Developmental Aspects of the Intracerebral Microvasculature and Perivascular Spaces: Insights into Brain Response to Late-Life Diseases

Miguel Marin-Padilla, MD and David S. Knopman, MD

Abstract

The development of the microvasculature of the human cerebral cortex offers insight into the response of the cerebral cortex to later-life brain injury. We describe the 3 basic and distinct components of the developmental anatomy of the cerebral cortical microvascular system. The first compartment is meningeal and, therefore, extracerebral. In addition to the major venous sinuses, arachnoidal arteries, and veins, the pial anastomotic capillary plexus that covers the surface of the developing and adult cerebral cortex represents the source of the penetrating vessels that become the second component, the intracerebral extrinsic microvascular compartment. During embryogenesis, sprouting vascular elements from pial capillaries pierce the brain’s external glial limiting membrane and penetrate the cortex. These vessels, which eventually differentiate into arterioles and venules, are separated from the cortical tissue by the extravascular Virchow-Robin compartment (V-RC) formed between the internal vascular and the external glial basal laminae. The V-RC remains open to the meningeal interstitial spaces and outside the blood-brain barrier (BBB) and acts as a prelymphatic drainage system for removal of substances that cannot be transported into the blood or catabolized intracellularly. The third element is the dense intracerebral intrinsic microvascular compartment. Intracerebral capillary vessels sprout from the perforating vessels, penetrate through the Virchow-Robin glial membrane, and enter the neuropil. Intracerebral capillaries lack smooth muscle and a basal lamina. Their role as the physiological BBB is the exchange of oxygen, glucose, and small molecules. This developmental perspective highlights 3 principles: (a) the V-RC is intimately related to the cortical penetrating arterioles and venules and represents an inefficient protolymphatic system that lacks the anatomic and physiologic constituents found in lymphatic beds elsewhere in the body; (b) the anatomic contiguity of the V-RC and the penetrating vascular compartment (arterioles and venules) implies that the pathology in 1 compartment could lead to dysfunction in the others; and (c) the anatomic localization of the immunologic BBB at the level of the penetrating venules might impose constraints on immunologically mediated transport involving the V-RC.

Key Words: Alzheimer disease, Chronic traumatic encephalopathy, Development, Microvascular, Neocortex.

INTRODUCTION

The development of the microvasculature of the human cerebral cortex offers insights into the response of the cerebral cortex to brain injuries and diseases. Vascular cognitive impairment due to arteriosclerosis (1–4) and cerebral amyloid angiopathy (5, 6) is an obvious example. Chronic traumatic encephalopathy (CTE) (7) and Alzheimer disease (AD) (8) both show a pattern of cerebral cortical degeneration that exhibits a perivascular distribution of pathology. Whereas observations in AD have raised the possibility that cerebral ischemia induces the pathology of AD (9, 10), there are other possible explanations for the perivascular distribution of pathology besides ischemia.

Here, we describe the development, anatomy, and physiology of the human cerebral cortex vascular system as well as its possible role in the evolving pathology (i.e., increasing number of lesions) of some encephalopathies. The developmental anatomy of the cortical vascular system offers insights beyond pediatric diseases into the evolving pathology and inexorable clinical deterioration that occurs with some adult brain disorders. Because the pathology of CTE has not been studied as extensively, most of the following discussion concerns AD and cerebrovascular disease.

Before the description of the brain vascular system, it is worth emphasizing that the mammalian cerebral cortex (and entire CNS) lacks a lymphatic system. This system evolved to transport cellular proteinaceous and nonprotein wastes away from the site of injury elsewhere in the body. The lack of a lymphatic system could help explain some of the pathologic alterations described in degenerative encephalopathies. Even nonneural tissues with an adequate lymphatic system fail to remove an excessive and/or recurring amount of necrotic debris. The accumulation, in various tissues of misfolded or damaged proteins and related substances, has been long recognized in some chronic infectious diseases characterized by extensive and recurrent cellular necroses, such as tuberculosis (11). It seems plausible that damage to the prenatal human brain results from unremoved necrotic residues with additional deleterious effects on its structural and functional maturation (12–15). Necrotic and/or calcified developing brain residues continue to elicit inflammatory responses without resolving the situation, which could eventually affect adjoining unaffected regions (14).
The key insights from the developmental anatomy of the cortical vascular system are (a) that the origin of the microvascular circulation of the cerebral cortex also carries the brain’s prelymphatic system with it (16–19) and (b) that it is composed of 2 distinct intracerebral extrinsic and intrinsic microvascular compartments. Unfortunately, in most embryology, neuroanatomy, neurohistology, and neuropathology textbooks, the description and composition of the intracerebral microvascular system are incomplete. The anatomic and functional differences between the intracerebral extrinsic and intrinsic microvascular compartments have not been clearly defined and remain poorly understood. Although the importance of cortical perivascular pathway as a drainage pathway to the subarachnoid space has been recognized previously for its role in AD (20–22), the link between these observations and cerebrovascular changes with aging is underestimated by many in the field because of the way knowledge is compartmentalized. The developmental perspective provides a blueprint for how the response to injury in the adult brain must involve both the vascular and the prelymphatic components.

