

The Blood-Brain Barrier in Neuroinflammatory Diseases

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I. The Blood-Brain Barrier

The existence of the blood-brain barrier (BBB)^b was revealed through studies by Ehrlich (1885) in the late 19th century, describing that brain tissue remained unstained after injection of a vital dye into the systemic blood circulation of rats. In contrast, the brain tissue was stained after direct injection of trypan blue into the brain ventricular system, indicating the existence of some kind of barrier at the site of the brain microvessels (Goldmann, 1909). At first it was generally believed that the BBB was formed by the glial sheets covering the brain capillaries (Dempsey and Wislocki, 1955). Administration of the electron-dense

marker horseradish peroxidase (HRP), however, revealed that the anatomical localization of the BBB could in fact be detected at the level of the cerebral endothelial cells (CEC). No HRP was found in the extracellular space surrounding the brain capillaries after intravenous injection. When administered directly into the brain ventricular system, HRP passed astrocytic end processes readily and was retained at the cerebral endothelial plasma membrane (Reese and Karnovsky, 1967; Brightman and Reese, 1969).

The homeostasis of the central nervous system (CNS) environment is maintained by the BBB, which separates the brain from the systemic blood circulation. The cerebral capillaries are organized such that the brain is protected from blood-borne compounds, since a strict homeostasis of the neuronal environment and an intact barrier are essential for optimal brain functioning. However, during various neurological diseases the permeability of the BBB may be changed. This review will discuss the role of the BBB and especially of the CEC in various neuroinflammatory diseases.

A. Morphology and Function

The BBB is formed by a complex cellular system of endothelial cells, astroglia, pericytes, perivascular macrophages, and a basal lamina (Bradbury, 1985). Astro-

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^b Abbreviations: BBB, blood-brain barrier; HRP, horseradish peroxidase; CEC, cerebral endothelial cells; CNS, central nervous system; Pgp, P-glycoprotein; MDR, multidrug resistance; CSF, cerebrospinal fluid; MS, multiple sclerosis; TNF, tumor necrosis factor; IL, interleukin; AA, arachidonic acid; PG, prostaglandin; ROS, reactive oxygen species; NO, nitric oxide; NOS, NO synthetase; cNOS, constitutive NOS; iNOS, inducible NOS; Ig, immunoglobulin; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; LPS, lipopolysaccharide; IFN, interferon; A4P, amyloid peptide precursor; AIDS, acquired immune deficiency syndrome; HIV, human immunodeficiency virus.

cytes project their end feet tightly to the CEC, influencing and conserving the barrier function of these cells. CEC are embedded in the basal lamina together with pericytes and perivascular macrophages (Graeber et al., 1989). Pericytes are characterized as contractile cells that surround the brain capillaries with long processes, and are believed to play a role in controlling the growth of endothelial cells. Due to their close contact with the endothelial cells, pericytes may influence the integrity of the capillaries and conserve the barrier function. Pericytes additionally limit the transport by the ability to phagocytose compounds which have crossed the endothelial barrier, as observed in a healthy BBB (Broadwell and Salzman, 1981). Finally, the lumen of the cerebral capillaries is covered by CEC in which the functional and morphological basis of the BBB resides (fig. 1).

CEC exhibit various functional and morphological differences in comparison with endothelial cells derived from peripheral organs. CEC possess narrow intercellular tight junctional structures. The tight junctions are composed of a complex of belt-like zonula occludens, which is localized close to the lumen of the capillary (Heimark, 1993). Electrical resistance in vivo across the barrier can increase to approximately $1200 \text{ ohm}\cdot\text{cm}^2$ due to these intercellular structures (Butt et al., 1990). These tight junctions hinder paracellular transport of hydrophilic compounds across the cerebral endothelium. The absence of fenestrae in the endothelial plasma membrane and the presence of high densities of mitochondria in the cytosol are other prominent morphological features of the CEC. In addition, pinocytotic vesicular activity seems to be virtually absent at the endothelial plasma membrane, which implies that fluid phase uptake is limited (Cervos-Navarro et al., 1988).

Recently, P-glycoprotein (Pgp) expression, which appears to be associated with multidrug resistance (MDR) in numerous tumors, has also been detected at the site of

the BBB. The discovery of its presence on the BBB has much contributed to the understanding of the penetration of various drugs into the brain (Cordon-Cardo et al., 1989; Thiebaut et al., 1987). Pgp is a 170-kDa glycoprotein and belongs to the superfamily of the ATP-binding-cassette transporters (Higgins et al., 1986). The system is comprised of two almost identical halves within a total of 12 membrane spanning domains and two ATP-binding sites. In humans the *MDR1* and the *MDR2* genes have been identified, which encode for the two different isotypes of Pgp (Gottesman, 1993). The *MDR1*-Pgp is mainly found in epithelial tissues of the intestine, kidney, pancreas, adrenal gland, and in the endothelium of various tissues like the endocervix, glomeruli, intestine, ovarian cortex, prostate, spleen, testes, and the BBB (Hegmann et al., 1992; Cordon-Cardo et al., 1989). In rodents there are three genes encoding for the *mdr1a*-, the *mdr1b*-, and the *mdr2*-Pgp (Schinkel et al., 1995a). The *mdr1a*- and the *mdr1b*- gene products fulfill the same function as the *MDR1* gene product in humans (Borst and Schinkel, 1996). *MDR2* and *mdr2*, however, do not seem to play an important role in the transport of drugs. It is abundantly expressed in the liver and it may function in the transport of phospholipids across the canicular membranes in hepatocytes into the bile (Smit et al., 1993).

