Review

Immunobiology of the blood-brain barrier

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The brain microvessel endothelial cells (BMVEC) that form the blood-brain barrier are uniquely positioned to influence immune responses within the central nervous system. As the biological interface separating the blood from the brain extracellular fluid, BMVEC regulate the entry of leukocytes into the brain. In addition, through the release of various soluble factors that affect immune responses, BMVEC may modulate immune responses in the brain. This review addresses the interplay between the immune system and the blood-brain barrier as it relates to the regulation of CNS defense and immunity.

Keywords: leukocyte migration; endothelial cells; modulation

Introduction

The brain has often been considered an immunologically privileged organ. This was based on early studies that found few antigen-presenting cells in the central nervous system. In addition, there was a perceived lack of a lymphatic system within the brain to carry immunogenic material in the central nervous system to lymph nodes where a humoral immune response could be initiated. And finally, the presence of the blood-brain barrier (BBB) was thought to prevent the entry of immune cells from the peripheral circulation into the brain.

However, there is increasing evidence to suggest that the brain is under immunological surveillance (see reviews by Cserr and Knopf, 1992; Hickey, 1999). First, the glial cells within the brain can secrete cytokines and chemokines as well as participate in antigen presentation (Ransohoff and Tani, 1998; O'Keefe *et al*, 1999). Furthermore, the failure of most long term central nervous system xenografts are the result of immune responses within the brain (Czech *et al*, 1997). From a disease perspective, multiple sclerosis (MS) and experimental allergic encephalomyelitis (EAE) clearly demonstrate the devastation brought about by cellular and humoral immune responses within the central nervous system.

The BBB was once considered to be a physical, static barrier to the passage of solutes and macromolecules from the bloodstream to the brain extracellular fluid. However, recent studies indicate the BMVEC of the BBB form a dynamic interface between the blood and the brain that can be influenced by drug regimens (Bartus *et al*, 1996; Greig, 1989) and endogenous factors such as bradykinin (Hurst and Clark, 1998) bile salts (Greenwood *et al*, 1991) and cytokines (Mark and Miller, 1999). Changes in BBB function are observed in several neurological disorders associated with immune responses (i.e. MS, meningitis, HIV-1-associated dementia) (Sharief *et al*, 1993; Spellerberg and Tuomanen, 1994; Epstein, 1998; Power and Johnson, 1995). The alterations in BBB function observed in the above neurological disorders result in a loss of integrity and enhanced permeability of the BMVEC. The extent to which BBB permeability is increased in neurological disorders such as MS and meningitis is positively correlated with the severity of the disease (Sharief *et al*, 1993; Spellerberg and Tuomanen, 1994). Therefore, understanding the interactions of the immune system with the BBB during both health and disease may provide insight into the treatment of various neurological diseases.

This review examines the interplay between the immune response and the BBB. Special emphasis will be given to examining leukocyte adherence to and penetration through the BBB. Although there are several types of leukocytes, the present review will focus on the neutrophils, monocytes and lymphocytes (T and B cells) and their movement across the BBB. In addition, the review will consider how the BBB can influence immune

responses within the central nervous system by controlling the entry of immunomodulatory agents into the brain. In this regard, the BBB will be treated as both a target site for the immune system, and a conduit for activation of immune responses within the central nervous system.

Components of the BBB

The schematic in Figure 1 compares the capillaries in the peripheral vasculature to those that form the BBB. In contrast to many of the endothelial cells found in the peripheral microvasculature, the BMVEC form a continuous layer of cells and extracellular matrix (Audus et al, 1992; Broadwell, 1989). There are also no detectable fenestrations and reduced levels of pinocytic activity in the BMVEC that form the BBB compared to endothelial cells in the peripheral microvasculature (Audus et al, 1992; Broadwell, 1989). In addition, the brain microvessel endothelial cells also form tight extracellular junctions, similar (but not identical) to those found in epithelial cells (Schneeberger and Lynch, 1984; Brightman, 1989). Together, these morphological features act to limit the passage of solutes, macromolecules and cells across the BBB.

Astrocytes are an additional cellular component of the BBB. The astrocytes are a type of glial cell that extend foot-like projections around the outside of the capillaries (see Figure 1). While the astrocytes themselves do not form the barrier, they do have an important role in the development and maintenance of the BBB (Goldstein, 1988). The importance of the astrocytes to the establishment of the BBB comes from the seminal work of Stewart and Wiley (1981). In these studies, the embryonic, avascular quail brain was implanted into the abdominal cavity of a chick embryo. Although the resulting microvasculature in the implanted quail brain originated from the systemic abdominal vasculature of the chick, distinct functional, morphological and biochemical features emerged characteristic of the BBB. These studies indicated that endothelial cells placed in the microenvironment of the brain, develop the characteristics of the brain microvessels that form the BBB (Stewart and Wiley, 1981).