DEVELOPMENT OF THE HUMAN BRAIN VASCULAR SYSTEM

The cerebral cortex vascular system is composed of 3 distinct compartments, namely, (a) the extracerebral or meningeal compartment with its important pial anastomotic capillary plexus, (b) the intracerebral extrinsic microvascular compartment with the Virchow-Robin prelymphatic channels, and (c) the intracerebral intrinsic microvascular compartment that represents the blood-brain barrier (BBB; Table).

Extracerebral or Meningeal Vascular Compartment

The development of the human cerebral cortex extracerebral vascular compartment starts around the sixth and seventh weeks of gestation with the establishment of the meninges. All essential vascular components of the meninges are already recognizable in 7-week-old human embryos. They include the dural venous sinuses, the arachnoidal main arteries and veins, and the pial capillary plexus, all embedded within the meningeal tissue that surrounds the cerebral cortex (16, 18, 23). By 7 weeks of gestation, the brain surface is covered by an impermeable membrane composed of glial end feet united by tight junctions and covered by basal lamina that covers the brain surface (and entire CNS) during its entire prenatal and postnatal development. This impermeable external glial limiting membrane (EGLM) maintains the brain anatomic integrity and its separation from surrounding tissues. Originally, the CNS is a neuroectodermal tissue and, as such, is separated from surrounding tissues by a basal lamina material manufactured by its cells. When this surface neuroepithelium is enclosed to form the neural tube (CNS anlage), its entire outer surface becomes covered by basal lamina. The original neuroectodermal (ventricular) cells supply the necessary end feet and the basal lamina needed to maintain the neural tube integrity. As the brain expands, its original ventricular cells are transformed into radial glial cells that remain attached to the ventricular wall and have several dichotomized endings, each one with end feet covered by basal lamina (16, 19, 24). During early prenatal development (from the 7th to around the 25th week of gestation), radial glial cells supplied the necessary end feet and basal lamina material to maintain the expanding brain integrity (24). After this time, special astrocytes become progressively incorporated into the cortex first lamina supplying the necessary end feet and basal lamina material for its later expansion. The incorporation of these first layer astrocytes coincides with the progressive radial glial cell disintegration and eventual disappearance (24). The EGLM is an essential and important component of the CNS throughout its entire development and functional maturation. This limiting glial membrane is only perforated by an active and complex process involving entering vessels and nerves and by exiting nerves.

The prenatal evolution of the various meningeal dural sinuses and the arachnoidal main arteries and veins has been described and beautifully illustrated in human embryos by Padget (25, 26), but there is no mention of its third component—the pial anastomotic capillary plexus. This important meningeal microvascular component has seldom been described in the literature and is not mentioned in most embryology, neuroanatomy, neurohistology, and neuropathology textbooks (27, 28). It plays a crucial role in the internal vascularization of the cerebral cortex during prenatal and early postnatal life and for as long as the brain is expanding (16, 18, 19, 23). It also participates in the establishment and maintenance of the cerebral cortex prelymphatic drainage system, and it could indirectly contribute in the brain’s response to injury.

A possible explanation for this apparent neglect is the fact that the pial capillaries are microscopic (their diameter ranges from 5 to 10 μm) and, hence, invisible to the naked eye. Also, because the removal of the meninges invariably carries the pial capillary plexus in toto, it leaves the cerebral cortex surface without any recognizable vasculature. A magnifying glass is needed to observe the innumerable microscopic openings covering its entire surface. These microscopic orifices are where the perforating arterioles enter and the venules exit; each has its corresponding Virchow-Robin prelymphatic compartment (V-RC) (16, 17, 19). Throughout the entire brain surface, the overall separation between these vascular orifices ranges between 400 and 600 μm (16). This distance determines the size of the intracerebral intrinsic microvascular compartment.

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*Both the extrinsic (directly) and the intrinsic (indirectly) intracerebral microvascular compartments evolve from the pial anastomotic capillary plexus.
Pial Anastomotic Capillary Plexus

The pial capillary plexus (PCP) is a short-linked anastomotic plexus that covers the entire surface of the cerebral cortex including gyri and sulci. A rich pial capillary plexus already covers the entire surface of the cerebral cortex of 6- and 7-week-old human embryos, even preceding the start of its internal vascularization; these early pial capillaries still have nucleated red cells (Figs. 1A, B). The PCP expansion is linked to that of the cortex and parallels its evolving, expanding, and changing configuration. Consequently, we presume that the pial anastomotic capillaries must undergo continuous expansion, remodeling, and adaptations by both angiogenesis and reabsorption. An expanding pial anastomotic capillary plexus covers the surface of the developing brain at all ages, and the number of perforating vessels increases accordingly. Its innumerable capillaries are separated from the cerebral cortex’s external glial limiting membrane by fibroblasts, a few meningeal cells and collagen fibers (Figs. 1A, B, 2A, B, and 3A).

