Neuronal and endothelial transglutaminase-2 expression in experimental autoimmune encephalomyelitis and multiple sclerosis

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Multiple sclerosis (MS) is a neurological condition characterized by the disruption of the blood-brain barrier, immune system activation, and inflammation that is accompanied by glial reactivity, neuronal cell death, axon demyelination, and axotomy. Pathological changes result in functional loss including paralysis, migraine, vision problems, spasticity, and neuropathic pain. Although the causative factor responsible for triggering MS remains to be identified, anti-inflammatory treatments have been translated to clinical use with favorable reductions in the frequency, severity, and duration of relapses in the relapsing-remitting form of MS. Among the identified therapeutic targets in MS, transglutaminase-2 (TG2) has been reported to be involved in disease pathogenesis (Chrobok et al., 2018).

TG2 is a multifunctional, calcium-regulated enzyme that exhibits protein transamidation, protein disulfide isomerase, serine/threonine-protein kinase, and GTPase activities. In addition, TG2 also acts as an important adaptor and scaffold, independent of its enzymatic activities, which occurs through noncovalent interaction with cell-surface and extracellular matrix proteins that are associated with cell survival, growth, migration, adhesion, differentiation, and extracellular matrix remodeling. In an experimental autoimmune encephalomyelitis (EAE) model of MS, global TG2 knockout has been shown to ameliorate disease severity (Oh et al., 2012; van Strien et al., 2015) and drug inhibitors of TG2 activity, including ERW1041E (Chrobok et al., 2018) and Cysteamine (Oh et al., 2012), or antagonists of TG2’s scaffolding functions with fibronectin, such as KCC009 (van Strien et al., 2015) can reduce clinical deficits in EAE. Following the induction of EAE, TG2 expression has been reported in subsets of macrophages-microglia and astrocytes in perivascular lesions of white matter in rodents and non-human primates as well as in the sclerotic plaques of human MS samples (Espitia Pinzon et al., 2014). Recent reports, however, have suggested that TG2 may play not only a pathological role but also a protective function in the diseased central nervous system (CNS) depending upon the cell type in which it is expressed and its specific activities (Quinn et al., 2018).

Converse to its pathological role in EAE, global TG2 knockout mice exhibit deficiencies in macrophage phagocytic activity and increased susceptibility to inflammatory pathologies (Toth et al., 2009) whereas the loss of TG2 in macrophages impairs myelin recycling and leads to deficits in remyelination after EAE (Giera et al., 2018). Additionally, TG2 seems to drive early oligodendrocyte precursor cell proliferation and differentiation (Espitia Pinzon et al., 2019) and is involved in remyelination repair through adhesion G protein-coupled receptor signaling as demonstrated in murine models of demyelination (Giera et al., 2018). Mice devoid of TG2 exhibit a defective mitochondrial ADP/ATP transporter adenine nucleotide translocator, elevated activity of the ATP/ADP carrier and increased mitochondrial membrane potential. These changes arise from abnormalities in the mitochondrial respiratory chain, a dysregulation of the respiratory complexes I and II and decreased ATP production in TG2 knockout mice (Battaglia et al., 2007). Mice lacking TG2 appear to also have a higher sensitivity to cardiac ischemia/reperfusion injury (Szondy et al., 2006), indicating that TG2 is involved in important physiological functions.

Ablation or inhibition of TG2 has been shown to be beneficial after EAE as well as in other neurological conditions despite the dichotomy of it possessing both pathological and protective activities. Elucidating its cellular expression and its involvement in signal transduction pathways based upon its multifunctional actions may therefore guide the development of improved therapeutics that target selectively its detrimental effects for maximal beneficial efficacy.

Cell-specific roles of TG2 in demyelinating neurological disease pathologies: The aim of our study (Pearse et al., 2020) was to determine the cellular localization of TG2 in perivascular lesions of the CNS gray matter temporally after EAE and MS. Combined histochemical examination of TG2 with markers of cell reactivity and cell death sought to further understand the pathological role of the enzyme in neural and immune cells at lesion sites. In the early active phase following EAE induction in rats and mice as well as in human MS tissue, TG2 was robustly expressed both in the cytoplasm and nucleus of neurons and endothelial cells, within and adjacent to perivascular lesions of the gray matter.