MDR1- and the *mdr1a*-Pgp are located at the luminal side of the cerebral endothelium and function as an efflux pump for several drugs. In particular cytostatic drugs such as anthracyclines, taxanes, epipodophyltoxins, and vinca alkaloids (Gottesman, 1993) are transported out of the cells. In addition, noncytotoxic drugs such as ivermectin, digoxin, cyclosporin A, dexamethasone (Schinkel et al., 1995a; Mayer et al., 1996), also other drugs such as domperidone, ondansetron, and loperamide (Schinkel et al., 1995b) are effluxed by this system. *MDR1*- and *mdr1a*-Pgp-mediated transport can

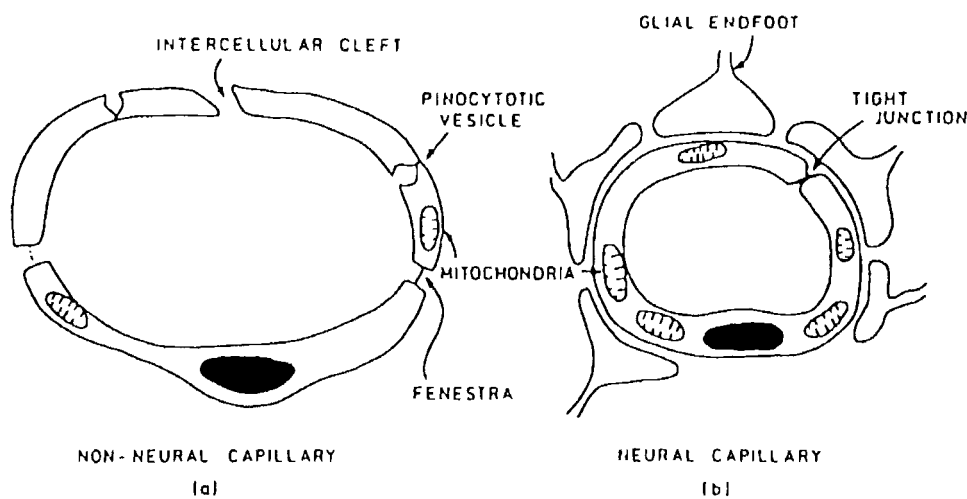


FIG. 1. Schematic representation of the morphological characteristics of neural (a) and non-neural (b) capillaries. Neural capillaries differ from non-neural ones by the presence of narrow tight junctions, paucity of pinocytotic activity, absence of fenestrae, and the relative high density of mitochondria. Neural endothelial cells are surrounded by a continuous basal lamina on which astrocytes oppose their end feet.

be inhibited by so-called reversal agents such as verapamil (particularly R-verapamil), cyclosporin A, SDZ PCS 833, but also by small peptides (Gottesman, 1993; Ford, 1996). In vivo, microdialysis studies showed considerably higher rhodamine concentrations in the brain of *mdr1a*-deficient mice in comparison to wild type mice. Normally *mdr1b-Pgp* is not detectable in vivo at the level of the BBB (Schinkel et al., 1995a). However, in BBB cell cultures, the expression of the *mdr1b-Pgp* has been demonstrated, indicating that changed circumstances induced by culture conditions may induce its expression (Barrand et al., 1995).

Certain enzymes which reside selectively in the CEC constitute a metabolic barrier, which also contributes to the protective function of the BBB. For instance, enzymes like monoamine oxidase A and B, catechol *O*-methyltransferase, or pseudocholinesterase are involved in the degradation of neurotransmitters present in the CNS. In addition, blood-borne compounds that have entered the CEC can be degraded by enzymatic activity (Maxwell et al., 1987; Baranczyk-Kuzma et al., 1986; Kalaria and Harik, 1987; Betz and Goldstein, 1984).

In more than 99% of the brain capillaries, a BBB is present, but in some areas of the brain a blood-cerebrospinal fluid (CSF) barrier can be found instead. This barrier is present in the circumventricular organs such as the median eminence, pituitary, choroid plexus, subfornical organ, organum vasculosum of the lamina terminalis and the area postrema (Hashimoto, 1992). The blood-CSF barrier is not as strict as the BBB but it also prevents the entrance of blood-borne compounds into the brain. Since the surface of the BBB is about 5000-fold larger than that of the blood-CSF barrier, the main route of entry for compounds from plasma into the brain is via the brain capillaries (Pardridge, 1986).

B. Mechanisms of Transport of Compounds Across the Blood-Brain Barrier

The presence of the BBB has major implications for the passage of relatively large and hydrophilic compounds into the brain. As a result the entry of certain endogenous compounds such as nutrients is restricted. Essential nutrients are transported into the brain by means of (selective) carrier mechanisms. Several transport systems have been characterized varying from passive transport (such as diffusion) to active and energy requiring processes.

The diffusion of compounds across the plasma membranes of the endothelial cells of the BBB is dependent on the physicochemical properties of these compounds, such as lipid solubility, molecular weight, electrical charge, or extent of ionization. Rapoport et al. (1979) described the correlation between diffusion across the BBB and lipid solubility of compounds. Lipid soluble substances penetrated the cerebral endothelial plasma membranes readily and also equilibrated easily between blood and brain tissue (Bradbury, 1985). In vitro studies

revealed also close correlation of the lipid solubility of compounds and their BBB permeability (Van Bree et al., 1988). In contrast to these observations, compounds which are a substrate for Pgp are less efficiently transported across the BBB as would be expected on the basis of their lipophilicity as discussed before (section A).

Specific carrier systems mediating active transport of certain compounds into the brain have been identified. A selective stereospecific glucose carrier system (GLUT-1 within the sodium-independent glucose transporter supergene family) has been characterized transporting 2-deoxyglucose, 3-*O*-methylglucose, mannose, galactose, and glucose with a high capacity. GLUT-1 (55 kDa) was expressed asymmetrically both at the abluminal and luminal membrane of the CEC (Farrell and Pardridge, 1991). Three high affinity amino acid carrier systems have been described for large neutral amino acids (LNAA system), for basic amino acids and for acidic amino acids, respectively. Furthermore, carrier systems for purine, nucleoside, thiamine, monocarboxylic acid, and thyroid hormones have been identified (Pardridge, 1986; Spector, 1990). Thus, a functional barrier is of great importance for the maintenance of a constant environment of the CNS and for its optimal functioning.

II. Pathophysiology of the Blood-Brain Barrier in Neurological Diseases

Under healthy conditions, the BBB not only regulates the entry of drugs or endogenous compounds into the brain, but also cellular infiltration is lower compared to peripheral organs. The normal endothelial cell layer provides a thromboresistant surface that prevents platelet and leukocyte adhesion and activation of any coagulation system. The highly specialized CEC form a tight barrier which isolates the brain from immune surveillance, and allow only a few mononuclear cells (such as activated T-cells) to migrate into the CNS. The low expression of major histocompatibility complex antigens, the low number of antigen-presenting cells in the healthy CNS, and the fact that the CNS is not properly drained by a fully developed lymphatic vasculature, make the brain an "immunosecluded" site (Hafler and Weiner, 1987; Wekerle et al., 1986).

