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Review

Physiology and pathology of the blood-brain barrier: implications for microbial pathogenesis, drug delivery and neurodegenerative disorders

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The blood-brain barrier (BBB) regulates the passage of solutes between the CNS and the blood. The BBB not only restricts the entry of serum proteins into the CNS, but it also controls the passage of nutrients, electrolytes, vitamins, minerals, free fatty acids, peptides, and regulatory proteins in both the brain to blood and blood to brain direction. The BBB performs these functions through a number of saturable and non-saturable mechanisms. For example, efflux (CNS to blood) mechanisms regulate the levels of nutrients and minerals in the CSF, detoxify the CNS, reinforce the impermeability of the BBB against circulating toxins and many drugs, secrete CNS-originating substances into the blood, and drain substances directly into the cervical lymphatic nodes. Influx mechanisms control the homeostatic environment of the CNS, supply the brain with nutrients, and help to integrate CNS and peripheral functions. These mechanisms are altered in and can be the basis for disease and many of these systems are altered in neuroAIDS. We review here examples of several diseases in which the functions of the BBB are altered, and some conditions, such as alcoholism, multiple sclerosis, obesity, and a subtype of mental retardation, where those altered functions may underlie the pathophysiology. Finally, we consider some of the ways in which these aspects of the BBB could be active in neuroAIDS, including the efflux of anti-virals, the transport of virus by adsorptive endocytosis, egress routes for HIV-1 via brain lymphatics, and the release of neurotoxins from brain endothelial cells.

Keywords: HIV; gp120; adsorptive endocytosis; transmembrane diffusion; peptides; aging; neuroaids

Introduction

The blood-brain barrier (BBB) is a monolayer of cells that regulates the passage of solutes between the CNS and the blood. The restrictive properties of the BBB are formidable, essentially equaling that of a continuous cell membrane. However, the BBB is much more than a physical barrier. The endothelial cells that form the barrier at the capillaries, venules, and arterioles and at the epithelial cells that form the barrier at the choroid plexus control the homeostatic environment of the CNS, determine the passage of peptides and regulatory proteins, and govern the entry of metabolic fuels, neurotransmitter precursors, and essential nutrients into the CNS. The cells forming the BBB are also enzymatically active, are a source of cytokines and nitric oxide, and can secrete toxic factors. As such, the BBB is appropriately viewed as a regulatory interface and increasingly found to be a source of disease and a target for therapeutic interventions.

Not surprisingly, each of the diverse aspects of the BBB has been associated with disease, either being altered by disease or producing it. Perhaps what is surprising, is that most of these aspects have an important connection with AIDS. This review will examine the physiology of the BBB, review some of the classic situations in which the BBB is involved in disease states, and examine selected aspects of AIDS BBB pathophysiology.

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A brief history of the BBB

In late 19th century Germany, Paul Erlich and others initiated studies that produced the first evidence for a separation between the circulation and the CNS (Davson, 1967). They found that some dyes given peripherally were able to stain most tissues in the body except for the brain and spinal cord. At first, explanations such as the presence of a very small interstitial fluid space within the CNS or the inability of CNS tissue to be stained by these particular dyes were advanced. Eventually, these explanations were disproved and it became increasingly clear that a hematoencephalic barrier existed. Elegant experiments in 1940s and 1950s by Davson and others characterized many of the physiological aspects of this barrier and found that it existed because of the restriction of serum proteins at the level of the endothelial cell (Davson, 1967). Ultrastructural studies in the 1960s showed that this restriction was due to tight junctions cementing together into a continuous monolayer the endothelial cells comprising the capillaries, and lining the venules and arterioles and the ependymal cells forming the choroid plexus (Reese and Brightman, 1968). Thus, the plasma ultrafiltrate produced by the Starlings forces is eliminated and large serum proteins are nearly excluded. The cerebrospinal fluid (CSF) to serum ratio for albumin of 1 : 200, one of the highest gradients in the body, demonstrates the effectiveness of the BBB.

The brain uptake index (BUI) introduced by Oldendorf in the early 1970s allowed a new level of quantitation of BBB permeability (Oldendorf, 1971). This method was used to establish that the entry rate of many compounds could be predicted from their lipid solubility, molecular weight, or other physicochemical characteristics. Some compounds cross the BBB to an extent greater than that predicted by their physicochemical characteristics because saturable systems transported them into the brain and other compounds crossed less than predicted because of efflux (brain to blood) transporters or other factors such as protein binding.

The 1970s and '80s saw the introduction and refinement of *in vitro*, of *in situ*, and of extremely sensitive *in vivo* methods (Blasberg *et al*, 1983; Patlak *et al*, 1983; Takasato *et al*, 1984; Zlokovic *et al*, 1986; Barrera *et al*, 1991; Raeissi and Audus, 1989). The introduction of these methods coupled with techniques such as high performance liquid chromatography, capillary depletion, and radioactive labeling has made it possible to study the permeability of the BBB to a host of substances which penetrate the BBB slowly such as viruses and viral products.

Physiological mechanisms of the BBB

The restrictive BBB

The BBB largely defines the operating environment of the CNS by regulating the movement of substances between the blood and the CSF and brain interstitial fluid. The BBB is often divided into the vascular, or endothelial, barrier and the epithelial barrier at the choroid plexus (also termed the blood-CSF barrier). The endothelial cells that comprise the capillaries and line the arterioles and venules constitute the barrier function of the spinal cord and in most areas of the brain (Rapoport, 1976). The endothelial cells are modified in that circumferential belts of tight junctions between contiguous nonfenestrated endothelial cells of the CNS preclude the leakage found in the capillary beds of peripheral tissues. Intracellular tight junctions comparable to those of the brain endothelium exist between contiguous epithelial cells at the choroid plexus (Johanson, 1988) and between arachnoid mater cells (Balin *et al*, 1986). The brain endothelia have other modifications as well. They do engage in endocytosis of blood-borne macromolecules and a recycling of the luminal plasmalemma but to a lesser degree than peripheral endothelia and choroid plexus (Broadwell and Banks, 1993). Secondary lysosomes hydrolyze many but not all macromolecules undergoing endocytosis within the BBB endothelia (Broadwell and Salcman, 1981; Broadwell and Banks, 1993). These modifications of the endothelia effectively eliminate the plasma ultrafiltrate characteristic of capillary beds in peripheral tissues and serve to define the restrictive permeability of the BBB.

Mechanisms of passage

Elimination of the production of the plasma ultrafiltrate may protect the CNS from circulating elements and from rapid fluid shifts but it also eliminates the traditional mechanism by which tissues are nourished. The BBB must, consequently, also facilitate the entry of vital substances into the brain. The BBB, therefore, is not a simple rigid or inert barrier. Instead, the endothelia, which form the vascular BBB and epithelial monolayers, that form the blood-CSF barrier, are dynamic, biologically active interfaces between the CNS and the blood. The BBB both restricts and regulates the exchange of substances between the blood and the CNS and, as will be demonstrated in other sections below, is involved in the pathophysiology of several disease states including AIDS.