Direct evidence also exists that confirms the importance of the astrocytes in influencing the barrier properties of brain endothelial cells. These are through the use of astrocyte co-culture and astrocyte conditioned media in cell culture models of the BBB (Rubin et al, 1991; Raub et al, 1992; Hurwitz et al, 1993; Rist et al, 1997; Hayashi et al, 1997). In these models, astrocytes were either grown on the underside of a semi-permeable membrane with the endothelial cells cultured on the opposite side of the membrane, or media was collected from astrocytes and then used in the media provided to the endothelial cells. The use of astrocyte co-culture and conditioned media have been reported to reduce the permeability of the BMVEC (Rubin et al, 1991; Raub et al, 1992; Rist et al, 1997). Even endothelial cells from the peripheral microvasculature (i.e. umbilical vein endothelial cells) displayed reduced permeability and BBB-specific characteristics following co-culture with astrocytes (Hurwitz et al, 1993; Hayashi et al, 1997). Identification of the astrocyte-released
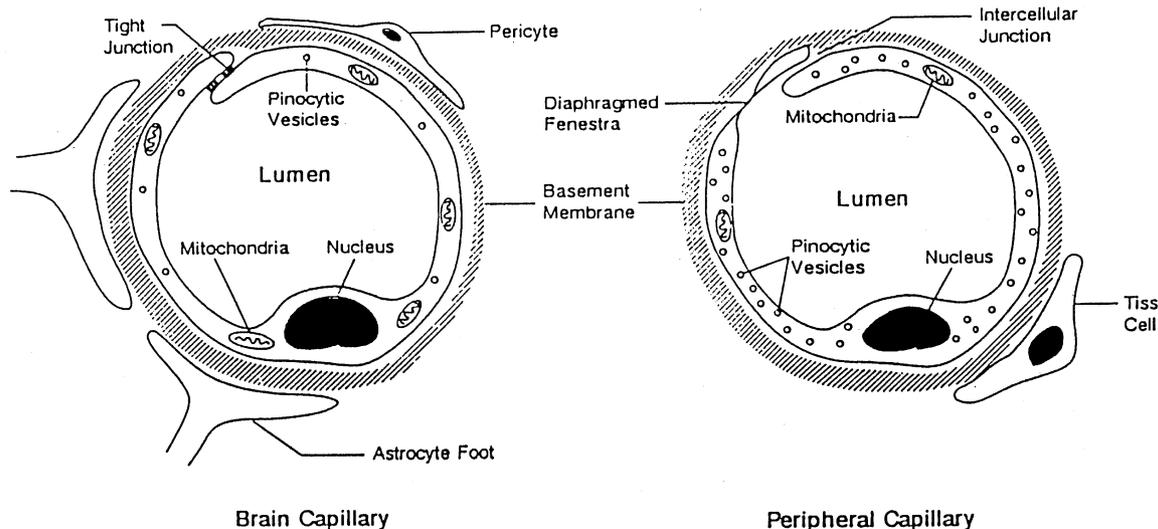
Figure 1 Cross-sectional diagram comparing brain capillary and peripheral capillary. Significant differences include the presence of tight junctions, reduced pinocytic vesicles, increased mitochondria organelles and the close association to astrocytes in the brain capillary.

factors involved in controlling the permeability properties of the brain endothelial cells, as well as the cellular mechanisms responsible for such permeability changes, remain unknown. However, it is clear that the astrocytes can influence the permeability characteristics of the BMVEC that form the BBB.

In addition to the effects of astrocytes on BMVEC permeability, it has also been suggested that the astrocytes may influence the expression of adhesion molecules in the brain microvessel endothelial cells that form the BBB (Engelhardt *et al*, 1997). Studies by Joseph and colleagues (1997), demonstrated an increased adhesion of T cells to both brain and peripheral endothelia cells following exposure to astrocyte conditioned media. In this regard, the co-culturing of human brain microvessel endothelial cells with human astrocytes may offer a more representative model for examining immune responses in the BBB.

Although they occur less frequently than the astrocytes, pericytes and perivascular cells can also be found scattered along the length of the brain capillary endothelial cells. The pericytes are smooth muscle cells that are in close proximity to the BMVEC (see Figure 1). Although the function of the pericytes in the BBB are still being debated, these cells produce and secrete several immuno-modulating agents that can influence immune responses in the CNS (Fabry *et al*, 1993). The pericytes may also provide a means for contraction of the brain capillaries. Perivascular cells are distinct from the smooth muscle derived pericytes. The perivascular cells are also believed to have an immune function in the CNS (Angelov *et al*, 1998). Studies suggest the perivascular cells, located near the basal lamina of the BMVEC, can act as macrophages capable of phagocytosis and antigen presentation (Angelov *et al*, 1998). Given the proposed function of these cells and their proximity to the brain capillaries, the cerebral perivascular cells may provide a first defense to infiltrating antigens in the CNS.