However, when inflammation occurs, an extensive leukocyte migration into the brain takes place, for instance during multiple sclerosis (MS) or encephalitis (Andersson et al., 1992; Lassmann et al., 1991). The migration of mononuclear cells into the CNS is often accompanied by an increased flux of serum proteins which are transferred to the CSF. Besides the CEC, other cell types such as microglial cells and perivascular macrophages may eventually be involved in the neuro-immune response.

The barrier function of the BBB can change dramatically during various diseases of the CNS i.e., during hypertension or seizures, or during cerebral inflammation such as MS or cerebral infections. Enhanced BBB

permeability is considered to be the result of either opening of tight junctions or of enhanced pinocytotic activity and the formation of transendothelial channels (Juhler et al., 1985)

The BBB itself may play an active role in the mediation of the neuroimmune response either by production of inflammatory mediators or by the expression of adhesion molecules. Various BBB-related factors involved in the development of CNS inflammatory diseases will be discussed in the following sections.

A. Inflammatory Mediators during Neurological Diseases

1. Cytokines. An early step in inflammation is the secretion of various mediators. Cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6) are of crucial importance in the development of the inflammatory response. Cells in the CNS that can produce cytokines upon activation include macrophages, microglial cells, astrocytes, and CEC. The cytokines TNF, IL-1 β , and IL-6 are predominantly present in the CNS after injury or inflammation. These cytokines play a major role in mediating the pathogenesis of a fever response (Hashimoto et al., 1991), in the host defense response (Beutler and Cerami, 1988), activation of the hypothalamus-pituitary-adrenal axis, and they may trigger the release of other cytokines in the CNS.

Cytokines may influence transport of compounds into the brain by opening the BBB. In vitro studies revealed that administration of TNF, IL-1, and IL-6 to monolayers of endothelial cells leads to an increase in the permeability (De Vries et al., 1996). Administration of TNF- α to an in vitro model for the BBB resulted in enhanced transport of inulin and sucrose, which was accompanied by the reorganization of actin filaments (Deli et al., 1996). Intracisternal administration of TNF in newborn piglets resulted in a constriction of the cerebral arteries and a dose- and time-dependent increase in the brain uptake of marker compound (Megyeri et al., 1992). Moreover, TNF administration (intracerebroventricular) to rats resulted in an increased number of white blood cells in the CSF and enhanced levels of radiolabeled albumin, indicating BBB disruption (Kim et al., 1992).

IL-1 β , the predominant form of IL-1 in CNS tissue, can be present not only after local synthesis by astrocytes or microglia but also after transport from the peripheral blood into the brain tissue (Banks et al., 1991). Production of IL-1 by CEC in a damaged BBB has also been reported (Plata-Salamán, 1991) and the permeability of the BBB is affected by IL-1. Intracisternal administration of IL-1 β to rats revealed an increase of BBB permeability to radiolabeled albumin with a peak concentration in the CSF after 3 h of inoculation. The alteration in BBB permeability was dose-dependent and could be inhibited by pretreatment of animals with antibodies directed against IL-1 β (Quagliarello et al.,

1991). Receptors for IL-1 β were detected in the cerebral vasculature (Ericsson et al., 1995) and in vitro on rat brain endothelial cells, which appear to be functionally coupled to the release of IL-6 and eicosanoids (Van Dam et al., 1996).

Hence, the production of cytokines by cells of the BBB, such as microglial cells, astrocytes, and endothelial cells contribute to the total inflammatory response of the CNS after injury or infection and also affect the function of the BBB.

2. Eicosanoids. The derivatives of arachidonic acid (AA), called eicosanoids, and their metabolism play an important role in the mediation of the inflammatory response and the pathogenesis of fever. AA is released from phospholipids present in cell membranes by the activation of phospholipase A₂. AA can be converted by two different enzymes. Through the cyclo-oxygenase pathway, AA is metabolized into prostaglandins (PGs) such as PGD₂, PGE₂, PGF_{1 α} (prostacyclin; PGI₂) and thromboxane A₂. Through the lipoxygenase pathway, AA is converted initially into the mono- or dihydroxyeicosatetraenoic acids and leukotrienes, lipoxins, and the peptidoleukotrienes (Shimizu and Wolfe, 1990) (fig. 2).

Eicosanoids are biologically very active compounds. The PGs and thromboxane B, for instance, induce vasoconstriction (Moncada et al., 1985) and the hydroxyeicosatetraenoic acids can act as chemotactics on leukocytes (Piper and Samhoun, 1987). Predominantly PGE₂ and PGI₂ are involved at the site of the inflammation and both are secreted by inflamed tissue and by vascular endothelium (Moncada et al., 1985).

During brain injury, eicosanoids play an important role in the pathogenesis of CNS inflammatory diseases. In ischemic human brain, for instance, high levels of PGE₂, PGI₂, and PGF_{2 α} have been detected. Increased

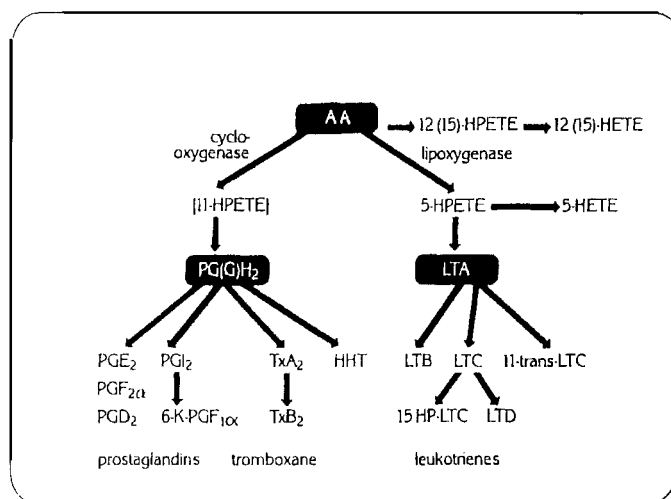


FIG. 2. Formation of cyclooxygenase and lipoxygenase products (eicosanoids) from arachidonic acid. PG, prostaglandin; LT, leukotrienes; HHT, hydroxyheptadecatetraenoic acid; Tx, thromboxane; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid.

levels of PGs were also found in the CSF of patients suffering from suspected intracranial disease (Saeed Abdel-Halim et al., 1980). Augmented levels of PGs could either be produced in the CNS itself or by entry into the CNS, as was shown for PGE₂ (Dascombe and Milton, 1979). One possible site of PG synthesis is the CEC, since these cells have been shown to produce PGE₂ and PGI₂ when exposed to traces of AA (Moore et al., 1988; De Vries et al., 1995).