Blood-borne substances use a limited number of well-defined pathways to enter the CNS: residual leakiness, membrane diffusion, saturable transport systems, and diapedesis. Major pathways for efflux are CSF readsorption at the arachnoid villi, saturable transport, membrane diffusion, and drainage by way of the brain's primitive lymphatics.

Blood-to-brain passage

Residual leakiness: Patent, extracellular pathways are found at the pial surface/subarachnoid space and allow passage between the blood and the CNS interstitial fluid/CSF (Broadwell and Banks, 1993; Broadwell and Sofroniew, 1993). These extracellular pathways may allow a quasi-equilibrium between plasma and the fluids of the CNS for otherwise impermeable proteins such as albumin (Broadwell and Sofroniew, 1993). The amounts of plasma proteins entering the CNS by this pathway are low, however, as exemplified by albumin which has a CSF/blood ratio of 0.005 and an unidirectional influx constant (K_i) of 10^{-5} to 10^{-6} ml/g-min. Although these routes are very slow, they may nonetheless have a significant physiological role and are available to any substance found in the blood.

The circumventricular organs (CVOs) are areas of the brain with a vasculature that often does not have barrier functions and, as such, have a more direct access to blood-borne substances. Neural connections between the CVOs and areas of the CNS with a BBB provide a way by which blood-borne substances that have entered the CVOs can affect deep brain function (Johnson and Gross, 1993). The CVOs in mammals include the pineal gland, the subfornical organ, the median eminence, the neural lobe of the pituitary, the area postrema, the subcommissural organ, and the organum vasculosum of the lamina terminalis (Weindl, 1973) and together make up less than 1% of the brain. The cells which delimit the CVOs impede diffusion into the rest of the brain and the CSF (Krisch and Leonhardt, 1989; Maness *et al*, 1998). Therefore, substances that have entered the CVOs do not have unrestricted access to the rest of the CNS.

Fluid phase endocytosis refers to the fluid that happens to be incidentally engulfed with the formation of vesicles induced by the more specific processes of receptor mediated endocytosis and adsorptive endocytosis. Any solute in the fluid will also be carried along in a non-specific manner. As discussed below, the fluid phase endocytosis that accompanies adsorptive endocytosis may form the basis for the minimal BBB disruption seen in neuroAIDS.

Membrane diffusion: Many endogenous molecules and drugs diffuse through the cell membranes that comprise the BBB. Lipid solubility/hydrogen bonding correlates directly and the square root of the molecular weight inversely with the rate of passage across the BBB (Chikhale *et al*, 1994; Rapoport, 1976; Oldendorf, 1974; Cornford *et al*, 1982). However, even water soluble molecules, as exemplified by morphine and some peptides, can cross to some degree by this mechanism to induce CNS effects (Banks and Kastin, 1985, 1994a; Begley, 1994; Oldendorf, 1974). Charge, protein binding, tertiary structure, or other factors can be influential or even predominant for specific substances (Rapoport, 1976).

Some have misinterpreted the work of Levin (1999) as showing that substances with molecular weights greater than about 400–600 Daltons are unable to cross the BBB by membrane diffusion. Subsequent work showed that the four substances examined by Levin with molecular weights over 400 Daltons are all substrates for the p-glycoprotein efflux system. Other studies have shown that substances with molecular weights over 5000 Daltons are capable of crossing the BBB by transmembrane diffusion (Banks and Kastin, 1985). As such, Levin's study illustrates the dangers of not considering efflux systems as a reason for a lower than expected penetration of the BBB.

Blood to brain saturable transport: The rate of entry into the CNS is limited for large, water soluble substances unless they are transported across the BBB (Bradbury, 1979; Davson and Segal, 1996c, 1996d; Rapoport, 1976). The presence of a transporter typically increases the rate of entry by tenfold or more (Oldendorf, 1971). Transport systems exist for almost every substance which the brain requires but cannot synthesize (Table 1), e.g., essential amino acids, vitamins, free fatty acids, glucose, minerals, nucleic acids, and electrolytes (Davson and Segal, 1996d). The hexose and the LNAA

Table 1 Selected saturable blood to brain transporters and examples of their ligands

Large neutral amino acids
Tyrosine, leucine, tryptophan
Hexoses
Glucose, fructose
Electrolytes
Potassium
Sodium
Minerals
Iron
Zinc
Magnesium
Peptides
Tyr-MIF-1/Met enkephalin
Ebiratide
Pituitary adenylate cyclase activating polypeptide
Insulin
Regulatory proteins
Interleukin-1α
Tumor necrosis factor-α
Leptin
Vitamins
Thiamine
Folic acid
Ascorbic acid
Biotin
Free fatty acids
Arachadonate
Palmitate
Docosahexaenoate

systems are passive diffusion systems that typically function as influx systems at the BBB because CSF levels of glucose and amino acids are kept lower than those in the serum by the active (energy requiring) transport of glucose and amino acids into neurons. However, if CSF levels become greater than serum levels or if radioactive ligands are given into the brain, saturable efflux can be demonstrated (Snodgrass *et al*, 1969; Davson *et al*, 1982; Betz and Goldstein, 1978).

An increasing number of transport systems are being described that are involved in communication between the brain and the peripheral tissues. For example, leptin, pancreatic polypeptide, and insulin are substances that are transported across the BBB to affect feeding (Baura *et al*, 1993; Banks and Kastin, 1998b; Banks *et al*, 1995; Friedman and Halaas, 1998; Spigelman and Flier, 1996).

Transporter activity can be modulated by allosteric regulators. For example, aluminum is a noncompetitive inhibitor (Banks *et al*, 1988) and leucine an uncompetitive inhibitor (Banks and Kastin, 1986b) of peptide transport system (PTS)-1. Transport systems may be globally distributed or localized. Not surprisingly, those transport systems for substances such as glucose and amino acids that are required by all regions of the brain are globally distributed. Many of the regulatory substances, such as interleukin-1α (Maness *et al*, 1995) and leptin (Banks *et al*, 1996a), show marked regional distribution.

