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Review

The molecular, cellular, and morphological components of blood–brain barrier development during embryogenesis

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ABSTRACT

The blood brain barrier (BBB) is a hallmark of blood vessels in the brain and functions to protect the brain from unwanted blood born materials, support the unique metabolic needs of the brain, and define a stable environment crucial for brain homeostasis. The temporal profile of BBB development was long debated until recent studies produced convincing evidence demonstrating that the BBB is established and functional during embryogenesis. Here we review research focused on the molecular, cellular and morphological characteristics of BBB development. Our review discusses the precise temporal profile of BBB formation, the development of endothelial cell ultrastructure and the molecular components that provide sealing and transporting properties, the molecular pathways involved in the induction of BBB specific endothelial cell differentiation, the signaling pathways driving developmental angiogenesis versus barrier-genesis, and finally the contribution of other cell types to BBB formation. We examine aspects of BBB development that are still unresolved while highlighting research tools that could provide new insight to answer these open questions.

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1. Introduction

In the brain, blood vessels form a sophisticated biological barrier between the blood and neural tissue that is crucial for proper brain function. Most of our current knowledge regarding BBB character and function was established via research examining the BBB in adult organisms while relatively little is known about BBB formation and activity during embryogenesis.

The BBB was initially identified when dye injected into the peripheral blood circulation of an adult animal reached a majority of the body tissues but failed to penetrate the brain (Ehrlich, 1885; Goldmann, 1909). Subsequently, very compelling evidence for the existence and function of this barrier in embryos and newborns was published (Saunders et al. [1]). A beautiful review by Norman R. Saunders et al. [1] weaves the history of experimental evidence for a viable embryonic BBB, side by side with artifacts, misconceptions and untested claims leading to the exact opposite conclusion – that the embryonic and newborn BBB is “immature”,

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poorly formed, leaky, or even absent. Saunders et al. analyzed the rationale that drove the majority of the scientific and medical community to ignore these early studies and adopt the notion that the BBB develops postnatally. Due to the common belief that the embryonic BBB does not exist and technical difficulties associated with studying the embryonic BBB, BBB research in the developing embryo grew stagnant.

In the late 1960s, Reese and Karnovsky [2] used horseradish peroxidase (HRP) as a tracer in conjunction with electron microscopy (EM) to discover that endothelial cells are the cellular basis of the BBB. Saunders and others (e.g. Risue et al. [3], Bauer et al. [4]) pioneered the use of similar techniques to re-visit the embryonic BBB and demonstrate its functionality. In addition to advancement in EM, immunohistochemistry, and tracer techniques, these studies also took advantage of unique animal models such as the sheep (prolonged pregnancy and endurance to surgical procedures) and opossum (embryonic development primarily occurs outside of the uterus).

In recent years, research examining the embryonic BBB has rejuvenated and deepened our understanding of the cellular and molecular mechanisms underlying BBB development. In parallel with uncovering the molecular and cellular underpinnings of angiogenesis, pathways specific for brain vascular development such as the Wnt or DR6/TROY pathways [5–8] and genes important for barrier-genesis such as norrin and Mfsd2a [9,10] have been identified. Advances in transcripotomics and proteomics have shed light on gene expression specific to the BBB and enabled us to translate physiological properties of the BBB into molecular determinants. Several different cell types have also been credited for their contribution to BBB development. Specifically, endothelial cells are at the heart of the BBB, pericytes control specific gene expression programs in endothelial cells, and astrocytes contribute to the maintenance of the barrier postnatally.

A functional BBB during embryonic development implies that the nervous system forms in a defined and restricted environment. Moreover, BBB dysfunction in the adult has been associated with the initiation and persistence of various neurological disorders, raising the question: How does BBB dysfunction during embryogenesis impact brain development? On the other hand, the intact BBB constitutes a major obstacle for drug delivery to the CNS. In this regard, does a functional embryonic BBB protect the embryonic brain from toxins that escapes the placental barrier or prevents drug treatment to embryonic brain? Here, we present an overview of research focused on molecular, cellular and morphological characteristics of BBB development. We will review and discuss aspects of BBB development that are still unresolved while highlighting research tools that could provide new insight to these open questions.