Synthesis and secretion of eicosanoids in the brain during cerebral inflammatory diseases denotes the importance of these mediators, besides cytokines, in modulation of the function of the BBB in such diseases. In addition, the cerebral endothelium itself may contribute to the total pool of PGs produced in the CNS after injury.

3. Free radicals. Upon activation, cells of the immune system can produce a range of free radicals, such as reactive oxygen species (ROS) or nitric oxide (NO), which can contribute to tissue damage. Free radicals are defined as ions with an electron that possess unusual chemical reactivity, including an ability to alter and to fragment membrane lipids (Fishman and Chan, 1980). In healthy conditions, the constantly produced oxygen-derived free radicals are scavenged by endogenous antioxidants such as, e.g. superoxide dismutase and glutathione peroxidase. During pathological conditions, such as ischemia and inflammation, however, this defense mechanism is perturbed and results in the overproduction of oxygen-derived free radicals (Chan et al., 1991).

ROS such as superoxide or hydrogen peroxide are reactive molecules that can interact and change the properties of other molecules. The respiratory burst of cells of the immune system upon activation leads to the reduction of oxygen to superoxide, followed by the formation of other ROS. ROS can cause considerable damage to the membrane lipids in the CNS. The polyunsaturated fatty acids after reacting with ROS can become peroxidated, destroying the structure of myelin and cell membranes. The integrity of the BBB can also be threatened by exposure of the endothelial cells to ROS (Griot et al., 1990; Kim and Kim, 1991).

Another free radical that can damage the BBB is NO. NO is synthesized in the presence of nitric oxide synthetase (NOS) out of the guanidine group of L-arginine. Two isoforms of NOS are known, the constitutive form (cNOS) and its inducible (iNOS) form, which can be induced by various cytokines. Endothelial cells of the BBB are known to possess the inducible form of NOS. Inflammatory mediators released in the CNS during viral or bacterial infections are able to induce iNOS, present in endothelial cells, astrocytes and brain macrophages (Feinstein et al., 1994; Morin and Stanboli, 1994). Studies on BBB opening during infections revealed the involvement of NO in this process. Increased permeability of the BBB, after endotoxin administration to rats, could be blocked in the presence of a inhibitor of NOS, N-nitro-l-arginine methyl ester. On the other hand

the effect could be potentiated after administration of L-arginine a substrate for NOS (Shukla et al., 1995). In addition to other mediators influencing the permeability of the BBB, ROS, and NO generated by BBB endothelial cells, can play an important role in the response to injury or inflammation in the CNS.

4. Adhesion molecules. Within minutes after the release of inflammatory mediators such as cytokines or eicosanoids, neutrophils arrive at the site of inflammation followed by the migration of antigen-specific B- and T-lymphocytes and monocytes into the inflamed site (Osborn, 1990). Three families of homologous adhesion molecules are responsible for the adhesion and migration of leukocytes into an inflamed site: the immunoglobulin (Ig) superfamily, the integrins, and the selectins (Osborn, 1990; Springer, 1990). The immunoglobulin superfamily comprises a large group of molecules, characterized by the presence of one or more Ig homology units (Springer, 1990). On endothelial cells this group is represented by intercellular adhesion molecule-1 (ICAM-1), intercellular adhesion molecule-2 (ICAM-2), and vascular cell adhesion molecule-1 (VCAM-1). These molecules recognize their leukocytic ligand and permit adhesion and migration of these cells out of the bloodstream (Osborn, 1990; Springer, 1990) (Table 1).

TABLE 1

Adhesion molecules involved in leukocyte-endothelium interactions

	Family	Ligand	Expressed on
CD11a/CD18 (LFA-1)	integrins	ICAM-1, ICAM-2	all leukocytes
CD11b/CD18 (Mac-1)	integrins	ICAM-1, iC3b, fibrinogen	monocytes, neutrophils, lymphocytes
CD11c/CD18 (p150,95)	integrins	iC3b	monocytes, neutrophils
VLA-4	integrins	VCAM-1, fibronectin	all leukocytes, except neutrophils
ICAM-1	Ig superfamily	CD11a/CD18 CD11b/CD18	various, including endothelial cells
ICAM-2	Ig superfamily	CD11a/CD18	endothelial cells
VCAM-1	Ig superfamily	VLA-4	activated endothelial cells
E-selectin (ELAM-1)	selectins	Sialyl-Le ^x	activated endothelial cells
P-selectin (GMP-140)	selectins	carbohydrate	degranulated endothelial cells and platelets
L-selectin (LAM-1)	selectins	carbohydrate	monocytes, neutrophils, lymphocytes

Brain endothelial cells are capable of expressing several adhesion molecules. For instance, ICAM-1 expression on cultured brain microvascular endothelial cells could be up-regulated time and dose dependently by the bacterial endotoxin lipopolysaccharide (LPS), interferon- γ (IFN- γ), TNF- α , and IL-1 (Fabry et al., 1992). Migration of lymphocytes across monolayers of CEC could be observed after treatment of the CEC either with IFN- γ or TNF- α . The migration of lymphocytes could subsequently be suppressed by antibodies directed against lymphocyte functional antigen-1 (LFA-1), indicating the relevance of the LFA-1/ICAM-1 pathway (Male et al., 1992). In addition, the involvement of very late antigen-4 (VLA-4)/VCAM-1 pathway in the adhesion of lymphocytes to CEC in vitro stimulated with LPS, IL-1, and IL-6 was also demonstrated (De Vries et al., 1994). Other studies illustrated that the barrier function of the cerebral endothelium is decreased during an inflammatory challenge and after the adhesion of lymphocytes (Huynh and Dorovini-Zis, 1993).

