A novel role for tissue-nonspecific alkaline phosphatase at the blood-brain barrier during sepsis

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Sepsis and the blood-brain barrier (BBB): Sepsis is a life threatening systemic inflammatory condition involving multi-organ dysfunction. The World Health Organization estimates that about 30 million people are affected by sepsis every year, and at least 6 million people die from sepsis each year. Out of approximately 24 million sepsis survivors, it is estimated that 70% of these individuals experience some form of long-term neurological impairment (Iwashyna et al., 2010). Thus, when combined with prevention, diagnosis, and therapeutic management strategies, a functional understanding of the mechanisms that promote sepsis-associated neurological impairment is necessary to address the clinical challenges and economic burdens faced when treating sepsis.

An emerging area in basic and translational sepsis research is the role of the BBB in sepsis pathophysiology. The BBB is an important dynamic interface between the brain and periphery. Its stringent structure regulates the transport of molecules from the brain to the periphery and vice versa (Varatharaj and Galea, 2017). However, in many neuroinflammatory and neurodegenerative diseases, e.g., Alzheimer’s disease, stroke, sepsis, the function and structure of the BBB are disrupted – a process which may result in acute and/or chronic permeations in neurological function (Varatharaj and Galea, 2017). Studies have suggested that approaches to mitigate the loss of BBB integrity seen in neuroinflammatory and injury conditions like sepsis must incorporate a functional understanding of proteins localized to brain microvascular endothelial cells (BMECs) in order to elucidate their role(s) in preserving BBB integrity (Varatharaj and Galea, 2017; Nwafor et al., 2019). This concept is exemplified in a recent study which showed that the sphingosine 1-phosphate receptor localized to BMECs is required for small-molecule-selective opening of the BBB (Yanagida et al., 2019). It is imperative that researchers learn to assess canonical as well as noncanonical functions of proteins localized to the BMECs to elucidate how they preserve or impair BBB integrity.

Tissue-nonspecific alkaline phosphatase in physiology - bone to brain: Alkaline phosphatases (APs) were first discovered in 1923 and are well known for their roles in bone and teeth mineralization. There are four human AP isoforms that include: tissue-nonspecific alkaline phosphatase (TNAP), germ cell alkaline phosphatase, intestinal alkaline phosphatase, and placental alkaline phosphatase. The latter three isoenzymes are expressed in tissues which they are aptly named while TNAP is expressed in multiple tissues types such as bone, liver, intestine, kidney, and brain. TNAP as well as other AP isoforms are ectophosphatases anchored to the cell plasma membrane by glycosylphosphatidylinositol (GPI) protein (Buchet et al., 2013). Although there is an extensive literature on the important role played by TNAP in skeletal mineralization, a paucity of data exists on the mechanisms underlying TNAP’s role in non-skeletal contexts. Despite being an enzyme involved in the metabolism of inorganic and organic phosphates, TNAP is expressed in multiple tissues and cell populations, including bone, the gonads, adipose tissue, and the brain (Buchet et al., 2013). Although the majority of studies have focused on the role of TNAP in bone mineralization, a paucity of data exists on the mechanisms underlying TNAP’s role in non-skeletal contexts. Despite being an enzyme involved in the metabolism of inorganic and organic phosphates, TNAP is expressed in multiple tissues and cell populations, including bone, the gonads, adipose tissue, and the brain (Buchet et al., 2013). Although the majority of studies have focused on the role of TNAP in bone mineralization, a paucity of data exists on the mechanisms underlying TNAP’s role in non-skeletal contexts. The primary objective of our study was to demonstrate that the decrease in TNAP activity in cerebral microvessels was not due to the loss of brain endothelial cells, as earlier studies reported that a decrease in TNAP activity was caused by the loss of cerebral microvessels (Brown et al., 2007; Fonta et al., 2015). We employed CD31 immunostaining and showed that cerebral microvessel density remained unchanged in the face of diminished TNAP activity, unlike those same vessels. Furthermore, it was apparent from our results that the loss of TNAP activity on CD31 positive vessels was complete in some vessel segments and incomplete in others (Nwafor et al., 2020).

To further elucidate a functional role for TNAP on BMECs, we carried out a series of complementary in vitro and in vivo experiments. We showed that pharmacological inhibition of TNAP with two highly specific inhibitors, TNAP inhibitor and SBI-425, significantly disrupted BBB remodeling following levamisole treatment in a model of human cerebral microvascular endothelial cell line (hCMVEC/D3 cells) and primary murine BMECs, respectively (Burchacek et al., 2019; Nwafor et al., 2020). Our results are consistent with the observations of our group that received vehicle (Nwafor et al., 2020). Our novel findings are addressed in the next section.

