The Blood-Cerebrospinal Fluid Barrier

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CRC PRESS

Boca Raton London New York Washington, D.C.

Library of Congress Cataloging-in-Publication Data

TO COME

XX-XXXXX

Catalog record is available from the Library of Congress

Visit the CRC Press Web site at www.crcpress.com

St. Lucie Press Lewis Publishers Auerbach is an imprint of CRC Press

No claim to original U.S. Government works
International Standard Book Number 0-4153-2074-7
Library of Congress Card Number XX-XXXXX
Printed in the United States of America 1 2 3 4 5 6 7 8 9 0
Printed on acid-free paper

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Preface

The first account of the cerebroventricular system was provided by Galen of Pergamon (ca. A.D. 129–210). It was not until the seventeenth century, however, that the physician Thomas Willis came up with an explanation of choroid plexus (CP) function that was surprisingly close to the current concept of cerebrospinal fluid (CSF) production. By that time the anatomy of the ventricular system, the CP, and the meninges had been well described. Despite some progress in understanding the workings of the CP-CSF system, it took researchers almost three more centuries to realize that the CP is not only the major source of CSF, but that it is also the main site of the blood-CSF barrier.

The tight and adherens junctions connecting the choroidal epithelial cells form a barrier between the blood and the CSF. The CP shares the properties of the blood-CSF barrier with the arachnoid membrane. However, unlike the latter barrier, the blood-CSF barrier in the CP plays an active role in maintaining homeostasis of the brain microenvironment and in regulating various central nervous system (CNS) functions. Rapid development of experimental methodologies, including molecular techniques, which has taken place over the last 20 years, has facilitated advancements in research on the blood-CSF barrier and greatly enhanced our knowledge of the CP-CSF system. The CP now emerges as an organ possessing multifaceted functions, including broad enzymatic activities and the ability to synthesize proteins and transport peptides, nutrients, xenobiotics, and their metabolites. Increasing evidence indicates that the CP/blood-CSF barrier plays an important role in neuroendocrine signaling, neuroimmune and neuroinflammatory responses, drug metabolism, and protection from chemical-induced neurotoxicity. The critical role of the CP in brain development is also well recognized, and ample data have been obtained to suggest that the CP/blood-CSF barrier is involved in the aging of the CNS and the progression of some neurodegenerative diseases.

The lack of a comprehensive and up-to-date overview of the CP and its barrier function has prompted us to assemble this book. The book is comprised of four sections. In the **first section**, the reader will find a description of the ultrastructure and molecular features of the blood-CSF barrier and an analysis of the morphological changes in the CP that occur in various CNS disorders. In this section, the ontogeny of the CP is also discussed. The **second part** of the book focuses on various physiological functions of the CP/blood-CSF barrier. These include the molecular mechanisms of CSF formation and the peptide-mediated regulation of this process, transport of nutrients and essential elements, transport and metabolism of drugs, production of polypeptides, and neuroendocrine signaling. The **third section** analyzes the involvement of the CP/blood-CSF barrier in various CNS disorders, such as cerebral ischemia, Alzheimer's disease, metal-induced neurodegenerative diseases, and neuro-AIDS. It also discusses aging of the CP-CSF system and the role

of the CP in immune signaling. In addition, this section contains chapters on the molecular mechanisms of hydrocephalus and the clinical aspects of CNS disorders involving the CP and CSF circulation. The **last part** of the book describes the methodologies currently employed in blood-CSF barrier research, such as the *in situ* CP perfusion technique, choroidal cell culture systems, and in vivo methods of CSF production measurement.

We believe it is time to take the blood-CSF barrier out from the shadow of the blood-brain-barrier and recognize its importance in controlling the microenvironment of the neuropil and functioning of the CNS. It is with this intention that we wish to provide the reader with an expert opinion on recent advancements in both basic and clinical research on the CP/blood-CSF barrier. We hope that this book will not only benefit those whose primary interest is in the CP-CSF system, but that it will also serve as an important source of reference to clinicians, neuroscientists, and toxicologists in academia, the pharmaceutical industry, governmental agencies, as well as graduate students.

We wish to thank our colleagues for the time and effort they have given in the preparation of the chapters for this book. We would also like to thank our families for their continuous support, without which our work could not have been completed.

Adam Chodobski
Wei Zheng

Introduction

Wei Zheng

The brain has four major fluid compartments: the blood that flows through entire brain structures; the interstitial fluid (ISF) bathing neurons and neuroglia; the cerebrospinal fluid (CSF), which circulates around brain ventricles and spinal cord; and the intracellular fluid within brain cells. There is no major diffusional barrier between the ISF and CSF. Thus, materials present in either of these two fluid compartments are free to exchange and reach their destination cells. However, for a bloodborne substance to enter the ISF or CSF, it must first pass across the cell layers that safeguard the milieu of the central nervous system (CNS). Such cell layers possess unique cell types that join tightly together through intercellular connections, forming two major barrier systems designed to protect the brain microenvironment from fluctuations in concentrations of ions, metabolites, nutrients, and unwanted materials in the blood. The barrier located in the cerebral endothelia that separates the systemic circulation from the ISF compartment is defined as the blood-brain barrier, and the barrier in the choroidal epithelia that separates the systemic circulation from the CSF compartment is known as the blood-CSF barrier.