The promotion of leukocyte adhesion to the cerebral endothelium may be mediated through the LFA-1/ICAM-1 as well as the VLA-4/VCAM-1 pathway during cerebral inflammatory diseases. These adhesion molecules are of importance for enabling the migration of lymphocytes across the BBB to battle the inflammatory process inside the brain tissue after injury.

B. Neurological Diseases Affecting the Blood-Brain Barrier

1. Multiple sclerosis and experimental allergic encephalomyelitis. MS is an autoimmune disorder directed toward demyelination of the CNS. The myelin sheaths, which normally surround the axons of the neuronal cells, are digested, and subsequently the conductive properties of the neurons are reduced distinctly. MS manifests itself in relapsing and remitting periods of illness. Early symptoms and signs are weakness and numbness in one or more of the limbs associated with tingling of the extremities. When the disease has settled, neurological disorders of the brainstem, optical nerve, cerebellum, and spinal cord and dysfunctioning of memory and attention become apparent (Adams and Victor, 1989).

The pathology of the disease is characterized by the presence of multiple lesions restricted to white matter of the CNS tissue, and primarily the brain capillaries located in the periventricular areas are affected. Lesions expose characteristics such as the formation of brain edema adjacent to the lesions, perivascular mononuclear infiltration, and the presence of myelin debris inside the macrophages. The vessel wall of the cerebral capillaries is damaged, which coincides with a disposition of complement and immune complexes and an increased number of activated macrophages in the capillaries. In time, oligodendrocytes and myelin disappear from the site of

the lesion and diffuse gliosis develops (Gay and Esiri, 1991; Morganti-Kossmann et al., 1992).

Inflammatory mediators play an active part in the mediation of the immune response during MS or in its corresponding animal model experimental allergic encephalomyelitis (Sharief et al., 1993a, b). IFN- γ , for instance, has been identified at the site of the lesions in the CNS tissue. It is suggested to be involved in the lesion growth during MS and may exert local immunosuppressive effects. Also TNF has been identified at the site of the lesions, and is likely to mediate cytotoxic effects on the oligodendrocytes which produce myelin (Sharief et al., 1993a, b). Elevated levels of cytokines like IL-1, IL-6, and TNF are observed in the CSF of MS patients (Sharief et al., 1993a, b; Gallo et al., 1989; Shaw et al., 1995) and also in CNS tissue of mice suffering from experimental allergic encephalomyelitis (Gijbels et al., 1990).

CEC are involved in the encephalitic processes during experimental allergic encephalomyelitis. They attract autoantigen-specific T-cells migrating into the brain (Raine, 1990). During experimental allergic encephalomyelitis, the expression of the adhesion molecule ICAM-1 was 6-fold higher on the CEC, perivascular cells, and some of the astrocytes than in controls. During remission, the level of ICAM-1 expression was similar to that before the onset of the clinical signs (Cannella et al., 1990). Wilcox et al. (1990) also reported an up-regulation of ICAM-1 on cerebral endothelia of the guinea pig suffering from acute experimental allergic encephalomyelitis. Furthermore, traces of soluble ICAM-1 in the CSF of patients with active MS were found, which correlated with BBB damage (Gijbels et al., 1990; Hartung et al., 1993). It was suggested that in addition to ICAM-1 other adhesion molecules are involved in these adhesion processes. The high expression of VCAM-1 on microvessels isolated from CNS tissue from MS patients was shown recently in addition to the presence of ICAM-1 (Washington et al., 1994).

During experimental allergic encephalomyelitis the integrity of the BBB itself is reduced (Hawkins et al., 1991). Transport studies using radiolabeled mannitol as a marker for the transport of compounds across the BBB revealed a 2-fold increase of transport at day 14 post inoculation, when the clinical signs of experimental allergic encephalomyelitis were most abundant (Daniel et al., 1983). Using several markers for the transport of compounds across the BBB, Juhler (1988) demonstrated enhanced transport of either radiolabeled sodium, chloride, sucrose, or inulin in all regions. Changes in BBB permeability preceded the occurrence of the clinical signs (Claudio et al., 1989). Transport of radiolabeled albumin and IgG was enhanced in the caudal region and increased transport occurred in the more cranial regions when the clinical signs became more severe. It was demonstrated that the BBB disruptions were near regions which are called "leaky areas," such as the entry zone of

the trigeminal nerve and the spinal roots, area postrema and choroid plexus (Claudio et al., 1989).

Changes in BBB permeability and the loss of BBB function may either be generated by the formation of pores in the CEC, or by an increase in the number of multivesicular bodies and transcytotic vesicles in the CEC. Claudio et al. (1989) demonstrated enhanced vesicular transport and a decrease in the number of mitochondria in the CEC during experimental allergic encephalomyelitis. Disruption of tight junctions may also occur, possibly induced by fibrinolysis mediated by endothelial derived plasminogen activator and by production of fibrin-fibrinogen products (Koh et al., 1992).

The current opinion on MS is that the BBB together with active inflammatory processes at the lesion sites, leading to enhanced BBB permeability, plays an important role in the development and progression of the disease (Moor et al., 1994; Poser, 1993). Long-standing MS lesions contain a permanent damaged BBB, detected by serum proteins leakage (Claudio et al., 1996). The continuous exposure of demyelinated areas to blood-borne compounds may have a negative influence on the oligodendrocyte regeneration and thus prevent remyelination of these areas (Kwon and Prineas, 1994). The opening of the BBB itself may also reinforce the progression of the disease.

2. *Bacterial meningitis.* A disease of bacterial origin can also affect the integrity of the BBB. Certain bacteria can cross the BBB and penetrate the CNS tissue and the CSF where they multiply and cause bacterial meningitis. The three most frequent causative bacterial species are *Hemophilus influenza*, *Necisseria meningitidis*, also called meningococcus, and *Streptococcus pneumonia* (Tuomanen et al., 1985).

Bacterial meningitis starts with nasopharyngeal colonization and invasion of bacteria, followed by CNS invasion. Subsequently, a generic intense subarachnoid space inflammation process can develop, leading to the release of various cytokines that induce inflammatory reactions. Consequences of bacterial meningitis are the formation of brain edema, increased intracranial pressure and alterations of cerebral blood flow. Once typical meningeal pathogens have crossed the BBB, host defense mechanisms in the subarachnoid space are inadequate to control the infection (Tunkel and Scheld, 1993).