Adsorptive endocytosis: Adsorptive endocytosis (AE) is a process likely to be involved in the uptake and transport of viruses by the BBB. Classically, AE is induced when toxic glycoproteins, such as wheatgerm agglutinin (WGA) and ricin, or polycations, such as poly-L-lysine and protamine, act as lectins by binding to negatively charged membrane-bound oligosaccharides, such as sialic acid, N-acetylglucosamine, and β-D-galactose (Vorbrodt, 1994; Villegas and Broadwell, 1993; Broadwell, 1989). AE occurs after chemical, anoxic, and physical insults (Dux *et al*, 1988; Lossinsky *et al*, 1983; Vorbrodt, 1994; Hardebo and Kahrstrom, 1985) and is largely a pathological process in BBB endothelia, although not necessarily in other endothelia. AE is distinguishable from the other vesicular processes of fluid phase endocytosis, which does not involve binding sites at cell membranes, and of receptor-mediated endocytosis (RME), which involves specific receptors for molecules such as insulin, transferrin, and lipoproteins.

The study of mechanisms of uptake and transcytosis of glycoproteins across brain endothelial cells is one of long standing interest in the field of BBB research. Most of this work has focused on WGA conjugated with horseradish peroxidase (WGA-HRP) (Broadwell and Banks, 1993; Broadwell, 1989, 1993) and can be used to highlight the aspects of the field relevant to neuroAIDS.

WGA-HRP binds to membrane surface glycoproteins with exposed sialic acid and N-acetylglucosamine oligosaccharide residues. The bound membrane is endocytosed with potential routing to several intracellular membrane compartments including lysosomes, endosomes, and the Golgi complex (Broadwell, 1989). As such, AE provides a mode of entry into brain endothelium. Membrane internalized by AE and routed to lysosomes is usually recycled to the luminal surface.

Vesicles originating from the Golgi complex and containing some of the contents of the original vesicles may be routed to the abluminal surface of the BBB endothelium (Broadwell *et al*, 1988; Villegas and Broadwell, 1993; Banks and Broadwell, 1994). Ultimately, WGA-HRP can be transcytosed so that 4 h after intravenous injection it is found in cells deep within the brain (Banks and Broadwell, 1994). The rate of entry of WGA-HRP is biphasic with the initial entry phase being about 30 times faster than the entry rate of albumin (Banks and Broadwell, 1994). Albumin slowly enters the CNS by leaking into the brain by a number of extracellular pathways (e.g., pial surface, Virchow-Robin space, subpial cortical grey matter) and by fluid-phase transcytosis (Broadwell and Sofroniew, 1993). The much faster entry rate of WGA-HRP shows that its entry is totally independent of the albumin pathways. In addition, treatment of animals with excess unconjugated WGA, a powerful inducer of AE, has little effect on the rate of albumin entry (Banks and Broadwell, 1994); this shows that induction of AE is associated with minimal disruption of the BBB.

With extreme insults, vesicles can lengthen and may even form canaliculi that extend through the endothelial cell allowing immunocytes and serum proteins to cross a now disrupted BBB (Lossinsky *et al*, 1983). At this extreme point, AE has resulted in a disrupted BBB and exhibits some similarities to diapedesis.

Diapedesis is the process by which cells cross cellular barriers (Lossinsky *et al*, 1991) and shows at least some similarities with classic AE. Mature immune cells are able to cross such cellular barriers, including the high epithelium (Tavassoli and Minguell, 1991; Chin *et al*, 1991) of the bone marrow and the endothelial BBB. Diapedesis involves a coordination between invagination of the endothelial cell and podocytosis of the immune cell that is mediated through glycoprotein adhesion molecules. This results in the immune cell tunneling through the endothelial cell or crossing between cells at the tight junctions. Cytokines, which affect AE (Banks *et al*, 1999a) at the BBB, can also modulate both the immune cell and the barrier cell actions in diapedesis (Chin *et al*, 1991; Tavassoli

and Minguell, 1991). Cytokines are involved at both the high epithelium of the bone marrow and the endothelium of the BBB, modulating both the immune and barrier cells (Male, 1995; Persidsky *et al*, 1997; Sharief *et al*, 1993; Nottet *et al*, 1996). Release of, and response to, other secreted substances by both the barrier and the immune cell result in an intimate communication which underlies diapedesis.

Brain-to-blood passage

The accumulation of blood-borne substances behind the blood-brain barrier is the net difference between influx and efflux rates. Efflux has long been recognized as important to the proper functioning of the CNS. Quantitative methods to study efflux in small animals have been devised, the first and still the most quantitative introduced about 15 years ago (Banks *et al*, 1986; Banks and Kastin, 1984). Efflux systems serve several functions. These include regulating levels of nutrients and minerals in the CSF, detoxifying the CNS, and reinforcing the impermeability of the BBB against circulating toxins. Efflux systems may also allow the CNS to contribute substances to the circulation in an endocrine-like fashion as illustrated by methionine enkephalin, tumor necrosis factor-α and corticotrophin releasing hormone (CRH). Major pathways for efflux are CSF reabsorption at the arachnoid villi, saturable transport, membrane diffusion, and drainage by way of the brain's primitive lymphatics. As discussed below, almost every one of these mechanisms is involved in some aspect of neuroAIDS.

Cerebrospinal fluid reabsorption: Any substance present in the CSF will enter the blood together with the usual reabsorption of CSF from the CNS at the arachnoid villi (Passaro Jr *et al*, 1982). Romero *et al* (1996) have shown that substantial blood levels can be achieved for substances that exit the CNS by this pathway and are not rapidly cleared from the circulation. For example, human TNF-α injected into the brain of the rat yields blood levels similar to those produced by i.v. injection (Bodnar *et al*, 1989; Chen *et al*, 1997).

Table 2 Selected saturable efflux systems and representative ligands

Efflux system	*Ligand*
p-Glycoprotein	Verapamil
Organic acid	Probenecid
Nucleoside (generalized)	Uridine
Nucleoside (limited)	Hypoxanthine
Hexose	3-O-methylglucose
Acid amino acid	Aspartate
Neutral amino acid	Phenylalanine
Basic amino acid	Arginine
PTS-1	Met-enkephalin
Thyroid	Thyroxine
Anionic	Iodide

Brain to blood saturable transport (efflux systems): For most substances, substantial efflux requires the presence of a saturable transporter (Table 2). Saturable efflux systems are responsible for the CNS to blood transport of electrolytes, free fatty acids, peptides, amino acids, metabolic degradation products, and toxins (Davson and Segal, 1996a; Martins *et al*, 1997a; Chen *et al*., 1997; Daniel *et al*, 1978; Tsuji *et al*, 1992; Begley, 1992; Banks *et al*, 1997a). For some substances, their efflux mechanisms are so robust that their levels in the blood are nearly identical after injecting them either into the lateral ventricle of the brain or infusing them intravenously (Davson and Segal, 1996a). Efflux systems can play important roles in establishing and maintaining the nutritive and homeostatic environment of the CNS (Davson and Segal, 1996a), be affected by disease states (Plotkin *et al*, 1997), and participate in the neuroimmune axis (Martins *et al*, 1997b; Cserr and Knopf, 1992).