In the vasculature, TG2 was localized both intraluminally and in endothelial cells. With EAE disease progression, the enlargement of blood vessels and the formation of perivascular lesions, TG2 expression increased and its cellular immunoreactivity extended outwards into the lesion penumbra, indicative of cell migration from the vasculature. TG2 closely juxtaposed that of fibronectin both within blood vessels as well as surrounding CNS tissue. Analogous to a previously published marmoset EAE paradigm (Espitia Pinzon et al., 2014), TG2 in active, acute lesions was not expressed in astrocytes or oligodendrocytes. Previous investigations have reported that TG2 was present in subsets of macrophages after EAE that transmigrate the blood-brain barrier or microglia within perivascular lesions (van Strien et al., 2015). We found that whereas the macrophage activation marker CD68 was expressed highly within and surrounding perivascular lesions, its co-localization with TG2 was limited. Cellular TG2 in regions of outward migration from blood vessels co-existed with activated endothelial cell proteins platelet-activating factor and E-selectin or fibrin, which is expressed when vascular integrity is compromised, such as in acute inflammatory injury. TG2 expression therefore may be indicative of the invasion of the CNS at perivascular lesions by endothelial cells, pericytes and perivascular fibroblasts. Whether this response contributes to the initiation of angiogenesis or the deposition of fibrous extracellular matrix and the formation of scar tissue remains to be elucidated and may be dependent upon the chronicity of the disease. In contrast, non-diseased control tissue showed a low basal expression of TG2 that was found predominantly in the nuclei of neurons and in endothelial cells.

TG2 expressing neurons after EAE showed induction of the cell death activator, cleaved caspase-3, and elevated levels of phosphorylated ERK1/2. Prior work has reported that nuclear expression of TG2 confers a pro-survival benefit to neurons under cytotoxic conditions, whereas coregulation of neurons with TG2 over-expressing astrocytes impairs neuronal survival under these conditions (Monteagudo et al., 2018). In our study (Pearse et al., 2020), basal TG2 expression was almost exclusively nuclear in neurons, albeit at very low levels, though strong cytoplasmic immunoreactivity was seen with EAE progression that may indicate a change in its function with the disease. Human MS samples exhibited an analogous induction pattern. To our knowledge, this was the first finding of robust neuronal TG2 induction following EAE or MS across species. Neuronal TG2 expression has been identified in a myriad of neurodegenerative conditions.
such as Alzheimer’s disease, Huntington’s disease, amyotrophic lateral sclerosis, and Parkinson’s disease. TG2 under conditions of Ca²⁺ dysregulation produces, through its transamidation activity, an accumulation of covalently cross-linked misfolded protein aggregates (Caccamo et al., 2010) that enhance neuronal vulnerability to stressors and apoptotic cell death. TG2 may also crosslink the apoptotic protein Bax, leading to the release of cytochrome c, activation of caspase-3, and cell apoptosis (Shawgo et al., 2008). Although our study (Pearse et al., 2020) suggests that TG2 neuronal expression may be involved in the pathology of EAE with the co-induction of caspase-3, it remains to be confirmed whether TG2 induction is involved in neuronal dysfunction and cell death or is expressed as a compensatory response to a loss in the capacity of the neuron to process aberrantly prepared proteins.

**Perspectives:** Understanding the role that TG2 plays in specific cells and the signal transduction pathways it is involved in during EAE and MS pathogenesis is important for leveraging its beneficial action towards a therapeutic application. Our study (Pearse et al., 2020) identified a strong induction of TG2 in endothelial cells and neurons in gray matter lesions after EAE in rats and mice and MS in humans. In endothelial cells and perivascular fibroblasts, TG2 could play a critical role in the transmigration of these cells into the CNS where they may be involved in angiogenesis or contribute to the formation of fibrous extracellular matrix and scar by externalization of TG2 and its association with fibronectin (fibrin). In neurons, the induction of TG2 with the apoptotic cell marker cleaved caspase-3 could be indicative of cell death or a compensatory mechanism to the failure of the neuron to process misfolded or aberrant proteins. To elucidate the cell-specific functions of TG2 in EAE, it will be important to generate cell selective TG2 knockout mice. It has been demonstrated that TG2 overexpression or knockout in one cell type can affect the function of another cell – TG2 overexpression in astrocytes impairs neuron survival under cytotoxic conditions and TG2 ablation in macrophages inhibits oligodendrocyte remyelination. Cell-specific knockout of TG2 in astrocytes using a GFAP transgenic line has been employed in a paradigm of spinal cord injury to demonstrate that astrocyte TG2 may play an important role in scar formation that leads to abortive axon growth after neurotrauma (Monteagudo et al., 2018). Generation of neuron and endothelial TG2 knockout transgenic lines will allow dissection of the role of TG2 in disease pathology and permit interrogation of downstream signal transduction pathways involved in cell survival, growth, differentiation, and migration. The availability of pharmacological inhibitors of specific TG2 activities has shown that both TG2’s transamidation (cysteamine) and fibronectin-binding (KCC009) activities play pathogenic roles in EAE and similar advancement in our knowledge is expected with the use of cell-specific knockout lines.

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