided into three compartments: the outermost epineurium, inner perineurium that surrounds nerve fascicles and the innermost endoneurium (Mizisin and Weerasuriya, 2013). As with all organs, peripheral nerve vascularization occurs during development and is maintained in health. Adaptations are expected dependent on tissue-specific functions and physiologic state. The peripheral nerve internal microenvironment is tightly controlled to facilitate coordinated and regulated axonal transmission. Peripheral nerves and nerve roots are perfused by extrinsic blood vessels called the vasa nervorum. These vessels penetrate the outer epineurium to give rise to epineurial arteries and arterioles and receive blood from epineurial venules and veins. Macroversicles subsequently transverse the inner perineurium, formed by multiple concentric layers of specialized epithelial myofibroblasts, to perfuse the innermost endoneurium, in which reside myelinated and unmyelinated axons in a loose array of collagen fibers. If this system breaks down, capillary-like microvessels exist within the endoneurium which are in direct contact with circulating blood. Thus, these microvessels are considered to form the blood-nerve barrier (BNB) (Ubogu, 2020). Tight junction-forming perineural myofibroblasts provide a critical interface between the endoneurial and epineurial intersitial fluid compartments that further regulate the endoneurial microenvironment; however, these cells are not in direct contact with circulating blood. Peripheral neuropathies affect over 100 million people worldwide, and a significant number of these individuals during their lifetimes. Understanding peripheral nerve molecular and biophysical interstitial fluid compartments that further interface between the endoneurial and epineurial tight junction proteins.

Human blood-nerve barrier tight junctional complex: The human BNB transcriptome consists of 133 intercellular junctional complex molecules (22 tight junction or junction-associated, 45 adherens junction or junction-associated and 52 cell junction-associated molecules). The transcriptome data from in situ human endoneurial smooth muscle protein expression of α1 catenin (CTNNAA1), catenin-5 (CHDS), catenin-6 (CDH6), claudin-4 (CLDN4), claudin-5 (CLDN5), claudin-6 (CLDN6), claudin-7 (CLDN7), claudin-8 (CLDN8), claudin-9 (CLDN9), claudin-13 (CLDN13), claudin-14 (CLDN14), claudin-18 (CLDN18), Claudin-20 (CLDN20), claudin-24 (CLDN24), and claudin-27 (CLDN27) was obtained from cryopreserved human peripheral nerve biopsies (Ubogu, 2020). These claudins and other cell junction-associated or adaptor molecules, such as adherens junction proteins cadherin-5 (CHD5), cadherin-6 (CDH6), cadherin-9 (CDH9), cadherin-11 (CDH11), and CTNNA1-phosphorylated CTTN-CLDN4 expression result in more continuous intercellular contacts. We ascertained that GDNF (1 ng/mL, 0.03 nM) sufficiently restores murine sciatic nerve BNB macromolecular function following injury is dependent on CREB1 transcription factor-driven synthesis and SEC31A transport of essential junctional complex molecules cortactin (CTTN), CTNNAA1 and CLDN4, as well as GDNF-mediated SRC kinase activation required to phosphorylate CTTN, downstream of RET-Tyrosine Kinase-MAPK signaling (Ubogu, 2020). We also observed that GDNF induced F-actin cytoskeletal filament reorganization, resulting in more continuous intercellular membrane contacts between pHetEnDCs, supporting the notion that restrictive junctional complex formation and cytoskeletal dynamics are intimately linked to BNB barrier function (Ubogu, 2020). In support of our in vitro human BNB work, we have also demonstrated that GDNF restores murine sciatic nerve BNB macromolecular impermeability within 14 days following non-translaminar crush nerve injury. We subsequently determined that Rasmitogen activated protein kinase (MAPK) signaling was employed for this biological effect at the human BNB in vitro (Dong and Ubogu, 2018). Our published initial data using RNA-interference support the hypothesis that the observed GDNF-mediated regulatory effect on the BNB function following injury is dependent on CREB1 transcription factor-driven synthesis and SEC31A transport of essential junctional complex molecules cortactin (CTTN), CTNNAA1 and CLDN4, as well as GDNF-mediated SRC kinase activation required to phosphorylate CTTN, downstream of RET-Tyrosine Kinase-MAPK signaling (Ubogu, 2020). We also observed that GDNF induced F-actin cytoskeletal filament reorganization, resulting in more continuous intercellular membrane contacts between pHetEnDCs, supporting the notion that restrictive junctional complex formation and cytoskeletal dynamics are intimately linked to BNB barrier function (Ubogu, 2020). In support of our in vitro human BNB work, we have also demonstrated that GDNF restores murine sciatic nerve BNB macromolecular impermeability within 14 days following non-translaminar crush nerve injury. 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References

Allt G, Lawrenson JG (2000) The blood-nerve barrier: enzymes, transporters and receptors—a comparison with the blood-brain barrier. Brain Res Bull 52:1-17

Dong C, Ubogu EE (2018) GDNF enhances human blood-nerve barrier function in vitro via MAPK signaling pathways. Tissue Barriers 6:1-22

Dong C, Helton ES, Zhou P, Ouyang X, d'Anglement de Tassigny X, Pascual A, Lopez-Barneo J, Ubogu EE (2018) Gial-derived neurotrophic factor is essential for blood-nerve barrier functional recovery in an experimental murine model of traumatic peripheral neuropathy. Tissue Barriers 6:1-22