Efflux systems can also allow the CNS to contribute to circulating blood levels. It has been suggested that about 50% of circulating methionine enkephalin may be of CNS origin (Banks and Kastin, 1997) and enough CRH is transported out of the brain to affect splenic production of β-endorphin (Martins *et al*, 1997b).

Detoxification of the CNS is illustrated by the efflux of HIAA by a probenecid-sensitive mechanism (Bulat and Zivkovic, 1978) and by the removal of neurotransmitters and neurotransmitter precursors, agonists, and antagonists such as glycine (Murray and Cutler, 1970), β-alanine, and taurine (Komura *et al*, 1997). The tobacco hornworm *Manduca sexta* has an efflux pump at its BBB for nicotine (Murray *et al*, 1994) allowing it to ingest the tobacco plant without neurotoxicity.

The presence of a saturable efflux system can be the major factor in determining the extent to which a drug will accumulate in the CNS and can underlie the apparent impermeability of the BBB to a substance by removing that substance from the CNS after it has entered. Efflux can prevent a drug from exerting a therapeutic effect within the CNS, as exemplified by penicillin. In other cases, efflux prevents the drug from being neurotoxic, as illustrated by the antifungal ivermectin, which is transported out of the brain by the p-glycoprotein (P-gp) transporter (Schinkel *et al*, 1994). In a strain of mice that lack the P-gp system, ivermectin is a lethal neurotoxin (Schinkel *et al*, 1994).

The increase in accumulation by brain of a ligand can be both dramatic and therapeutically important when its efflux system is inhibited. Traditional examples include increased efficacy of penicillin and cephalosporins in the treatment of CNS infections by blockade of the organic acid pump with probenecid or other agents (Spector,

1987; Spector and Lorenzo, 1974). In mice with the P-gp transporter knocked out, sensitivity to ivermectin is increased 100-fold and to vinblastine by threefold (Schinkel *et al*, 1994). Loperamide is an opiate drug used to treat diarrhea because it does not have CNS effects (e.g., analgesia) due to its poor accumulation by the CNS. Schinkel *et al* (1996) showed that loperamide is a substrate for P-gp and, with blockade of efflux by P-gp, loperamide uptake by brain increased by about sevenfold and it had analgesic effects. Others have demonstrated increased uptakes of up to 15 times when verapamil was used to inhibit efflux by P-gp (Chikale *et al*, 1995). Increases in brain accumulation also occur with inhibition of CSF reabsorption, probably in the range of 50–100% (Reed *et al*, 1965).

To date, no transporter has been isolated and fully characterized from BBB tissue. Transporters present in the BBB and in other tissues have been isolated from non-BBB tissues. The hexose transporter Glut-1 and p-glycoprotein transporter have been shown to be pore-forming, membrane-spanning proteins. Receptor mediated transcytosis, long known to occur in choroid epithelium (Davson and Segal, 1996b), has been suggested to also occur in brain capillaries for larger substances (Duffy and Pardridge, 1987) and the transport of some peptides has been shown to be dependent on vesicles (Shimura *et al*, 1991; Terasaki *et al*, 1992). It has been assumed that the gene product expressed as a receptor in non-BBB tissues is co-opted by the brain endothelium to act as a transporter, as suggested for insulin (Duffy and Pardridge, 1987). However, the BBB transporter for interleukin-1α is immunologically distinct from the T-cell type I receptor (Banks *et al*, 1991; Banks, 1999b) and PTS-1, which transports Tyr-MIF-1 and Met-Enk, is distinguishable from the receptors for either of its peptide ligands. The transporter for corticotrophin releasing hormone is energy and calcium channel dependent, involves microtubules, is acutely regulated by steroids, cytokines, and endogenous opioids, but is distinguishable from the P-gp system (Martins *et al*, 1997a).

Membrane diffusion: Lipid solubility, an important determinant for the rate at which a substance can enter the CNS, can also be an important determinant for the rate at which a substance exits the CNS (de Lange *et al*, 1995). This, in turn, affects diffusion distances within brain tissues and in CSF with more lipid soluble substances diffusing less far in brain tissue (de Lange *et al*, 1995; McQuay *et al*, 1989).

Lymphatic drainage: The least studied pathway of CSF reabsorption occurs via primitive brain lymphatics, which then drain along routes of cranial and spinal nerves to the cervical lymph nodes (Davson and Segal, 1996a). Although typical lymphatic channels are not found in brain tissue, a significant fraction (14–47%) of the CSF can drain into cervical lymphatics (Davson and Segal, 1996a; Yamada *et al*, 1991). The mechanisms that dictate which substances are drained by the lymphatics, which provides a direct pathway to the cervical lymphatic nodes, is unclear but can have important consequences for immune responses (Knopf *et al*, 1995; Yamada *et al*, 1991). This pathway has been associated with specific alterations in immune responses termed CNS immunization (Knopf *et al*, 1995).

Non BBB factors important in BBB permeability
Characteristics other than those of the BBB can affect how much of a blood-borne substance enters the brain. For a substance with an extremely high permeability, cerebral blood flow (CBF) will determine how much of that substance will enter the CNS (Kety, 1987); uptake of such a substance is termed flow-dependent. These substances cross the capillary bed so rapidly that their concentrations are depleted before they traverse the capillary. Enhancing CBF increases the amount of the substance delivered to and taken up by the brain. For such highly permeable substances, the amount taken up by the brain is a direct function of CBF.

Binding to elements circulating in the blood may also affect their rates of passage across the BBB. Binding proteins and soluble receptors are the most well known of such elements, but other forms of binding also occur. Binding may be homologous (aggregation), non-homologous (substances which are not identical but belong to the same class), or by the cellular elements in blood (Banks and Kastin, 1993b). For example, erythrocytes can take up glucose and amino acids from the circulation and release them as they pass through the brain (Drewes *et al*, 1977).

Pathology of the BBB

The above has been a brief review of some of the major aspects of the BBB. How disease affects the functioning of the BBB is an area of great interest that is accumulating an increasing number of examples. Often the questions arise whether altered functions of the BBB are responsible for the clinical manifestations of the disease process and whether the BBB should be a target of therapeutics. The rest of this review will examine some of the alterations that the BBB undergoes in disease states. The purpose is to highlight the diverse pathophysiology of the BBB. In addition, some of the specific aspects of BBB pathophysiology relating to neuroAIDS will be discussed.