In animals with experimental meningitis, it was shown that the bacteria multiply in the CSF, and then at sufficiently high concentration, fever and inflammation develop, accompanied by the release of cytokines, predominantly IL- β and TNF- α (Jacobs and Tabor, 1990). There upon, an increase in the number of white blood cells migrating through the BBB into the CNS tissue was observed. These events may lead to degeneration of neurons and permanent damage of the visual and hearing system, even after treatment with penicillin (Tuomanen, 1993).

During bacterial meningitis the permeability of the BBB may be altered (Tuomanen et al., 1985). Morphological changes, observed in animals after intracisternal administration of bacterial endotoxin, LPS, as a model for bacterial meningitis, revealed an enhanced pinocytotic activity in the CEC. A progressive opening of tight junctions was observed from 4 to 18 h after inoculation, which resulted in enhanced transport of radiolabeled albumin into the CSF. Time-dependent effect of LPS on BBB permeability was postulated to be partly mediated by cytokines, released after intracerebroventricular inoculation of LPS (Wispeley et al., 1988).

3. *Ischemia.* During and after stroke or cerebral injury, ischemic processes will occur in the brain due to insufficient blood circulation. Stroke is a form of cardiovascular disease that affects the cerebral arteries. Cerebral thrombosis and cerebral embolism are the most common types of stroke, caused by clots that plug an artery. Cerebral and subarachnoid hemorrhage can also appear, caused by ruptured arteries. Stroke may damage the neurons and lead to the loss of speech, memory, or motility, and may change behavior dramatically. During ischemia an overproduction of free radicals, such as superoxide and NO has been observed, functioning as mediators in the ischemic process. The release of these factors in the CNS can lead to cellular injury of glia or neurons by membrane disruption and increasing regional cerebral blood flow (Chan et al., 1984; Koide et al., 1986; Pfister et al., 1990; Ikeda et al., 1989; Chan et al., 1991).

After an ischemic seizure, damage to the BBB occurs and may be followed by an opening, as revealed by leakage of serum proteins into the ischemic brain tissue. Occlusion of the cerebral artery in the rat forebrain revealed an increased permeability of the BBB for sucrose (Preston et al., 1993) and HRP (Pluta et al., 1994; Tanno et al., 1992). Ischemic sites remain permeable to HRP up to 72 h after induction of the injury.

The opening of the BBB itself will probably not contribute to the progressive damage observed after the ischemic event, although injury to the cerebral microvasculature may lead to the formation of brain edema (Preston et al., 1993). However, increased infiltration of monocytes and macrophages into damaged regions can lead to perivascular inflammatory reactions, in which the BBB may play a role by production of chemotactic substances. The increase in BBB permeability after an ischemic event could be significantly inhibited by superoxide dismutase and catalase, acting as scavengers for superoxide. These observations indicate that superoxide, released in the CNS after an ischemic event, may influence the BBB permeability (Nelson, 1992). In addition, the BBB can contribute to ischemic damage by the production of NO and the up-regulation of adhesion molecules responsible for platelet aggregation as observed in the ischemic site (Said et al., 1993).

4. *Brain edema.* Brain edema can be classified into two different types on the basis of morphological characteristics: (1) Vasogenic or “wet” edema, the result of an increased BBB permeability, and (2) cytotoxic or “dry” edema, the result of the actual swelling of the cells of the brain parenchyma (Klatzo, 1967). Vasogenic edema is the type of edema most often present in the brain after injury, induced by ischemic stroke, brain tumors or inflammatory lesions. The BBB expresses morphological changes during the onset of vasogenic brain edema, such as the opening of tight junctions and a damaged endothelial cell membrane, followed by migration of leukocytes into the CNS (Klatzo, 1987).

In addition, increased BBB permeability detected during vasogenic brain edema may be the outcome of enhanced pinocytotic activity in the CEC. It has been suggested that intensified vesicular activity is the result of an increase in the serotonin levels surrounding the capillaries. During brain vasogenic edema, the serotonin accumulates within the microvessels. Serotonin may affect the plasma membrane of the CEC and induces the formation of pinocytotic vesicles and the transfer of compounds across the BBB (Westergaard, 1980). Histamine may also contribute to the change of pinocytotic activity in the CEC. Joó (1990) assumed that after brain damage excessive amounts of histamine are released from damaged histaminergic nerves and mast cells. Released histamine may activate the histamine (H_2) receptor on the CEC, which is coupled to adenylate cyclase. The rise in intracellular cyclic AMP may result in an increase in the number of pinocytotic vesicles. In addition to cyclic AMP, other second messengers such as cyclic GMP and AA may be involved in augmented pinocytotic activity (Joó, 1990; Joó and Klatzo, 1989; Wahl et al., 1988; Michiels et al., 1993). Other mediators in the formation of vasogenic edema may be leukotrienes (Baba et al., 1992) and NO (Nagafuji et al., 1992). The synthesis of secondary messengers by the CEC may contribute to the local changes of the BBB permeability as observed in vasogenic brain edema (Joó, 1990).

Cytotoxic brain edema is the most prominent clinical disorder after ischemic processes in the CNS and is characterized by an increase in the water content of the cells of the CNS, which may be caused by a disturbance in the transport systems for potassium and sodium rather than from changes in the permeability of the BBB.

5. *Alzheimer's disease.* Alzheimer's disease is a chronic neurodegenerative condition that affects approximately 10% of the individuals in age over 65 years. Five percent of this group of patients suffers from severe dementia. Memory decline as well as the inability to absorb new information are the most prominent clinical signs. Imaging techniques revealed disturbances in cerebral blood flow and glucose metabolism. Until now a deficiency in the neurotransmitter acetylcholine has been hypothesized to be involved in Alzheimer's disease, since

cortical acetylcholine synthesis is markedly diminished in Alzheimer's patients. Other neurotransmitters which may be involved in the course of the disease are dopamine, γ -aminobutyric acid, vasoactive intestinal peptide, and glutamate (Struble et al., 1985).