Delineating TNAP’s role in BMECs: Elucidating the mechanisms underlying TNAP biology outside of teeth and bone has been challenging due to its presence in numerous tissues, e.g., liver, kidney, skeletal muscle, presence in numerous cell types in the brain, e.g., BMECs, neurons, astrocytes, and microglia. Mice with a global knockout of TNAP, known as Alpl−/− or Apk0−/− mice, die a few weeks after birth from hypophosphatemia and seizures (Buchet et al., 2013; Fonta et al., 2015). Thus, TNAP’s redundancy in numerous tissues makes the global knockout model an insufficient tool to delineate tissue-specific TNAP functions. The main objective of our study was to address our research questions by establishing an in vivo role for TNAP in BMECs. We employed a sepsis disease model since we aimed to understand how brain endothelial dysfunction affects brain function and we knew that sepsis targets the microcirculation (Nwafor et al., 2019). Our study utilized the gold standard sepsis model ofecal ligation and puncture to study the effects of both early sepsis (24 hours post-sepsis) and late sepsis (7 days post-sepsis). Our results showed that TNAP activity was diminished at 24 hours and 7 days post-sepsis in the cortex, striatum, and spinal cord. Interestingly, the sustained decrease in TNAP activity at 7 days did not extend to the hippocampus (Nwafor et al., 2020). These findings are supported by earlier studies which have shown that different brain regions have specific TNAP expression patterns. For example, human and rodent cortical regions express the highest level of TNAP and demonstrate a descriptive yet functional role for TNAP in BMECs through the maintenance of brain endothelial cell integrity. An additional set of experiments addressed whether the observed decrease in TNAP activity was coupled to canonical neuroinflammatory pathology such as microgliosis and astrogliosis seen in systemic inflammation (Varatharaj and Galea, 2017). We found that these two indices of neuroinflammation, i.e., microgliosis and astrogliosis, were present and elevated in a pattern consistent with observed spatial and temporal changes in TNAP activity and increased cerebral microvascular permeability (Nwafor et al., 2020). Our final experiments explored a critical functional outcome in neuroscience research – behavior. We hypothesized that sensorimotor function would be impaired since the cortex and spinal cord both show a loss in the high density of TNAP activity on and near the neuronal membrane. Conversely, we also hypothesized that our mice would not exhibit any deficits in learning and memory since the hippocampus showed no alterations in TNAP activity at 7 days post-sepsis. However, our results revealed no deficits in learning and memory at 7 days post-sepsis; however, septic mice did exhibit decreased spontaneous movement, diminished exploratory behavior in a novel environment, and hypoalgesia compared to controls. Interestingly, evoked locomotion and coordination were preserved as early as 3 days post-sepsis (Nwafor et al., 2020), a time period when mice typically exhibit the most severe sickness behavior. Collectively, the behavioral deficits we observed closely mimic clinical features observed in human sepsis patients. The presence of significant locomotor impairment along with the absence of evoked motor deficits suggests that septic mice exhibit altered mood and motivational behaviors previously described in humans.
The translational implications of our findings are summarized in Figure 1. We demonstrated that TNAP's enzymatic activity is novel modulator of BBB integrity in sepsis. Under healthy conditions (Figure 1A), TNAP activity in BMECs is maintained at basal levels and this activity is important for maintaining barrier integrity and cerebral homeostasis. However, during sepsis, key brain regions (Figure 1B) undergo a loss of TNAP activity earlier than others. This loss of TNAP activity occurs as early as 24 hours and is sustained up to 7 days post-sepsis. Following a loss of TNAP activity in BMECs, the disruption of junctional proteins enhances the infiltration of leukocytes which release pro-inflammatory cytokines into the parenchyma. This process, in turn, increases numbers of activated microglia and reactive astrocytes that release additional pro-inflammatory cytokines in the brain parenchyma. Thus, an acute systemic inflammatory disorder such as sepsis may sustain a chronic state of neuroinflammation that eventually leads to neurological impairment and increased mortality and morbidity in sepsis survivors (Figure 1C).

Concluding remarks: Our results demonstrate a novel role for TNAP in maintaining BBB integrity and add to the only existing scientific literature which had previously examined TNAP's role in maintaining barrier function (Deracinois et al., 2015). Furthermore, the contributions from our study highlight the emerging importance of TNAP in other neuroinflammatory and neurodegenerative diseases. Most importantly, our study lends support for TNAP as a therapeutic target to mitigate the long-term cognitive impairment symptoms seen in septic patients via the maintenance of BBB integrity. Conversely, TNAP activity could be manipulated to allow pharmacological therapies to penetrate the brain and target cancerous cells. Nevertheless, we must stress that much research is needed to fully elucidate the mechanisms and signaling pathways through which TNAP is able to maintain BBB integrity. Additional studies are also needed to understand how other TNAP expressing cell types communicate with brain endothelial cells to preserve BBB integrity in the face of injury.

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