Role of the blood-brain barrier in trafficking of immune cells

The perceived inability of bone marrow derived cells to access the brain during steady-state conditions lead to the notion that the brain was sequestered from the immune system by the BBB. However, more recent studies have demonstrated that various immune cells in the CNS, most notably, activated monocytes, lymphocytes and B cells, can cross the BBB (Andersson *et al*, 1992; Nottet *et al*, 1996; Hickey *et al*, 1991; Knopf *et al*, 1998). Such studies have necessitated a re-thinking of the role of the BBB in immune cell trafficking into the central nervous system.

Monocyte and neutrophil trafficking across the BBB
An important part of the body's defense of bacterial or viral infections is the ability of monocytes and neutrophils to invade the tissue and begin phagocytosis of the foreign material. Indeed, infiltration of the monocytes and neutrophils from the bloodstream into the infected tissue is part of the initial immune response. However, there are notable differences in the penetration of monocytes and neutrophils in the BBB and the peripheral microvasculature. First, trafficking of activated leukocytes across the BBB display differences that are dependent on cell type, with monocytes being more capable than neutrophils in moving through the BBB. This was demonstrated in mice injected with various pro-inflammatory stimuli directly into the hippocampus (Andersson *et al*, 1991, 1992). Under normal homeostatic conditions, there was minimal margination and diapedisis of neutrophils and monocytes observed in the BBB. Following hippocampal injection of kianic acid or LPS challenge, a dramatic increase in the margination of neutrophils and monocytes to the BMVEC was seen (Andersson *et al*, 1991, 1992). Although there was an increased attachment of both neutrophils and monocytes to the BMVEC, only the monocytes underwent diapedisis and were able to move through the BBB into the brain. Infiltration of neutrophils through the BBB was only observed following breakdown of BBB integrity, and was in contrast to the rapid and substantial accumulation of neutrophils observed in the CSF through the choriod plexus (Andersson *et al*, 1991, 1992). The relative inability of neutrophils to move across the BBB, suggests that it is the initial monocyte response that is an important mediator of the immune response in the CNS.

Additional evidence in support of monocyte movement across the BBB can be found in the studies of Persidsky and colleagues (1997) and Fiala and co-workers (1997) examing the passage of the normal and stimulated monocytes across an *in vitro* model of the BBB. In these studies, the migration of HIV-infected monocytes was examined in a BBB model consisting of co-cultured BMVEC and astrocytes. As was observed *in vivo*, few monocytes were able to migrate across the BMVEC monolayer under normal conditions (Persidsky *et al*, 1997; Fiala *et al*, 1997). However, following activation of the monocytes with pro-inflammatory mediators such as LPS or tumor necrosis factor, significant increases in monocyte migration across the brain microvessel endothelial monolayers was observed (increases of up to 20-fold over control monolayers). Interestingly, there was no difference between HIV-infected or control (uninfected) monocyte migration across this BBB model, suggesting it is the generalized activation state of the monocyte that is important for penetrating the BBB.

Evidence for selectivity of migration of monocytes versus neutrophils in the *in vitro* BMVEC culture model has also recently been observed (Gan *et al*, 1999). In these studies the adhesion and migration of neutrophils and monocytes were examined in human BMVEC co-cultured with astrocytes. Following exposure of the monolayers to cocaine, there was an increase in adherence of both neutrophils and monocytes to the brain microvessel endothelial cells. Consistent with the *in vivo* studies, while both neutrophils and monocytes attached to the brain endothelial cells, only the monocytes exhibited significant migration across the endothelial cell monolayers (Gan *et al*, 1999).

These studies are in contrast to the early reports demonstrating significant adherence and migration of neutrophils across cultured bovine BMVEC monolayers (Dorovini-Zis *et al*, 1992). However, it should be noted that neutrophil migration was significantly increased in these studies through the use of specific chemotactic gradients produced by formyl-methionyl-leucyl phenylalanine, leukotriene B4 or acetyl-glyceryl-ether-phosphorylcholine. Such studies suggest that the activity of the monocyte migration observed *in vivo* may not be a function of the brain endothelial cells, but rather, the chemotactic agents presented in or around the CNS.