Altered residual leakiness: disruption of the BBB
A classic model of BBB disruption is that which occurs with hypertension and the most classic method of study has been by induction of hypertension with epinephrine. Interestingly, the disruption of the BBB is not great in magnitude and often cannot be demonstrated unless the vascular space of the brain is washed free of vascular contents (Johansson, 1989). In addition, the disruption occurs primarily by the induction of vesicles and pores rather than through the tight junctions (Hansson *et al*, 1975; Mayhan and Heistad, 1985). Therefore, the disruption of the BBB appears to be minimal and by a vesicular process and so has similarities to the disruption seen with AE and in AIDS dementia.

Altered saturable transport
After disruption, saturable transport systems have probably been the most studied aspect of the BBB in disease states (Table 3). Alterations in the function of BBB transporters for substances have long been appreciated and have often been correlated with altered metabolic demands of the CNS in healthy maturation, aging, and disease. As discussed below, such alterations in BBB function can result from disease, as well as being its cause. Unlike disruption, however, alteration of saturable transport is not a monolithic event; rather, each transporter must be individually assessed. Therefore, investigation of this area is necessarily targeted and limited. Nevertheless, a number of transporters and disease states have been investigated. A few of the following are chosen for their illustration of principles and their application to neuroAIDS.

Table 3 Selected disease states and examples of altered transport function

Alcoholism
Influx
Large neutral amino acid (increased)
Glucose (increased)
Sodium (increased)
Potassium (decreased)
Phosphorus (increased)
Thiamine (variable)
Efflux
Peptide transport system (PTS)-1 (decreased)
*Spinal cord trauma**
Influx
PTS-6 (variable)
TNF-α (increased)
*Multiple sclerosis/EAE**
Influx
TNF-α (increased)
Obesity
Influx
Leptin (decreased)
Mental retardation (selected cases)
Influx
Glucose (decreased)
Aging
Influx
Glucose (decreased)
Choline (decreased)
Palmitate (increased)
Efflux
PTS-1 (decreased)
Phenylalanine (increased)
Glucose (decreased)

*Also associated with BBB disruption and immune cell invasion

Alcoholism: Several transport systems are affected in alcoholism, as reviewed elsewhere (Banks and Kastin, 1993a). The blood to brain transport of large neutral amino acids, glucose, sodium and phosphorus are increased, whereas the blood to brain transport of potassium is decreased. Evidence for both a decrease and an increase in thiamine transport into the brain has been presented. Early in the thiamine deficiency that develops with alcoholism, the BBB is able to continue to sequester sufficient thiamine to maintain CNS function. Later, CSF/serum levels drop, the CNS manifestations of the Wernicke-Korsakoff syndrome appear, and the BBB becomes disrupted (Greenwood and Pratt, 1983). Several studies have suggested that ethanol ingestion is largely controlled by brain levels of methionine enkephalin (Met-Enk), with low levels of Met-Enk inducing drinking (Banks and Kastin, 1994b). In addition, seizure activity is related to either resistance to or low levels of Met-Enk in the brain. The level of Met-Enk is partially controlled by the BBB which possesses a saturable efflux system for removing Met-Enk from the brain. This transporter becomes deranged during alcohol addiction and may contribute to seizure activity (Banks and Kastin, 1993a).

Immunologic and traumatic insults to the CNS: BBB disruption and enhanced diapedesis are two aspects of MS and its animal model, experimental allergic encephalomyelitis (EAE), that have interesting parallels to neuroAIDS. These aspects are variously reviewed elsewhere, including in other articles in this issue. In addition, the transporter for tumor necrosis factor-α (TNF) has been shown to be enhanced in EAE with the enhanced transport mirroring disease activity anatomically and temporally (Pan *et al*, 1996). In spinal cord transection, TNF-α transport is also enhanced about 2 h after injury and is especially robust proximal to the site of injury (Pan *et al*, 1997). These correlations between TNF-α transport and immunologic and traumatic CNS injury suggests that bloodborne TNF-α could play a role in the progression of injury by being transported into the CNS.

Other transport systems are also affected with traumatic spinal cord injury, although the pattern of alteration differs with the compound studied. PTS-6 (Banks *et al*, 1993) transports pituitary adenylate cyclase-activating polypeptide, a peptide related to the VIP family (Arimura, 1992; Kimura *et al*, 1990)

with potent neurotropic properties (Uchida *et al*, 1994, 1996), across the BBB. Its transport is altered in a complex manner after spinal cord injury, showing a decreased transport rate most obvious in the brain and cervical spinal cord early after injury and an increase in transport seen throughout the CNS by day 7 of injury (Banks *et al*, 1998). The transport of ebiratide, a small peptide analog of MSH/ACTH that is rapidly transported across the BBB (Shimura *et al*, 1991; Terasaki *et al*, 1992), is unaffected by traumatic injury of the spinal cord (Pan *et al*, 1997).

Hypoglycorrhachia: Defects in the ability of the BBB to transport nutrients can lead to CNS dysfunction. A classic example of this is a family born with decreased levels of Glut1, the transporter responsible for delivering glucose to the CNS (De Vivo *et al*, 1991). Affected members of this family have developmental delays and mental retardation. The role of inadequate nutrient delivery, especially glucose, in the pathogenesis of Alzheimer's disease has been an area of intense interest (Harik and Kalaria, 1991). Glucose transport across the BBB is altered in humans infected with HIV-1 with the striatum showing relative hypermetabolism early in the disease (Rottenberg *et al*, 1996). Mice infected with a retrovirus show regional variation in the glucose use by astrocytes (Vann *et al*, 1999).

Obesity and anorexia: Leptin is a 16 Kd protein produced by fat cells that controls body weight by affecting feeding and thermogenesis at sites within the brain (Friedman and Halaas, 1998; Spiegelman and Flier, 1996). Leptin must cross the BBB to be active and it does so due to the presence of a saturable transport system specific to it (Banks *et al*, 1996a). Several lines of evidence have shown that obesity in humans occurs because of resistance to leptin and several authors have suggested that the resistance is due to a defect in the leptin BBB transporter (Caro *et al*, 1996; Schwartz *et al*, 1996). Recent work has confirmed that the leptin transporter in obese animals is indeed impaired (Banks *et al*, 1999b). Other peptides with effects on feeding that are transported across the BBB include insulin, pancreatic polypeptide, and amylin (Baura *et al*, 1993; Banks and Kastin, 1998b; Banks *et al*, 1995). Whether altered transport of any of these compounds underlies the anorexia of AIDS has not been addressed.

Alterations in aging

The BBB undergoes extensive morphological changes with aging, as previously reviewed (Banks and Kastin, 1986a). Many of these changes are regional and variable. For example, capillary lumen diameter has been reported to be increased in the frontal cortex, decreased in the hippocampus, and unaltered in other areas. The number of mitochondria per endothelial cell was reported to be decreased in aged Macaque monkeys but not in aged rats. Most authors have found a decreased number and increased length of endothelial cells with aging.