The PCP, an essential component of the meningeal extracerebral vascular system, represents the source of all perforating vessels that will enter the developing cerebral cortex and establish its intracerebral extrinsic microvascular compartment. Indirectly, these pial capillaries also participate in the establishment of the intracerebral intrinsic microvascular compartment (Table).

The pial capillaries have to perforate through the EGLM to enter into the cerebral cortex. Some pial capillaries have already started to perforate cerebral cortical proximal (ventral) region in 7-week-old embryos. At this age, the internal vascularization of striatum and pyriform lobe are already underway as well as that of older brain and CNS structures. By the eighth week of gestation, perforating vessels are recognized throughout the human cerebral cortex entire surface.

Consequently, the 4 early developmental stages (neuroectodermal, marginal zone, primordial plexiform and pyramidal cell plate early appearance) of the human cerebral cortex evolve within a still avascular tissue (16). The human cerebral cortex in 6- and 7-week-old embryos is at the marginal zone and the primordial plexiform stage of evolution, respectively, and is already covered by a rich pial capillary plexus (Figs. 1A, B). At these ages, the human cortex is composed of a few and loosely arranged neurons and fibers scattered, without specific locations, below the EGLM and above the matrix zone (Figs. 1A, B). Probably, the close proximity of the pial capillaries to these developing cortical elements permits the necessary oxygen diffusion for their early functional evolution.

The EGLM represents an anatomic boundary that delimits and isolates the cerebral cortex from the surrounding tissues. It is composed of the end feet of radial glial cells that are united by tight junctions cover the entire surface of the cortex (Figs. 2A, B, D, and 3A). The EGLM is also covered by basal lamina material manufactured by the glia end feet (Fig. 3A). This outer brain membrane is and remains impermeable and must be actively perforated by entering capillaries (16, 18, 29–34). During late prenatal and postnatal development, when the radial glia begin to disintegrate, first lamina astrocytes will supply the necessary additional glial end feet for its expansion and maintenance (35).

The capillary perforation of the human cerebral cortex EGLM commences between the seventh and eighth weeks of gestation. It starts through proximal (ventral) regions and progresses toward distal (dorsal) ones, paralleling the arrival and entrance of early neurons and fibers (from extracortical sources) under the EGLM (16, 30). By the end of the eighth week of gestation, the entire cerebral cortex has perforating vessels with their corresponding extravascular V-RC, as well as
interconnecting intrinsic capillaries plexuses between them (16). At this age, the human cerebral cortex weighs less than 2 g, and therefore, the number of perforating pial vessels entering the cortex is still very small. Because the human brain growth from more than 2 g (9 to 10 weeks’ gestation) to approximately 1350 g (adult age), the number of additional perforating vessels entering the cerebral cortex increases exponentially, although maintaining their constant intervascular distances.

**EGLM Pial Capillary Perforation**

The pial capillary perforation of the EGLM of the cerebral cortex is a complex developmental process. One of us

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**FIGURE 2.** Composite figure of electron photomicrographs showing various aspects of the perforation of the embryonic cerebral cortex external glial limiting membrane (EGLM) by vessels from the pial capillary plexus from 12-day-old hamster embryos. The pial capillaries (*) are small, with a diameter ranging between 5 and 10 μm, and have tight junctions (small arrows) separated from the cortex EGLM by pial (arachnoidal) cells, collagen fibers, and by their respective basal laminae. (A) Small arrows indicate tight junctions, which cover the cortical EGLM (vertical arrows) composed of radial glial end feet (G) united by tight junctions. The endothelial cells of the approaching capillary show considerable membrane activity with both internal (1 arrows) and external (2 arrows) filopodia. Some have already penetrated into the neural tissue (thick arrow). F, fibroblasts; N, neurons. (B) A high-power view of endothelial cell filopodia (E), from the approaching capillary, establishing direct contacts with the cortical EGLM (G) with fusion of both vascular and glial basal laminae. The fusion of both basal laminae precedes the filopodia perforation of the cortex EGLM. (C) Low-power view of a pial capillary (*) perforating through the cortical EGLM (thick arrows) showing the establishment of the extravascular Virchow-Robin compartment (V-RC) and the penetration of a meningeal (pericyte) cell (curved arrow), a possible precursor of vascular smooth muscle. (D) Detail of the perforating pial capillary (*) showing the establishment of the V-RC (arrows) and its external glial wall (G, arrows), which appears to be an extension of the cortex EGLM. The V-RC remains open to the meningeal interstitial spaces throughout both prenatal and postnatal cortical maturation, permitting the exchange of fluid and inflammatory cells between brain and meninges. A meningeal pericyte (P), a possible precursor of vascular smooth muscle, has penetrated into the V-RC. P, pericytes; PC, pial capillary; RBC, red blood cell. Scale bars = 5 μm.
advancing filopodia (PF).