Baines CE, Andreoso ID (2017) Biology of GDNF and its receptors—Relevance for disorders of the central nervous system. Neurobiol Dis 97:80-89

Mizisin AP, Weerasuriya A (2011) Homeostatic regulation of the endoneurial microenvironment during development, aging and in response to trauma, disease and toxic insult. Acta neuropathol 121:291-312

Ouyang X, Dong C, Ubogu EE (2019) In situ molecular characterization of endoneurial microvessels that form the blood nerve barrier in normal human adult peripheral nerves. J Peripher Nerv Syst 24:195-206

Palladino SP, Helton ES, Jan P, Dong C, Crowley MR, Crossman DK, Ubogu EE (2017) The human blood-nerve barrier transcriptome. Sci Rep 7:17477

Reddy CL, Yosef N, Ubogu EE (2013) VEGF-A165 potently induces human blood-nerve barrier endothelial cell proliferation, angiogenesis, and wound healing in vitro. Cell Mol Neurobiol 33:789-802

Ubogu EE (2020) Biology of the human blood-nerve barrier in health and disease. Exp Neurol 328:113272

Yosef N, Ubogu EE (2010) GDNF restores human blood-nerve barrier function via RET tyrosine kinase-mediated cytoskeletal reorganization. Microvasc Res 81:298-310

Yosef N, Xia RH, Ubogu EE (2010) Development and characterization of a novel human in vitro blood-nerve barrier model using primary endoneurial endothelial cells. J Neuropathol Exp Neurol 69:82-97

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Figure 1 | Human blood-nerve barrier in situ and in vitro.

(A) Human blood-nerve barrier (BNB) junctional complex. Digital high resolution indirect immunohistochemistry confocal photomicrographs of normal adult endoneurial microvessels stained with Rhodamine Phalloidin to detect microvessel F-actin cytoskeleton (pseudocolor blue), and fluoresceininated Ulex Europaeus Agglutinin-1 (UEA-1) to detect microvessel cell membranes were generated to determine cellular localization of hypothesized junctional complex molecules (red) claudin 4 (CLDN4), cadherin 5 (CDH5), α-1 catenin (CTNNA1), cadherin 5 (CLDN5), zona occludens-1 (ZO-1), α-parvin (PARVA) and cadherin 2 (CDH2). The punctate CLDN4 expression and membrane co-localization suggest that this molecule is an essential component of BNB tight junctions. This molecule is also expressed in the multilayered perineurium (P), implying that it may be an essential component of its restrictive barrier. The linear, continuous CDH5 expression and membrane co-localization in endoneurial microvessels is consistent with an intercellular adherens junction protein. The plaque-like linear CTNNA1 expression and strong membrane and cytoskeletal co-localization suggest a role as an adapter molecule that links the endoneurial microvessel membrane to the cytoskeleton. CLDN5, ZO-1, PARVA and CDH2 demonstrate diffuse intracellular expression with additional strong continuous membrane and cytoskeletal co-localization, implying that they are not structural components of BNB tight junctions. Low endoneurial background expression levels of ZO-1 and PARVA suggest possible roles in maintaining structural integrity in normal adult peripheral nerves, while high focal CDH2 expression suggest expression in surrounding axons, as previously published with cadherin 6. The figure is sourced from the authors’ laboratory. (B) Human BNB junction complex formation: The GDNF-CREB1-SRC-SEC31A-CTTN/ CTNNA1/CLDN4 hypothesis. This figure illustrates a hypothesized pathway by which paracrine GDNF secretion by Schwann cells mediates restrictive human BNB formation dependent on GFRα1-RET-MAPK signaling, including specialized junctional complex protein transport for further study. Potential redundancy is demonstrated by bFGF, TGF-β1 and glucocorticoids. +P indicates a phosphorylation and P indicates an activated phosphorylated protein. bFGF: Basic fibroblast growth factor; BNB: blood-nerve barrier; CLDN4: claudin-4; CTTN: cortactin; GC: glucocorticoids; GDNF: glial-derived neurotrophic factor; TGFB1: transforming growth factor-β1.

BNB formation during development and rapidly restore BNB function after injury, with potential redundancy mediated by bFGF, TGFB1 and endogenous glucocorticoids (Figure 1B). We have demonstrated that GDNF is a sufficient essential molecular regulator of the human and mouse BNB in vitro and in situ following injury (Yosef and Ubogu, 2012; Dong and Ubogu, 2018; Dong et al., 2018). We advocate that restoring BNB function through GDNF-mediated mechanisms may be a necessary prerequisite to re-establish the tightly regulated microenvironment required to support functional axonal regeneration needed for normal physiologic signal transduction in peripheral nerve disorders. Failure to restore endoneurial homeostasis may result in chronic persistent neuropathic pain with associated morbidity and mortality.

Conclusions: The BNB is required for peripheral nerve homeostasis. Its molecular structure and determinants, as well as signaling pathways responsible for normal function are incompletely understood. We have made early significant advances, guided by nonbiased pHEndEC and endoneurial microvessel transcriptome and proteome bioinformatics. These are further supported by in situ observations of normal and well-characterized diseased adult peripheral nerve biopsies. In addition to comprehensively understanding human BNB function in health and how it adapts or fails to adapt in different disease states, a major goal of our dedicated efforts, considering the unique biology of the BNB, is to discover molecular targets for peripheral neuropathies and chronic neuropathic pain that enhance peripheral nerve axonal and microvascular regeneration and restore normal peripheral nerve and nerve root function in humans.

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Perspective

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