Despite the existence of two barrier systems in the brain, the focus of brain barrier research in the last century seems to be rather unbalanced, favoring the blood-brain barrier. This is not entirely surprising, taking into consideration the blood-brain barrier's intimate contact with neuronal constituents, its large surface area that covers nearly every part of brain organization, and its practical association in the development of CNS-effective therapeutic drugs. Nonetheless, inadequate research efforts on the blood-CSF barrier may somewhat reflect the general underestimation of the function of this barrier, and, to a certain extent, the insufficient understanding of the function of the choroid plexus, the tissue that comprises the blood-CSF barrier.

The choroid plexus is developed primarily from spongioblasts. In the human brain at autopsy during which the CSF is usually completely drained, the choroid plexus can be seen to extend along the floor of the lateral ventricles, hang down from the roof of the third ventricle, and overlie the roof of the fourth ventricle. The size of the tissue in one ventricle when it is in a dry, condensed state appears to be nothing more than that of an index finger. These autopsy impressions, which have over the years become the popular perception among many neuropathologists, can be dreadfully misleading and may lead to a misjudgment of the function of this tissue. Recent advancement in technology has enabled a micro-video probe to be inserted directly into the lateral ventricles. The vivid image illustrates that the live choroid plexus pervades the ventricles, stretching in concert with the heart pulse.

To better understand this, it may be helpful to make an analogy between the choroid plexus and a fishnet. The fishnet occupies barely one corner of a fishing boat; yet upon spreading in the water, it extends to cover a fairly large area. Likewise, in the life situation where the brain ventricles are full of the CSF, the choroid plexus expands to fill nearly all the cerebral ventricles. Unlike the fishnet, however, the plexus tissue possesses well-developed brush-type borders (i.e., microvilli) on the apical epithelial surface. These brush borders further protrude into the CSF and increase the choroidal epithelial surface area, enabling rapid and efficient delivery of the CSF as well as other materials included with the CSF secretion.

The brush borders of the choroidal epithelia also function to trap, take up, and enzymatically degrade the metabolites spilled into the ISF. A common notion regarding the brain's clearance of metabolites is that the metabolites are released into the ISF between neurons and glial cells; they are further drained into the CSF, where the bulk flow of CSF originating from the choroid plexus carries the metabolites to the subarachnoid space and further up to the dural sinuses for elimination. One should keep in mind that brain metabolites in the ISF would ultimately enter the CSF in the ventricles, if they are not transported to the bloodstream en route to the blood-brain barrier. Once in the ventricle, the metabolites first encounter countless brush borders of the choroid plexus. Clearly richer plexus borders would retain metabolites more so than do ependymal lining cells, which possess fewer foldings and microvilli, and, accordingly, the brushes of choroidal epithelia literally filter the CSF. Thus, from a neuroanatomical viewpoint, it is tempting to believe that the choroid plexus serves as a primary site for efflux or clearance of neuronal metabolites.

The lack of understanding of the blood-CSF barrier has finally come to an end in the face of a growing body of evidence that suggests a critical role of this barrier in monitoring the CNS homeostasis (Davson and Segal, 1995). In addition to its role in the movement of materials between the systemic circulation and the CSF, the choroid plexus avidly participates in various aspects of brain function, including involvement in the early stages of brain development, neuronal functional maturation, brain immune function, and neuroendocrine regulation. More recently, a great research effort has been devoted to the understanding of the pathophysiological influence of the blood-CSF barrier in neurological disorders, including its involvement in chemical-induced neurotoxicity, aberrant brain development, and, possibly, the initiation of neurodegenerative diseases (Strazielle and Ghersi-Egea, 2000; Zheng, 2001; Zheng et al., 2003). It is quite possible to say that the cerebral microcapillary injury (i.e., dysfunction of the blood-brain barrier) could be the cause of numerous brain homeostasis disorders; yet attention must be equally given to the blood-CSF barrier, without which, however extensive the research would be, it will remain difficult to explain the ultimate alteration in CNS milieu.

As a barrier between the blood and CSF, several unique anatomical and physiological features of the choroid plexus make the tissue a focal point of brain homeostasis research. First, the choroid plexus possesses a fairly large surface area despite its relatively small weight. The mature choroid plexus is typically less than 5% of brain weight (Cserr et al., 1980). For the adult human brain weighing 1.3 to 1.5 kg, the choroid plexus weighs about 65 to 75 g. The rat lateral ventricle choroid

plexus weighs typically ~3 mg. Although the tissue weight is relatively small, the total apical surface area of the choroidal epithelium approximates 75 cm^2, about one half that of the blood-brain barrier (155 cm^2) (Keep and Jones, 1990). A recent study by Speake and Brown (2004) has estimated that the membrane area of a choroidal epithelial cell is at least ten times greater than what had been estimated by histological studies. This translates into a total surface area of the blood-CSF barrier that is within the same order of magnitude as that of the blood-brain barrier.