Also illustrated are the penetration of a meningeal pericyte (P) into the cortex with the formation of the perivascular (lower left), and the penetration of a pial perforating capillary (PC) into the brain tissue (Figs. 2 and 3A). The funnel formed by the fusion of vascular and glial basal laminae at the entrance site (lower left), and the penetration of a pial perforating capillary (PC) into the cortex with the formation of the perivascular Virchow-Robin compartment (V-RC) around it (Fig. 2D). This extravascular space, known as the V-RC, accompanies the perforating vessel through its entire length and remains, for life, extravascular as well as open to the meningeal interstitial spaces (16, 18, 19, 36). The V-RC is lined internally by the perforating vessel basal lamina and externally by a glial basal lamina formed by the progressive incorporation of additional glial end feet. The V-RC external glial wall represents an extension of the surface EGLM (Figs. 2D and 3A). The V-RC is outside the BBB. Even in normal conditions, fluids as well as a few macrophages may fill these perivascular spaces (Fig. 3B). The V-RC is the only open communication between the brain tissue and the meningeal interstitial spaces permitting the penetration of meningeal elements into it, such as pericytes and the perforating vessel smooth muscle cell precursors, as well as the exchange of fluids and inflammatory cells between brain and meningeal tissues (Figs. 2D and 3A). The perforating vessels (entering arterioles as well as exiting venules) are the only vascular component of the brain with smooth muscle cells that are capable of pulsations. Their size (diameter) varies depending on their length and functional commitments (Figs. 4A and 5A, B).

Intracerebral Extrinsic Microvascular (Prelymphatic) Compartment

Pial vessels perforate the cortex EGLM and enter the neural tissue simultaneously and at many locations during its expansion. Most of them enter the cortex perpendicularly and penetrate down to the paraventricular zone (Figs. 2C and 3A). At the paraventricular zone, an intrinsic capillary anastomotic plexus is established that interconnects all perforators throughout the zone. This will be the first of numerous intrinsic capillary anastomotic plexuses that will be established progressively between contiguous perforators, paralleling the cerebral cortex ascending stratification and functional maturation (16). During the gray matter’s functional maturation and during postnatal development, numerous additional perforators enter into the cortex, reaching only its maturing neuronal systems. The number of perforators reaching the gray matter is considerably larger than that reaching the white matter. This difference plays a significant role in establishing the anatomic integrity of the extrinsic microvascular compartment around it (Fig. 2D). This extravascular space, known as the V-RC, accompanies the perforating vessel through its entire length and remains, for life, extravascular as well as open to the meningeal interstitial spaces (16, 18, 19, 36). The V-RC is lined internally by the perforating vessel basal lamina and externally by a glial basal lamina formed by the progressive incorporation of additional glial end feet. The V-RC external glial wall represents an extension of the surface EGLM (Figs. 2D and 3A). The V-RC is outside the BBB. Even in normal conditions, fluids as well as a few macrophages may fill these perivascular spaces (Fig. 3B). The V-RC is the only open communication between the brain tissue and the meningeal interstitial spaces permitting the penetration of meningeal elements into it, such as pericytes and the perforating vessel smooth muscle cell precursors, as well as the exchange of fluids and inflammatory cells between brain and meningeal tissues (Figs. 2D and 3A). The perforating vessels (entering arterioles as well as exiting venules) are the only vascular component of the brain with smooth muscle cells that are capable of pulsations. Their size (diameter) varies depending on their length and functional commitments (Figs. 4A and 5A, B).

(M.M.P.) has described this process using the rapid Golgi procedure and electron microscopy (16, 18, 19, 22). It consists of 3 sequential processes: (a) EGLM pial capillary contact, (b) EGLM endothelial cell filopodia perforation, and (c) EGLM pial capillary perforation with penetration of the vessels into the brain tissue (Figs. 2 and 3).

The growing sprouts of some pial capillaries surrounded by vascular basal lamina approach the EGLM basal lamina. The space between the approaching capillary basal lamina and that of the EGLM becomes progressively reduced (Figs. 2A and 3A). The approaching capillary leading endothelial cells show considerable membrane activity with formation of both external as well as internal filopodia (Fig. 2A). Some external endothelial filopodia establish direct contacts with the EGLM and fusion of vascular and glial basal laminae occurs at the site (Figs. 2B and 3A). Subsequently, the contacting filopodia penetrate the EGLM fused basal laminae and penetrate into the tissue (Figs. 2A and 3A). The penetrating filopodia become surrounded by the fused vascular and glial basal laminae, thus reestablishing the EGLM anatomic integrity (Fig. 3A).

Eventually, the approaching vessel-growing sprout enters into the tissue followed by the penetration of the entire vessel (Figs. 2C, D, and 3A). The funnel formed by the fusion of vascular and glial basal laminae accompany the perforating vessels thus establishing a perivascular (extravascular) compartment around it (Fig. 2D). This extravascular space, known as the V-RC, accompanies the perforating vessel through its entire length and remains, for life, extravascular as well as open to the meningeal interstitial spaces (16, 18, 19, 36). The V-RC is lined internally by the perforating vessel basal lamina and externally by a glial basal lamina formed by the progressive incorporation of additional glial end feet. The V-RC external glial wall represents an extension of the surface EGLM (Figs. 2D and 3A). The V-RC is outside the BBB.