The BBB remains intact with normal aging despite these morphological changes in the brain vasculature. The vascular space of the brain and the number of tight junctions, vesicles, and gap junctions per endothelial cell are unchanged with aging, findings that are consistent with maintenance of BBB integrity. However, the changes that do occur in the vasculature with aging may make the BBB more susceptible to subsequent pathological insults.

The vessel in the aging brain shows increased tortuosity, kinking, looping, spiraling, and corkscrewing. Such angioarchitectural distortions are even more pronounced in Alzheimer's disease. The resulting hemorrheological aberrations have been proposed to interfere with the passage of oxygen, glucose and other substances across the BBB (de la Torre and Mussivand, 1993). Angioarchitectural abnormalities also occur in the brains of patients with AIDS, evidencing as arteriovenous shunts, vascular displacement, and neovascularization (Nelson *et al*, 1999).

Saturable transport systems have been studied in aged rodents, humans and dogs with a variety of methods, including magnetic resonance imaging and PET scanning. Most transporters studied are decreased in the range of 20–40% with proportionate decreases in K_m and V_{max}. Unaltered K_m/V_{max} ratios suggest an uncompetitive form of inhibition; that is, effects not mediated through changes in transporter number or ligand affinity. Some transporters have been shown to be reduced much more than the typical 20–40%. The V_{max} and K_m for choline transport are each reduced by over 95% and the V_{max} for glucose is reduced about 75% (Mooradian *et al*, 1991). The decrease in glucose transport begins in midlife for some brain regions and continues for other regions into senescence.

Aging has a significant interaction with AIDS. Once infected, the elderly die more rapidly from AIDS due in part to a more rapid loss of $CD4^+$ helper cells (Phillips *et al*, 1991; Adler and Nagel, 1994; Adler *et al*, 1997). AIDS-related dementia is also more likely to develop in the elderly (Ferro and Salit, 1992). However, the permeability of the BBB to gp120 is unchanged in aged mice (Banks *et al*, 1999c).

Opiates, drugs of abuse, and BBB permeability

The literature on the effects of opiates and drug addiction on the functions of the BBB is small but tantalizing. We have shown that adult mice exposed to opiates during their perinatal period have altered transport systems for enkephalins (Harrison *et al*, 1993; Banks *et al*, 1996b). Others have shown that *in vitro* models of the BBB exposed to enkephalins

have increased permeability to sugars and to sodium fluorescein, a fluid phase marker, but not larger markers (Thompson and Audus, 1994; Thompson *et al*, 1994) suggesting partial disruption of the BBB. The increased permeability to fluorescein is most powerfully induced by mu receptor agonists and can be blocked by naloxone (Baba *et al*, 1988). Opiates could act directly on the BBB or by releasing histamine and cytokines.

Fiala *et al* (1996) have shown that cocaine or cocaethylene affects T cell transport and HIV penetration across the BBB, possibly by induction of cytokines and chemokines (Zhang *et al*, 1998). Opiates given i.c.v. enhance macrophage migration into the CNS. Saland *et al* (1983) and Ting *et al* (1994) found that opiate exposure enhanced the disruption to the BBB induced by ischemia. Similarly, drug addition may increase the susceptibility of the BBB to other insults, such as assault by gp120. An opiate effect on the BBB mediated through cytokines would involve opiates in the many theories about the interactions of HIV with cytokines.

Efflux of drugs

One of the major obstacles in the treatment of AIDS is the ability to accumulate significant amounts of anti-viral drugs in the CNS. Ineffectual drug delivery means that it is difficult to reverse the CNS symptoms of AIDS and that the CNS is a potential reservoir of virus for reinfection. Significant evidence suggests that the main obstacle to accumulation of anti-virals within the CNS is not the inability of the drugs to cross the BBB, but rather that efflux systems remove them after they have entered the CNS.

Two of the 11 anti-virals currently used clinically, AZT and saquinavir, have evidence for significant CNS efflux. Since AZT is a nucleoside reverse transcriptase inhibitor (NRTI) and saquinavir is a protease inhibitor, two of the three classes of anti virals are represented. AZT uptake by brain is limited (Terasaki and Pardridge, 1988). Influx into brain of AZT is by nonsaturable diffusion (Masereeuw *et al*, 1994), whereas efflux is saturable (Wang and Sawchuck, 1995; Takasawa *et al*, 1997b; Masereeuw *et al*, 1994). The saturable efflux system is probenecid sensitive (Wang and Sawchuck, 1995; Takasawa *et al*, 1997b; Masereeuw *et al*, 1994).

Saquinavir was found in an *in vitro* model of the BBB to be a substrate for the P-gp transporter (Glynn and Yazdanian, 1998). The p-glycoprotein (P-gp) transporter is constitutively expressed by the BBB (Cordon-Cardo *et al*, 1989) and is the earliest known marker of BBB endothelial cell differentiation to be expressed (Qin and Sato, 1995). It has a large number of seemingly unrelated ligands, including verapamil, cyclosporin, vinblastine, chlorpromazine, quinidine, progesterone, ivermectin, colchicine, doxarubicin, sandostatin, loperamide, and digoxin (Schinkel *et al*, 1994, 1996; Tsuji *et al*, 1992, 1993; Cordon-Cardo *et al*, 1989; Begley, 1992; Tsuji and Tamai, 1997) and is the major mechanism in restricting blood to brain accumulation for most of them (Sakata *et al*, 1994). The affinity of these ligands for the P-gp transporter varies tremendously and, accordingly, inhibition of the P-gp transporter results in varying degrees of increased uptake and CNS effects among the ligands (Schinkel *et al*, 1994, 1996; Chikale *et al*, 1995; Kakee *et al*, 1996; Drion *et al*, 1996).

Of several antivirals examined, only saquinavir was found to be a substrate for the P-gp transporter in an *in vitro* model of the BBB (Glynn and Yazdanian, 1998). However, the *in vitro* model as used in that study is ideal for measuring nonsaturable permeability or saturable systems with low affinities but can mask high affinity saturable transport systems (Audus *et al*, 1992; Weber *et al*, 1993). High affinity systems would be the easiest to inhibit in a clinical setting and so represent a very important therapeutic target. In comparison, Lee *et al* (1998) examined a human carcinoma transfected with the transporter and found that three of the protease inhibitors (saquinavir, ritonavir and indinavir) were substrates for P-gp. This is consistent with the work of Kim *et al* (1999) who found that the uptake of protease inhibitors was increased 7–36-fold in mice that did not possess the P-gp transporter.