The surface area of the choroid plexus can be further increased by the unique structure of the microvilli. As mentioned earlier, the choroidal epithelial cells possess the apical microvilli and multiple basolateral infoldings (Johanson, 1995). This feature shall be expected with respect to its physiological role in the secretion of CSF and in the delivery of substances (e.g., drug molecules) to the cerebral compartment. From a pharmacological and toxicological point of view, the extended surface area increases the chances of the tissue being exposed to drugs or toxicants from either side of the barrier.

Second, unlike the endothelial cells in the blood-brain barrier, the endothelia in the choroid plexus are extremely fenestrated and quite leaky, possibly lacking tight junctions between adjacent cells. Large molecules such as proteins can readily pass from the blood through the fenestrated capillary and then into the connective tissue. Yet, most of these materials are prevented from further entering the CSF by the tight junctions between the epithelial cells. To produce the CSF and nurture the brain, the fenestrated phenotype of choroidal endothelial cells allows the materials to enter and, subsequently, enrich in the choroidal epithelia; from there the tissue may select the needed substances to be delivered to the CSF. The leaky choroidal endothelia also allows xenobiotics and toxicants to gain access to the choroidal epithelia, some of which ultimately build up in the epithelia, either due to strong protein binding or a weak back-flux to the blood.

Third, in comparison to other brain regions, the choroid plexus has fast blood flow. The blood supply of the choroid plexus is derived from two posterior choroidal arteries, which branch from the internal carotid arteries. On the basis of data from experimental animals, the blood flow rate to the choroid plexus is about 4 to 6 mL/min per gram of tissue (Maktabi et al., 1990), whereas the average blood flow to brain is about 0.9 to 1.8 mL/min per gram of tissue. The rapid blood flow to the choroid plexus warrants an efficient influx of chemicals to the cerebral compartment or the efflux in the opposite direction. Understandably, both the rich blood supply of the choroid plexus and the leaky endothelial layer help increase the chance for the choroidal tissue to be insulted by toxic materials in the blood circulation.

Fourth, the tight junctions between the epithelial cells in the blood-CSF barrier seem less effective, or somewhat "leakier," than those between endothelial cells of the blood-brain barrier (Davson and Segal, 1995; Johanson, 1995). The electrical resistance is the parameter commonly used to reflect the tightness of the barrier in the sense of the barrier's resistance to ionic conductivity. When the cells are grown on a permeable membrane, the cells with barrier characteristics produce tight junctions and form a cellular barrier between the two compartments. The trans-endothelial or epithelial electrical resistance (TEER) can then be determined to estimate the

tightness of the barrier. The TEER value for an established endothelial barrier (i.e., the blood-brain barrier) usually exceeds 1,300 ohms-cm^2, whereas the value for an established epithelial barrier is about 80 to 200 ohms-cm^2. Thus, the limited tightness of the choroidal barrier may provide a pathway for materials in the CSF to enter the brush borders of the choroid plexus, serving as a cleansing mechanism in the CNS. Some investigators have referred to the choroid plexus as a "kidney" to the brain (Spector and Johanson, 1989). The real kidneys remove unwanted materials from the blood to urine; the choroid plexus removes the materials from the CSF to the blood, should the CSF be analogously viewed as the "blood" to supply nutrients to neurons and glial cells in the CNS.

Finally, the unique anatomical location of the choroid plexus within the cerebral ventricles may be associated with certain chemical-induced neurotoxicities. For example, the hippocampal formation forms the wall of a certain part of the lateral ventricles. As discussed earlier, the live choroid plexus is loosely floating in the CSF and fills the spaces of all ventricles. The choroid plexus in the lateral ventricle is closely adjacent to the hippocampal formation and other neuronal structures. It remains unknown whether the close contact between the choroid plexus and some brain structures makes it easier for substances derived from the choroid plexus to come into contact with brain tissue and therefore affect certain brain functions. Nonetheless, it will be an interesting subject for future investigation.

Because of these anatomical and physiological characteristics, the choroid plexus is clearly a vital physiological compartment in the brain. This book, the first of its kind exclusively devoted to the blood-CSF barrier, does not attempt to be nor is it suitable for encyclopedic collection of all aspects of advancement in this area. Nonetheless, it is my belief that we must establish between the blood-brain barrier and blood-CSF barrier a cohesive relationship, which will allow, at the minimum, a clearer conception, so that before we can explain brain homeostasis in any meaningful sense, we must first be aware what causes and manages it.

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