2. When does the blood brain barrier form?

For almost a century the medical and scientific community held a strong belief that the BBB is not functional in embryos or perinatal animals. This belief flourished due to a blatant disregard for early studies providing evidence for the existence and function of an embryonic BBB. For the past three decades, methodological and technical advancements have allowed researchers to fight this antiquated dogma and firmly establish the view of a functional embryonic BBB.

The original and pervasive method used to test barrier function in animal models is through a tracer injection into the blood stream and an examination of tracer leakage into the brain parenchyma (the tissue 'outside' the blood vessels). In the adult, tracer injection is fairly straightforward but this method encounters four significant challenges in the embryo:

1. Extracting an embryo from the uterus detrimentally impacts embryo viability and brain tissue health, and alters the blood circulation.
2. Establishing an entry point into the blood stream is difficult especially in early embryogenesis.
3. Lower blood volumes require lower tracer volumes to be used in order to avoid massive changes in blood pressure.
4. Newly formed capillaries in the developing brain are more fragile then those of the adult and more susceptible to bursting. This reduced vessel strength might be attributed to the lack of mechanical support provided by tight-junctions, the basement membrane, and supporting cells such as pericytes, smooth muscle cells, and astrocytes. Without the ability to distinguish between a leaky barrier and mechanical assault, testing embryonic BBB functionality proves impossible.

Early tracer injection studies that demonstrated the existence of the embryonic BBB include Wislocki [11] with guinea pig embryos and Gröntoft [12] with both rabbit and human fetal material. Later studies attempted to determine the temporal profile of barrier formation. First, Dziegielewska et al. [13] used intravenously injected alcian blue in fetal sheep and found an embryonic barrier maturation process. Next, Risau et al. [3] intracardially injected HRP in mouse embryos and tested permeability after 5 min in circulation with light microscopy. This study revealed a decrease in vascular permeability from E13 to E16 and a fully functional barrier at E16. Similarly, Bauer et al. [4] injected both HRP and trypan bleu into the umbilical vein to test BBB development in mouse embryos and concluded that "the bbb is established very early in CNS development, probably in the course of intraneural neovascularization". More recently, Ek et al. [14] performed an intraperitoneal tracer injection in the opossum and determined that "as soon as vessels grow into the neocortex, their tight junctions are functionally restrictive". Finally, in 2010, Daneman et al. transcardially perfused tracers to demonstrate that most of the rat BBB is functional at E15 (comparable to mouse E13) [15]. The authors stated that "a functional BBB is present during embryogenesis before astrocyte generation. At each age tested (E15, E21, P1, P15 and P20) CNS vessels excluded the tracer from the CNS parenchyma". The study does find parts of the cerebral cortex to be leaky at E15 but attribute this leakiness to originate from the pia.

In addition to tracer injections, three other research tools are frequently used to study how barrier properties develop during embryogenesis. First, ultrastructure cellular properties of endothelial cells are examined by EM. Second the onset of specific BBB marker expression is examined by immunohistochemistry. Third, the presence of endogenous serum proteins in brain parenchyma is assessed. These approaches do not replace the functional assays mentioned above but provide additional indications and will be discussed in detail in the next sections.

Building upon these prior studies, we developed a highly sensitive and accurate injection method (Ben-Zvi et al. [10]) into the embryonic liver. Liver blood vessels are highly permeable allowing very fast absorption of the tracer into the blood circulation. Moreover, the liver successfully buffers the injection by preventing blood pressure fluctuations and allows the embryonic heart to control the natural perfusion of the tracer into the brain vasculature (see Fig. 1). The availability of new dyes with very high fluorescence intensity allows us to inject very small volumes and detect the tracer at very high spatial resolution via fluorescence and confocal microscopy. With this new methodology, we were able to test barrier function at very early embryonic stages and found that the majority of the BBB is functional before E13.5 while the cerebral cortex BBB becomes functional at E15.5 (see Fig. 2).