Inhibition of anti-viral drug efflux could lead to a significant advance in the treatment of HIV-1 CNS infections. Even an increase of threefold in peak antiviral uptake should be meaningful in the treatment of HIV-1 in the CNS. For example, a single dose of 40 mg of stavudine, which has an intermediate penetration of the CNS for an NRTI, resulted in a peak concentration in CSF of 61 ng/ml (Haworth *et al*, 1998), whereas the ED_{50} for stavudine is 52 ng/ml. Inhibition of efflux decreases the half-time disappearance rate from the CNS which prolongs residence time in the CNS and, after multiple doses, increases the steady-state concentration. A threefold increase in peak CNS concentration indicates that the half-time disappearance from brain is increased threefold. Therefore, even a modest inhibition of efflux would likely result in concentrations sustained well above therapeutic levels for most of the time between doses.

Secretions by the brain endothelial cell

A few studies have suggested that brain endothelial cells are a source of neurotoxic and neuroactive substances, including cytokines, nitric oxide, and prostaglandins (Terracina *et al*, 1994; Mandi *et al*, 1998). Recently, evidence has shown that the brain endothelial cell can secrete a neurotoxic substance when protein kinase C levels are decreased (Grammas *et al*, 1997). Similar studies have shown that brain endothelial cells from

patients with Alzheimer's disease, which have a defect in protein kinase C (Grammas *et al*, 1995), secrete the same or a similar neurotoxin (Grammas *et al*, 1999). NOS activity is increased in brain microvessels from Alzheimer patients (Dorheim *et al*, 1994).

HIV-1 Tat can induce the release of interleukin-6 through a cAMP dependent pathway (Zidovetzki *et al*, 1998). Thus, endothelial cells secrete many of the substances that, when secreted from microglia and astrocytes, have been postulated to induce neuronal death in AIDS. Like microglia and astrocytes, the release of those substances from brain endothelial cells can be stimulated by viral products. Therefore, the endothelial cell could be a third source of these neurotoxic agents. Unlike the microglia and astrocytes, however, the endothelial cell can be stimulated by virus and viral products that are circulating in the blood as well as those found within the CNS, since half of the endothelial cell membrane faces the circulation and half the brain interstitial fluid. Therefore, the brain endothelial cell has the potential for being both an early and a robust source of neurotoxins.

Adsorptive endocytosis

How HIV-1, either as free virus or within infected immune cells, binds to and crosses the BBB is unknown as the brain endothelial cell is CD4 and galactosylceramide negative. The answer in part, at least for passage of infected immune cells, may lie in effects of cytokines on the brain endothelial cell. LPS, TNF, IL-1β, other cytokines, and HIV-1 Tat increase chemokine receptors and cell adhesion molecules on brain endothelial cells, which are glycoproteins related to lectins as their name (se**lectins**) indicates (Persidsky *et al*, 1997; Nottet *et al*, 1996; Frigerio *et al*, 1998; Tamaru *et al*, 1998; Defazio *et al*, 1998; Shaw and Greig, 1999). We have shown that gp120, itself a glycoprotein, can also act as a lectin at the BBB to induce adsorptive endocytosis (AE) and so provide a direct mechanism for transcytosis of free virus and of infected immune cells expressing gp120 on their cell surface. HIV is not destroyed in lysosomes and can escape from them to enter cytoplasm (Bourinbaiar and Phillips, 1991); therefore, AE can provide a route for infecting the BBB endothelium (Moses *et al*, 1993; Wiley *et al*, 1986) as well as crossing the BBB.

We have also shown that AE induced by gp120 in the murine BBB *in vivo* and in murine and human brain endothelial cells *in vitro* allows gp120 to enter endothelial cells and cross the BBB (Table 4). The AE induced by gp120 is not a passive event for the BBB but involves five identified stages: (i) surface binding, (ii) internalization, (iii) post-internalization fusion, and exocytosis either (iv) to the luminal membrane or (v) transcytosis (exocytosis to the abluminal membrane). Internalization involves cytoskeletal binding, is glucose and cAMP dependent, calcium channel independent, and inhibited by potassium (Banks *et al*, 1998; Banks, 1999a). Internalization is also potentiated by WGA, the glycoprotein often used to study AE in brain endothelium, and by LPS, presumably through cytokine release. Fusion is glucose dependent, partially inhibited by potassium, stimulated by cAMP, calcium channel independent, and involves a protamine sulfate-sensitive co-receptor (Banks *et al*, 1998; Banks, 1999a).

Our *in vivo* and *in vitro* work shows that gp120 is a weak inducer of AE that binds to sites similar to those of WGA (Banks *et al*, 1997b, 1998). Multiple-time regression analysis measured the unidirectional influx constant (K_i) for I-gp120 as 4.62 (10^{-5}) ml/g-min, while permeation across the BBB of simultaneously injected albumin (labeled with ^{99m}Tc; Tc-Alb) could not be shown (Banks *et al*, 1997b). This significant ($P<0.001$) difference between I-gp120 and the smaller Tc-Alb shows that gp120 crosses by a pathway other than the extracellular routes. The per cent of an i.v. injected dose of I-gp120 that enters the brain is about 0.15% (Banks and Kastin, 1998a). This is a moderate level of uptake for a substance and far exceeds that of some substances that clearly have effects on the CNS. For example, less than 0.02% of an i.v. dose of morphine is taken up by brain (Advokat and Gulati, 1991; Banks and Kastin, 1994a).

WGA enhances the uptake of I-gp120 by brain endothelial cells by about 17-fold without disrupting the BBB in both *in vivo* studies (Banks *et al*, 1997b, 1999a; Takasawa *et al*, 1997a; Cashion *et al*, 1999) and *in vitro* studies in isolated brain endothelial cells (Banks *et al*, 1997b, 1998; Banks, 1999a). As would be predicted for AE, WGA did not induce uptake of the nonglycosylated version of gp120 (ngp120) either *in vivo* (Banks *et al*, 1997b; Banks and Kastin, 1998a) or *in vitro* (Banks *et al*, 1997b, 1998). The most likely explanation for the ability of WGA to mediate the uptake of gp120 is that gp120 binds to sites near or similar to those that bind WGA and most likely contain sialic acid or N-acetylglucosamine oligosaccharide residues. Con-

Table 4 Characteristics of GP120 adsorptive endocytosis

Slow rate of entry
Enhanced by WGA, LPS and cAMP
Post-internalization fusion (protamine sulfate-sensitive co-receptor)
Cytoskeletal component to binding
Internalization and fusion glucose dependent
Internalization and fusion partially inhibited by potassium
Dependent on glycosylation of gp 120
Brain uptake varies with region
Not dependent on clatharin, caveolae, endosomal acidification, or calcium channels
Independent of P-glycoprotein transporter
Independent of receptor mediated endocytosis and fluid phase endocytosis

sistent with this is the findings that other viruses bind to sialic acid (Yu *et al*, 1994; Marsh, 1984) and that HIV patients have decreased binding sites on brain endothelial cells for WGA suggesting down regulation (Büttner *et al*, 1996).