The pathology of Alzheimer's disease is characterized by the extracellular deposition of a particular form of β -amyloid protein, derived from a larger precursor protein. This amyloid peptide induces the release of the so-called β -peptide or the A4 peptide, which becomes stacked into a β -pleated sheet structure with a high degree of intermolecular hydrogen bonding. Usually, the amyloid peptide precursor (A4P) with an apparent molecular mass of 112 kDa is found in the brain (Masters et al., 1985). During Alzheimer's disease, the A4P is processed abnormally into a 43-amino acid amyloiditic peptide that arises from the near C terminus of the A4P (Marotta et al., 1992). Pathology of Alzheimer brains reveals granulovacuolar degeneration of neurons within the region of the hippocampus. Furthermore, neurofibrillary tangles are detected which are intraneuronal accumulations of dense non-membrane bound fibrillary material forming paired helices. Other lesions are composed of amyloid, predominantly associated with microglial cells (Cras et al., 1990; Marotta et al., 1992).

Inflammatory reactions, surrounding the cerebral microvasculature, are often observed during Alzheimer's disease. The number of perivascular macrophages increases and hypertrophy of astrocytes and microglia is observed in brain sections of the Alzheimer's disease dementia patients. IL-1 is found in the CNS of patients suffering from Alzheimer's disease, and its synthesis is postulated as an early event in the onset of Alzheimer's disease. IL-1 affects the synthesis of β -amyloid precursor protein and its subsequent deposition of β -amyloid (Glenner and Wong, 1984). ICAM-1 accumulates in the brain of Alzheimer's disease patients in the microvascular endothelial cells as well as in the senile plaque (Verbeek et al., 1994), indicating that infiltration of lymphocytes into the brain tissue is involved in inflammatory reactions occurring during Alzheimer's disease.

Brain capillaries themselves are also affected in the course of Alzheimer's disease. Evident is the cerebral microvascular angiopathy located at the level of the brain capillaries (Vinters et al., 1990). Vascular amyloid surrounds the microvessels of the Alzheimer's disease brain on the antiluminal surface of the vessels (Vinters et al., 1994). Brain capillaries may degenerate by the deposition of amyloid at the basement membrane. The thickened capillaries reduce vessel elasticity, which may disturb the cerebral blood flow, which eventually influences the transport of compounds into the brain (Vinters, et al., 1994).

Albumin concentrations in the CSF of Alzheimer patients are enhanced significantly at the early onset of Alzheimer's disease and result from an increase in BBB permeability (Mecocci et al., 1991). Two-dimensional gel

electrophoresis showed the presence of a high molecular weight protein, haptoglobin, molecular mass of 13.5 kDa) in the CSF of Alzheimer's disease patients. The presence of this protein was suggested to be the result of an increased penetration of this protein across an impaired BBB (Mattila et al., 1994). Alzheimer's disease patients have a diminished density in mitochondria and features of interendothelial junctions in the CEC, suggesting leakiness of the vessels (Stewart, et al., 1992). The diminished cerebral metabolic rate observed during Alzheimer's disease can be partly explained by a decreased density of glucose transporters (GLUT-1) in the brain capillaries (Harik, 1992). Changes in the intracellular signal transduction in the cerebral microvessels of Alzheimer's disease patients, in comparison with age-matched controls, have also been observed. Protein kinase C activity is strongly diminished in the brain microvessels, which lead to altered protein phosphorylation. Protein kinase C activity during Alzheimer's disease is of importance in the inhibition of the processing of β -amyloid precursor protein into soluble A β protein. The intracellular signaling in the cerebrovasculature may be one of the targets in Alzheimer's disease, and the balance of various second messengers may be disturbed (Grammas et al., 1995).

Perivascular inflammatory reactions during Alzheimer's disease are likely to cause additional changes in the function of the BBB. Recent studies showed that the BBB is indeed disrupted in brains of Alzheimer's disease patients. In addition, cultures of endothelial cells derived from brain capillaries of Alzheimer's disease patients revealed production of the β -amyloid precursor protein. In addition, apolipoprotein E4 which is considered as a risk factor for the development of Alzheimer's disease, was also produced by these cells (Wells et al., 1995). Changes in the perivascular environment were also found, like increased collagen content of the cerebral microvessels (Kalaria and Pax, 1995; Kalaria, 1992). Confirmation of these observations were made by Claudio (1996), who detected in addition to an increased collagen content in the vascular basement membranes also focal necrotic changes in endothelial cells. Furthermore, abnormalities in the microvasculature include profound irregularities in the course of the vessels and the basement membrane, and changes in specific receptors or proteins associated with the cerebral endothelium were reported (Kalaria, 1992).

These studies provide evidence that the function of the BBB is impaired in Alzheimer's disease patients. The disruption of the BBB can lead to an entry of neurotoxic environmental factors into the brain or circulating amyloid. One hypothesis is that BBB opening is the initial insult that may cause Alzheimer's disease, although more research needs to be conducted to confirm this hypothesis. Until then, it can be concluded that opening of the BBB is a characteristic feature of Alzheimer's disease.

6. Acquired immune deficiency syndrome dementia complex. Acquired immune deficiency syndrome (AIDS) severely affects the CNS. About 60% of the patients develop AIDS dementia complex and suffer from neurological dysfunctions (Gulevitch and Wiley, 1991). Neuronal disorders related to the AIDS dementia complex include opportunistic brain infections due to immunodeficiency, neoplasms of the CNS and meningeal infections. Multinucleated cell encephalitis is marked by the presence of infiltrates, consisting of macrophages and multinucleated cells with some lymphocytes and microglia. Cellular infiltrates are predominantly found in the white matter, and infiltration is accompanied by demyelination by macrophages, and finally vacuolization will occur at these sites. This process is most noticeable in the white matter of the thoracic spinal cord with predominant involvement of the posterior and lateral columns (Navia et al., 1986).

Cytokines are assumed to play an active role in the development of the AIDS dementia complex. The levels of the cytokines TNF, IL-6, IL-1 β in the CSF of AIDS dementia complex patients are elevated. The cytokines may be derived from activated mononuclear cells (Liuzzi et al., 1992). In tissue samples and CSF of patients suffering from AIDS dementia complex, increased levels of PGE₂ and thromboxane B₂ were detected (Griffin et al., 1994). Presence of adhesion molecules was also assessed by the detection of traces of soluble VCAM-1 in CSF of animals infected by simian immunodeficiency virus, serving as an animal model for AIDS (Sasseville et al., 1992). Furthermore, Nottet et al. (1996) suggested that soluble factors derived from HIV-infected monocytes are able to induce the expression of the adhesion molecules E-selectin and VCAM on brain microvascular endothelial cells.