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role in both normal as well as pathologic conditions involving the developing cerebral cortex (13–15). The additional gray matter–perforating vessels establish rich intrinsic capillary plexuses that parallel the growth and complexity of the developing cortical gray matter (16). The gray matter intrinsic capillary plexuses are more numerous and complex than those of the white matter, reflecting their greater functional commitment (Fig. 5).

Pial capillaries perforate the cortex (and CNS) EGLM, only to enter but not exit it (16, 17, 19). Eventually, circulatory dynamics will determine which pial vessels become entering arterioles and which ones exiting venules (Figs. 4A and 5A, B). Throughout the cortex, every exiting venule becomes surrounded by 8 to 10 entering arterioles. During prenatal and postnatal development and functional maturation, additional new perforating vessels continue to enter into the cortex between previous ones and maintaining a nearly constant intervascular distance. The PCP vessels play an essential role in the brain intracerebral microvascularization and retain, throughout life, a remarkable activity, with continued remodeling and readaptation to local functional needs. Every perforating vessel carries with it an extravascular V-RC that represents a prelymphatic drainage channel open to the meningeal interstitial spaces.

The distance between contiguous perforating vessels, throughout the entire brain surface, ranges between 400 and 600 μm and remains unchanged through both the developing and the adult cerebral cortex. (B) Higher magnification of the cortex IMVS formed between contiguous perforators showing the 3-dimensional organization of its capillaries and the relatively small intercapillary spaces between them, where neurons reside. There is a large stellate basket cell (BC) in one of them and some pericellular baskets formed around the unstained bodies of pyramidal cells.

FIGURE 4. Photomicrographs, from rapid Golgi preparations of a newborn infant motor cortex, showing the composition and 3-dimensional organization of its extrinsic (A) and intrinsic (B) microvascular compartments. (A) Equidistance of the pial perforators, including 2 entering arterioles (A) and an exiting venule (V) and the intrinsic microvascular capillary plexus (IMVS) established between them. The perforating vessels intervascular distance ranges between 400 and 600 μm and remains unchanged through both the developing and the adult cerebral cortex. (B) Higher magnification of the cortex IMVS formed between contiguous perforators showing the 3-dimensional organization of its capillaries and the relatively small intercapillary spaces between them, where neurons reside. There is a large stellate basket cell (BC) in one of them and some pericellular baskets formed around the unstained bodies of pyramidal cells.

FIGURE 5. Composite figure showing the nearly identical composition and 3-dimensional organization of the intracerebral extrinsic and intrinsic microvascular compartments from a newborn infant (A) and an adult brain (B). (A) A rapid Golgi preparation from the motor cortex showing an entering arteriole (A) distributed through the gray matter and an exiting venule (V), as well as the rich intrinsic capillary plexus formed between them that extends throughout both gray and white matter. The horizontal terminals of Cajal-Retzius cells axons (C-R at) are also illustrated. (B) The intravascular casting of the temporal lobe from a 66-year-old man showing an entering arteriole (Number 1 in the figure) that branches through the gray (GM) matter and an exiting venule (Number 5 in the figure) that reaches the white matter (WM). (From Figure 29 in Duvernoy et al [42]. Reproduced with permission from Brain Research Bulletin.) 2, recurrent arteriolar branch; 3, deep arteriolar branch coiling around the parent arteriole; 4, arteriole of the subcortical white matter; 6, pial vein. Considering that the adult brain is at least 3 times larger than that of the newborn, the similarities between the brain extrinsic and intrinsic microvascular compartments that remain unchanged from prenatal to adult life are striking and are undoubtedly physiologically important.
600 μm (Figs. 4A and 5A, B). This intervacular distance is maintained essentially unchanged throughout the brain pre-natal and postnatal development and functional maturation and for as long as the brain expands (11). Consequently, the numbers of new pial perforating vessels entering into the developing cerebral cortex progressively increase. The equidistant pial vessel perforations imply significant rearrangements between those that will become entering arterioles and those that will become exiting venules (Fig. 4A). The intervacular distance between contiguous perforators and, consequently, the dimension of the intrinsic capillary plexus established between them may represent a mammalian brain physiological constant necessary and adequate for the delivery of oxygen and other nutrients. The penetrating arterioles are the physiological locus of regulation of blood flow in response to changing metabolic demands of local neurons (10).

The length of perforating vessels, and hence that of its V-RC prelymphatic channels, increases progressively as the cortex thickness increases during its functional maturation. This active elongation will not affect the distance between perforating vessels nor the dimension of the intrinsic capillary plexus formed among contiguous ones (Figs. 4A, B, and 5A, B). It must be recognized that the increasing width of the cerebral cortex also increases the inefficiency of its prelymphatic drainage system for the removal of recurrent and/or excessive necrotic debris.