About 90% of the I-gp120 taken up by isolated brain microvessels *in vitro* is internalized, and about 90% of that is fused to the cell membrane (Banks *et al*, 1998). Internalization is clatharin independent and involves cytoskeletal elements. This is consistent with WGA also binding to cytoskeletal anchored glycoproteins in various types of cells (Mehta *et al*, 1999; Suchard and Boxer, 1989; Lehto and Virtanen, 1983; Kalomiris and Bourguignon, 1988) and altering F-actin (Sjölander and Magnusson, 1988). Internalization and fusion are energy dependent and potassium sensitive. Studies have also shown that the AE of gp120 is not dependent on clatharin, caveolae, or calcium channels (Banks *et al*, 1997b). Uptake is also not dependent on endosomal acidification (Banks *et al*, 1997b). This latter observation is consistent with the finding that retroviruses fuse at neutral pH in other cell types (Hernandez *et al*, 1996).

Protamine sulfate and other polycations induce AE in brain endothelial cells (Hardebo and Kahrstrom, 1985; Westergren and Johansson, 1993). However, protamine sulfate had no effect on internalization of I-gp120, suggesting that AE induced by polycations may be differentiated from that induced by glycoproteins (Banks *et al*, 1998). Protamine sulfate did decrease the cell membrane fusion of gp120. Protamine sulfate had no effect on any parameter of nonglycosylated gp120 uptake (Banks *et al*, 1998). Protamine sulfate is known to bind to mucopolysaccharides such as heparin and heparan sulfates; this suggests that there may be a negatively charged, heparan sulfate containing co-receptor (Salmivirta *et al*, 1996) responsible for membrane fusion that activates after internalization of gp120. Such receptors have been postulated to include chemokines which are involved in HIV-1 internalization by other cell types (Gilat *et al*, 1994; Pleskoff *et al*, 1997; Feng *et al*, 1996). Thus, fusion at the brain endothelial cell may differ from other cells studied in that gp120 rather than gp41 may be responsible for HIV fusion (Hernandez *et al*, 1996).

The uptake of I-gp120 is influenced by LPS (Banks *et al*, 1999a). LPS is a potent releaser of cytokines, including IL-1, IL-6 and TNF (Laye *et al*, 1994; Gonindard *et al*, 1996; Ogle *et al*, 1994). LPS increases gp120 penetration by two mechanisms that operate simultaneously: (i) it increases leakage across the BBB of gp120 and albumin; that is, by disruption of the BBB and (ii) it increases uptake of gp120 by AE in the presence and absence of WGA. BBB disruption did not occur in every experiment, but LPS always increased AE by 50–100%. Indomethacin blocked the loss of body weight that was associated with LPS administration but potentiated the effect on BBB penetration of I-gp120 (Banks *et al*, 1999a). This suggests that the enhancement of BBB penetration is mediated through a prostaglandin E-independent pathway such as that shown to occur for TNF (Pettipher and Wimberly, 1994). Therefore, I-gp120 uptake by brain endothelial cells is enhanced by LPS, consistent with a role for cytokines in the enhancement of HIV-1 passage across the BBB (Fiala *et al*, 1997).

Lymphatic drainage

The efflux from brain of I-gp120 occurs by a non-saturable pathway at a rate exceeding that due to reabsorption of the CSF (Cashion *et al*, 1999). The discrepancy in brain to blood efflux was explained by drainage from brain to the cervical nodes through the brain's primitive lymphatic system. Both spleen and cervical nodes concentrate I-gp120 from blood, but lymphatic drainage allows I-gp120 to concentrate to a level in the cervical nodes almost 20 times higher than in the spleen.

Whether HIV-1 also takes this route is unknown. If it does, then it would support the idea of the CNS as a reservoir of HIV-1. Lymphatic drainage would allow active virus direct access to the cervical nodes without entry into the blood and exposure to circulating antiviral agents. Because lymphoid tissues are the early sites of viral infection and replication and are a major reservoir of virus (Donaldson *et al*, 1994; Tenner-Racz *et al*, 1998), such direct access from the CNS to the lymph nodes could greatly facilitate relapse.

Future directions for the BBB and HIV-1

This review and the other articles in this issue illustrate some of the many avenues down which BBB and neuroAIDS research could travel. Some of these are obvious, such as continued investigations of how infected immune cells cross the BBB and resolving the role of endothelial cell infection. Some perhaps less obvious avenues are:

(1) What is the cell biology of HIV penetration? Few studies have investigated the intracellular machinery of immune cell and viral penetration of the intact BBB. A knowledge of such machinery could likely be used to devise treatments to block the entry of virus into brain.
(2) What are the earliest interactions between the BBB and HIV? The use of astrocyte co-culture models of the BBB relegates those studies to that period in which neuroAIDS is already established. It would be important to know how endothelial cells react to HIV before astrocyte and microglial activation and infection; that is, that early period when virus is making its initial attempts to enter the brain; only at this time would blockade prevent neuroAIDS.

(3) Is the endothelial cell a source of neurotoxins in AIDS? Brain endothelial cells, like astrocytes and glial cells, can secrete cytokines, NO, and neurotoxins into the CNS. Endothelia are in the unique position of responding to agents either in the blood or the CNS. As such, the BBB could be an early source of neurotoxins, even predating viral entry into the CNS.

(4) Do blood-borne toxic viral products other than gp120 (e.g. tat) cross the BBB? If so, this provides another pathway by which neuroAIDS could occur without the necessity of virus entering the brain.

(5) Does AIDS alter the transport systems of the BBB? These transporters are largely responsible for maintaining the homeostatic and nutritive environment of the CNS and have been shown to be altered in numerous other disease states in ways that affect CNS functioning. Alterations of these transporters could underlie the pathophysiology of neuroAIDS. For example, upregulation of TNF transport from blood to brain might underlie AIDS anorexia.

(6) How can antiviral drug delivery across the BBB be improved? Saturable efflux systems apparently play a major role in restricting the accumulation of anti-virals into the CNS. Based on the literature reviewed here, inhibition of transporters would be expected to increase brain concentrations between 3–100-fold which should be enough to increase therapeutic effect.

(7) How does HIV reenter the circulation from the CNS? Brain efflux systems for viruses are even less well studied than influx systems but are clearly of great relevance to understanding the reinfection of peripheral tissues from CNS reservoirs of virus. Our work has shown that gp120 could direct virus directly from brain to cervical nodes through the lymphatic system, bypassing the blood and blood-borne anti-virals. Other questions relate to whether infected microglial cells can enter the circulation or whether infected endothelial and choroid plexus cells might shed virus directly into the circulation.

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