The function of the BBB is dramatically changed during the onset of AIDS dementia complex. Lenhardt et al. (1989) examined the role of humorally mediated damage within the CNS of HIV⁺ patients, and observed perivascular inflammation and immunoreactive fibrinogen and immunoglobulin in the cortex and basal ganglia, probably caused by leakage of the BBB. Rhodes (1991) detected leakage of serum proteins across the BBB into the brains of AIDS patients. Local production of cytokines by BBB endothelial cells may cause a disruption of the BBB, leading to increased permeability (Moses and Nelson, 1994).

Enhanced permeability will not only lead to changes in transport of compounds across the BBB during AIDS dementia complex but also facilitate the entry of HIV-1 infected monocytes into the CNS tissue (Hurwitz et al., 1994). Furthermore, due to the enhanced expression of adhesion molecules on the endothelial cells surface, increased infiltration of macrophages or monocytes infected with HIV-1 will be able to enter the CNS which will lead to additional infection of the CNS tissue. The exact role of the BBB in the course of AIDS dementia

complex is not yet known, although perivascular inflammation may play a key role in the development and onset of this complex.

III. Final Considerations

Several pathological conditions of the brain are associated with structural and functional abnormalities of the cerebral microvasculature. In most diseases described an inflammatory process involves cerebral microvasculature, and severe CNS inflammatory diseases affect BBB permeability and its structure. Until now, most attention has been paid to the inflammatory activities of the glial cells during such diseases and the secretion of inflammatory mediators by these cells.

The role of the endothelial cells of the BBB in these processes can, however, not be ignored. Enhanced expression of adhesion molecules on the CEC in vivo during a pathological state facilitates the entry of leukocytes into the cerebral tissue. The possibility that the endothelial cells may secrete inflammatory mediators may indicate an important "gate" function for these cells between the immune system and the brain. In that respect, the BBB endothelial cells may and will contribute both to the onset and the progression of the disease. For instance, the production of enhanced levels of IL-6 and PGE₂ by CEC, suggests that these mediators can be involved in the transmission of the inflammatory signal to other cell types in the brain, such as astrocytes, microglia, pericytes, and perivascular macrophages. The CEC may be the connection of the CNS and the peripheral immune system resulting in the neuro-immune response (fig. 3).

The presence of cytokines in the CSF and the brain has also been described for diseases like Parkinson and schizophrenia. It may be that in the case of these disorders, the production of cytokines influences the permeability of the BBB. Future research may elucidate the specific role of the BBB and in particular that of the CEC in those types of cerebral pathology which are not of an inflammatory origin.

A more passive role for the endothelial cells in the increased transport of compounds across the BBB, either by the opening of tight junctions, or by an increased vesicular transport, may also be of importance for the progression of the disease. Changes of the capillaries may impair nutrition of the parenchyma. The effect of disease on the functioning of the BBB will secondarily affect the cerebral blood flow and the vascular tone in the brain, which also influences transport across the BBB.

The presence of various antigen presenting cells in the CNS after infection of disease may lead to novel therapeutic strategies to fight neuroinflammatory diseases. The use of antibodies directed against adhesion molecules expressed on the brain endothelial cells, could be a possible mechanism to block the infiltration of blood-borne macrophages or encephalitic T-cells into the CNS.

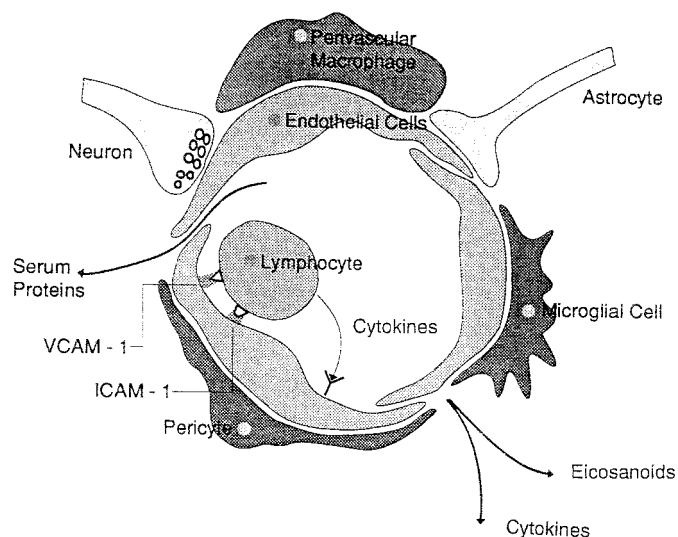


FIG. 3. Hypothetical view on the characteristics of BBB endothelial cells in neurological diseases. The ability of BBB-endothelial cells to secrete inflammatory mediators such as eicosanoids or cytokines in response to an injury of inflammation in the CNS illustrates that these cells play an important role in the pathogenesis of the neuroinflammatory response. In addition, the expression of adhesion molecules during neurological diseases facilitates the entry of leukocytic cells into the CNS. These factors can be involved in the transmission of the inflammatory signal to other cell types of the CNS, such as perivascular macrophages, microglial cells, astrocytes, neurons, and pericytes.

Thus, inhibiting the inflammatory reactions in the cerebrovasculature. For instance, antibodies directed against the very late antigen-4 could efficiently block the clinical signs in the experimental allergic encephalomyelitis model (Yednock et al., 1992). Recently, the Food and Drug Administration has approved the use of IFN- γ as a therapeutic agent for the treatment of ambulant relapsing-remitting MS patients. The mechanism of action may be the influence on macrophage actions, like the expression of glucocorticoid receptors, and down-regulation of cytokine production.

In conclusion, in neuroinflammatory diseases changes at the BBB, like the production of cytokines, free radicals or eicosanoids, and the expression of adhesion molecules at the cell surface, may contribute to the onset and progression of various neuroinflammatory diseases. The suppression of the inflammatory events at the site of the BBB should be further explored as a therapeutic strategy against neuroinflammatory diseases.

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