All V-RCs are open to the meningeal interstitial spaces, thus permitting the exchange of fluids and of cells between brain and meninges. Eventually, their content will reach the meninges and drain through the perivascular lymphatics (16–19, 37, 38). The large number and close proximity of these prelymphatic channels, throughout the entire cerebral cortex, might compensate for the physiological inadequacy of the system. The system lacks features found in other the lymphatic systems of other organs; therefore, the movement of fluids and of cells through these extravascular channels likely is slow. The absence of valves (and/or similar structures) found in lymphatic vessels in other organs would also contribute to the inefficiency of the V-RC for clearing debris and/or postinflammatory residues. The only possible mechanism for helping the movement of fluids and of cells through these prelymphatic channels must be the regular pulsations of the perforating vessels and the presence of a single superficial meningeal opening (21, 22).

One might speculate that arterioles that have endothelial cell damage due to diabetes or hypertension might worsen the process of draining residues into the meningeal subarachnoid space. Consequently, the prolonged or permanent residence of damaged proteins in the V-RC might result in the gradual appearance of perivascular inflammation and neurodegeneration. Moreover, unremoved necrotic residues within the V-RCs as well as within the contiguous tissue would remain in situ and undergo progressive enzymatic protein degradation. Unremoved necrotic (proteinaceous) residues would continue to elicit repeated inflammatory responses with the additional accumulation of new macrophages and of reactive local glial cells, perpetuating the unresolved pathologic lesions and possibly induce the appearance of others. This mechanism might explain the increasing number of similar lesions throughout the affected brain as well as the progressive cognitive deterioration characteristic of some neurodegenerative encephalopathies.

**Intracerebral Intrinsic Microvascular (Blood-Brain Barrier) Compartment**

An intrinsic anastomotic capillary plexus is progressively established between contiguous perforators throughout the development of the mammalian cerebral cortex (Figs. 4A, B, and 5A, B). The intrinsic capillary plexuses start to form throughout the cortex paraventricular zone and progressively ascend, paralleling the ascending and stratified functional maturation of its neurons (16). In the embryonic brain, the matrix zone is the first to vascularize because of its early germinal activity. Subsequently, the white matter, the subplate zone, and, finally, the maturing gray matter establish intrinsic capillary plexuses. The intrinsic capillary plexuses are first recognized during the ascending neuronal maturation of the subplate zone, which precedes that of the gray matter. The intrinsic capillary plexuses are established and extensively elaborated between contiguous perforators during the subsequent ascending and stratified functional maturation of the gray matter (Figs. 4 and 5). The formation of the intrinsic capillary plexuses follows an ascending progression, from deeper and older strata to superficial and younger ones (16, 18, 19).

The capillaries must perforate through the V-RC outer glial wall and basal lamina to enter the brain tissue in a manner similar to the perforation of the cortex EGLM by pial capillaries. Emerging capillaries from contiguous perforators establish contacts and form a short-linked anastomotic plexus between them (Figs. 4A, B, and 5A, B). These intrinsic capillaries are approximately 5 μm in diameter and consist solely of endothelial cells; they are covered by a single basal lamina of glial and vascular origin. Glial end feet can be observed on the abluminal surface of the capillaries. Although there are no smooth muscle cells surrounding cortical capillaries, pericytes located on the outer capillary walls are very abundant and might serve a similar role of inducing pulsatile flow (39). There is no V-RC at the level of the intrinsic capillaries. This intrinsic capillary plexus represents the physiological BBB (40, 41). Interactions between endothelial and glial cells that are not yet well understood participate in these processes. This intrinsic capillary plexus undergoes continuous remodeling by angiogenesis and reabsorption as it adapts to each zone’s growing and functional needs in development. Both processes have been described in rapid Golgi preparations of the developing cortex (16–19, 22). The physiological role of the intrinsic capillaries is exclusively committed to the metabolic and anabolic activities of the cerebral cortex (40).

The intrinsic microvascular compartment size and 3-dimensional organization remain essentially unchanged throughout prenatal and postnatal cerebral cortex development (Figs. 4A, B, and 5A, B). Rapid Golgi preparations of 200-μm-thick sections provide a unique view of the 3-dimensional nature of the density of the developing intrinsic capillary plexus. We are not aware of Golgi studies in adult human brain, but casts of the vascular system in adults (Fig. 5B) suggest that the density of intracerebral capillaries is comparable to prenatal examples (42). Throughout the gray...
matter, the intrinsic plexus intercapillary spaces are small and uniform, measuring between 80 and 100 μm in diameter. These spaces are occupied by the cortical neurons (Fig. 4B), whereas these intercapillary spaces are larger and more irregular throughout the white matter. The intrinsic capillary plexus undergoes continuous remodeling by angiogenesis and reabsorption throughout life. In the gray matter, they are more complex and have more capillaries than those of the white matter, reflecting their greater functional commitment (Figs. 5A, B). These intrinsic capillaries are undoubtedly among the most active microvascular systems of the human body and are not likely directly involved in inflammatory processes. The anatomic and functional development and expansion of the intrinsic capillary plexus (and of its important role in human brain physiology and pathology) have not been fully appreciated and/or recognized.

**Inflammation and the Brain Microvasculature**

The intracerebral extrinsic and intrinsic microvascular anatomy places anatomic constraints on inflammatory responses in the cerebral cortex. It is now believed that microglia migrate into the cerebral cortex during embryological development and do not enter the CNS during adulthood (43). Activation of intrinsic microglia in response to brain pathologies occurs within the neuropil (44, 45) and is thought not to be directly regulated by the microcirculation. What is relevant to the current discussion is how activated microglia come to interact with the immune system that is extrinsic to the brain. In response to brain injuries, leukocytes (and perhaps macrophages [44]) from the vascular space enter the brain as part of an inflammatory process. The passage of inflammatory cells does not occur through the intrinsic capillaries because of their dedicated physiologic role as well as their anatomy. Rather, it is through the penetrating venules (44) where the leukocytes first pass through the endothelium and vascular lamina of the venules. Penetrating arterioles presumably do not participate in the trafficking of inflammatory cells because their anatomic specialization of smooth muscle cells for regulation of blood flow. The concept that there is an immunologic BBB at the penetrating venule level that is distinct from the physiological one reflects the physiological specialization of the cerebral microcirculation (44). Passage of inflammatory cells laden with necrotic debris back into the vascular space must also occur at the penetrating venule locus. Although leukocytes might penetrate into the brain parenchyma through a damaged EGLM in some disease states such as infections, it is unclear whether that occurs in neurodegenerative diseases.

Thus, the V-RC acts as a buffer between the bloodborne immune surveillance system and the immunologically isolated cortical parenchyma. Macrophages in the neuropil itself might pass back through the EGLM and into the V-RC to transport their ingested debris out of the brain (44). That could be a rate-limiting step in the clearance of misfolded proteins and other debris. Alternatively, processes occurring in the V-R, including interactions between necrotic debris, misfolded proteins, and inflammatory cells, could also fail to keep up with the neurodegenerative process. For a long period, the net accumulation of proinflammatory elements in the V-RC as well as within the adjoining neural tissue could trigger additional inflammatory reactions and further neurodegeneration in cortical regions adjacent to the overburdened V-RCs. Unremoved necrotic residues might elicit additional inflammatory responses perpetuating the unresolved original lesions as well as establishing of new ones. Because penetrating venules are less abundant than penetrating arterioles (and presumably because inflammatory cells from the vascular space cannot penetrate to the V-RC surrounding arterioles), clearance of necrotic debris and misfolded proteins may be further complicated by the anatomic relationships of the V-RC to cerebral microvasculature.

**CLINICAL IMPLICATIONS**

The specific and unique intracerebral extrinsic and intrinsic microvascular compartments of the human brain determine its physiology as well as its pathologic responses to injury. The developmental perspective on the cerebral microvascular system highlights the following principles: 

1. The V-RC is intimately related to the cortical penetrating arterioles and venules and is an inefficient protolymphatic system that lacks the anatomic and physiological constituents found in lymphatic beds elsewhere in the body. 
2. The remarkable density of the penetrating arterioles and the extensive pial and intracortical collateral network implies that generalized arteriolar disease of the cerebral cortex will be a diffuse process and not one linked only to macroscopic infarctions. 
3. The anatomic contiguity of the V-RC and the penetrating vascular compartment (arterioles and venules) implies that pathology in one compartment could lead to dysfunction in the other compartment. 
4. The anatomic localization of the immunologic BBB at the level of the penetrating venules might impose constraints on immunologically mediated transport.

An imbalance between production and clearance of β-amyloid is likely to be a key mechanism in the pathogenesis of AD (46, 47). An obvious candidate for the anatomic locus of inefficient clearance of β-amyloid is the V-RC (6, 18, 19-22). Because of absolute or relative overproduction, excess extracellular β-amyloid in the case of AD or other proteins such as tau may accumulate slowly in the V-RC. In individuals with the enabling biochemistry, such as a carrier of the APOE ε4 allele, a pathologic imbalance between production and clearance would cause increasing concentrations of β-amyloid to reside in the V-RC. Indeed, previous studies have shown that the V-RC is the site of interaction between apolipoprotein E and β-amyloid (20, 48). By virtue of its relationship with penetrating arterioles and venules, inflammatory cells have access to the V-RC. Astrocytic and microglial ingestion of excess β-amyloid in AD can be observed in the V-RC, presumably those associated with penetrating venules (49). By serving as both the portal for inflammation and a conduit for soluble degraded peptides, the V-RC acts as a repository for damaged proteins that could, in turn, serve as a nidus for a chronic low-grade inflammatory process. The perivascular predilection of plaque pathology in AD is consistent with this interpretation (8). A similar observation in CTE (7) has led us to speculate on similarities between AD and CTE. The chronic presence of perivascular pathology could represent a process that could long remain dormant before
inducing neurodegeneration that then accelerates autonomously, similar to the protracted pulmonary consequences of tuberculosis (16). A recent hypothetical model of the pathogenesis of AD is based on the observations that brain β-amyloidosis exhibits a very long presymptomatic phase (50).

Arteriosclerotic microvascular disease causes intimal and medial damage to penetrating arterioles resulting in loss of smooth muscle cells and thickening of the vessel walls that can lead to infarcts (1–5). From the developmental anatomic perspective (16, 18, 19), the density of penetrating vessels and of the pial and intracortical plexi would determine the impact of ischemia. Because an individual penetrating arteriole provides blood flow to a radius of only 200 to 300 μm, microinfarcts rather than grossly visible infarcts might be the major consequence of microvascular arteriosclerosis. The importance of microvascular pathophysiological process in late-life cognitive disorders is well recognized (1–5, 51), but the antemortem imaging correlates of microvascular disease, such as white matter hyperintensities and subcortical infarcts (52), may be only modestly correlated with involvement of the penetrating arterioles of the cerebral cortex. Cortical atrophy might be a better marker (53, 54), but it is nonspecific.

Although reduced access to oxygen and nutrients would be expected with pathology in the penetrating arterioles, the anatomic contiguity between the cortical vascular system and the prelymphatic system suggests that arteriolar disease might have broader consequences. The mechanistic relationship between atherosclerotic/arteriosclerotic cerebrovascular disease and AD pathophysiology and whether they are completely separate processes or are somehow related is an open question. Several reviews highlight different hypotheses about how cerebrovascular pathology and AD pathology might interact (2, 6, 9, 10, 22, 47). The effects of hypoxia on amyloid precursor protein cleavage or tau phosphorylation have also been advanced to account for the relationship (10). Some, but not all, clinicopathologic and epidemiological studies have suggested that vascular risk factors increase AD pathology independent of cerebrovascular pathology (55).

The developmental perspective suggests that the anatomic contiguity of the V-RC and the penetrating arterioles of the cortex is an important part of the relationship between cerebrovascular disease and the pathology of AD. As we have shown, the V-RC exists wherever there are penetrating microvessels in the cerebral cortex. It has been postulated that flow through the V-RC could be retarded or diminished if the penetrating arterioles become hyalinized or denuded of smooth muscle cells (21, 22). Either by reduction of the physical capacity of the V-RC due to arteriolar wall enlargement or by the diminished pumping action of damaged arterioles, drainage functions of the V-RC would be compromised by arteriolar pathology. In addition, endothelial cell damage could allow the passage of serum proteins into the perivascular spaces (48), thereby further interfering with the V-RC bulk flow capability. The anatomic pairing of the penetrating arterioles and venules with the V-RC suggests that it is likely that microvascular and neurodegenerative processes interact.

By analogy, the extent to which the pathology of CTE is perivascular (7) suggests that one possible mechanism in its pathogenesis involves the transport of one or more damaged peptides in the V-RC. Whether the protein is β-amyloid, α-synuclein, tau, or TAR DNA-binding protein-43 (or some other as yet unidentified protein) is unclear. Because β-amyloid is released in interstitial fluids after traumatic brain injury, and its route of clearance involves the V-RC, it may be that multiple instances of acceleration-deceleration injuries to the cerebral cortex with associated overproduction of β-amyloid eventually lead to dysfunctional transport in the V-RC. That APOE ε4 genotype also plays a role in risk for CTE (56) suggests that transport of β-amyloid in the V-RC also plays a role in this condition.

Our observations suggest several insights into the processes involved in the pathophysiology of AD and CTE. Evaluation of the interactions between the inflammatory process and neuronal cell death (57) must also include consideration of how necrotic debris and misfolded proteins are transported out of the cerebral cortex. Why is it that other neurodegenerative diseases do not exhibit a perivascular pattern to their pathology? Are there differences in how those proteins interact with others in the V-RC? Are there differences with how inflammatory cells interact with prion protein, hyperphosphorylated tau proteins, or α-synuclein compared with β-amyloid? To the extent that late-onset sporadic AD often occurs in the setting of coexistent cerebrovascular disease (54, 58), is the presence of disease of the penetrating arterioles somehow necessary for the expression of AD-related neurodegeneration? Given the selective anatomic distribution of AD pathology, might there be differences in the anatomy of the cerebral microcirculation or their V-RCs between the AD signature regions, such as posterior cingulate or lateral parietal regions (59), and areas such as primary motor and sensory cortices that are typically the least affected by neurodegeneration in AD? How does the pial capillary plexus change with aging and might it play a role in later-life brain diseases? The insights from the perspective of the development of the cerebral microvasculature suggest a number of new avenues of investigation.

REFERENCES