The time course required for the infiltration of monocytes and neutrophils into the CNS is also different from peripheral tissues. Following an inflammatory response outside the brain, there is a rapid infiltration of neutrophils and monocytes that occurs within minutes of the inflammatory stimulus. Peak migration rates for neutrophils and monocytes outside the brain typically occur within 4 h (Cybulsky *et al*, 1988). This is in stark contrast to the time course of infiltration observed in the CNS following acute inflammatory responses. Following direct injection of LPS into the parenchyma of the central nervous system of mice, Andersson and colleagues (1992) observed a delayed appearance of macrophages in the brain. In these studies there was no significant migration of monocytes into the brain parenchyma until 2 days post LPS injection in the hippocampus. While there was a significant increase in the number of macrophages in the brain 2 days after LPS stimulus, the number of macrophages were significantly less than observed in non-CNS tissue following LPS challenge (Andersson *et al*, 1992). The observation that the number of macrophage cells in the brain (following LPS injection) was reduced when peripheral circulating monocytes were depleted, suggests that a significant number of the macrophages in the brain parenchyma were attributable to the infiltration of monocytes across the BBB (Andersson *et al*, 1992). These studies indicate that the time course for entry of the monocytes into the brain is significantly delayed compared to other tissues outside the BBB.

Lymphocyte trafficking across the BBB
Under normal conditions, there are minimal numbers of lymphocytes detected within the CNS (Hauser *et al.*, 1983; Hickey *et al*, 1991; Hickey and Kimura, 1987). This is the result of reduced penetration of lymphocytes across the BMVEC and the low levels of major histocompatibility complex expression in the normal CNS (Wong *et al*, 1984). However, during inflammatory conditions such as viral, bacterial or parasitic infections in the CNS, there is a significant accumulation of lymphocytes in the brain.

The ability of T lymphocytes to penetrate the BBB is illustrated in the studies of Hickey and colleagues (1991). In these studies, the penetration of T lymphocytes were examined in rat brain. The findings of these studies were that non-activated T lymphocytes had a minimal penetration into the CNS. However, activated T lymphocytes (i.e. lymphoblasts) were quite capable of penetrating the BBB (Hickey *et al*, 1991). The activated T lymphocytes crossed the BBB in a random fashion, and were not influenced by the specific antigen, major histocompatibility compatability, or phenotype of the T cell. Passage into the CNS occurred over a period of hours and, unless the specific antigen was recognized, the T lymphocytes exited the CNS within 24 – 48 h (Hickey *et al*, 1991). These studies suggest that activated immune cells can readily penetrate the BBB. However, without recognition of specific antigen targets within the brain, the immune cells are recycled back to the blood.

There have been several *in vitro* studies demonstrating the penetration of T lymphocytes across BMVEC (Lou *et al*, 1996; Joseph *et al*, 1997; Adamson *et al*, 1999; Wong *et al*, 1999). In the studies of Wong and colleagues the penetration of lymphocytes was shown to occur both through movement across the cytoplasm of the BMVEC and through the tight junctions between the BMVEC. The lymphocyte penetration occurred without any apparent disruption of the integrity or solute permeability of the BMVEC monolayer (Wong *et al*, 1999). While the actual mechanism of penetration of immune cells across the BBB is discussed below, it should be noted that the *in vitro* studies are in agreement with the *in vivo* observations regarding lymphocyte penetration into the brain in that adherence and penetration is dependent on the generalized activation state of the immune cell and the BMVEC.

Less is known about the migration of B cells into the CNS. Evidence that B cells do enter the brain can be found in the studies of Hatalski *et al* (1998a,b) and Knopf *et al* (1998). In the studies by Hatalski

and colleagues (1998a,b) the role of B cells in the humoral response to Borna disease was examined. The rats infected with Borna disease showed an acute immune response characterized by the migration of T cells and macrophages into the sites of infection in the CNS. There was also a chronic immune response which was observed in the rats infected with Borna disease. In the chronic stage, there was, among other things, a migration of B cells into the brain parenchyma (Hatalski et al, 1998a). The purpose of the B cell migration in the Borna virus infected rat was proposed to be the production of antibody in the CNS, as the BBB was intact and would not allow peripherally circulating antibodies to enter into the brain (Hatalski et al, 1998b).

Similar observations have recently been made regarding the movement of activated B cells into the brain by Knopf and co-workers (1998). In these studies, antigen was injected into the caudate nucleus of naive rats and rats pre-immunized against the antigen. Around the site of the cannula where the antigen was injected into the brain parenchyma of naive rats, the authors noted the presence of a scattering of B cells, as well as other leukocyte and lymphocyte cells (Knopf et al, 1998). However, in the pre-immunized rats, there was a much greater accumulation of antigen specific B cells, macrophages and T cells at the injection site within the caudate nucleus. In addition to the immune cells detected at the injection site, antibodies were also present within the CNS of the rats. Since there was no evidence of BBB disruption, the authors concluded that the antibodies detected in the CNS were the result of the movement of B cells into the brain (Knopf et al, 1998).

Importance of adhesion molecules in immune cell trafficking across the BBB
Identifying the cellular mechanisms by which the BBB allows the movement of the immune cells into the CNS is critical for understanding the immunology of the BBB. In this regard, attention has focused on the adhesion molecules found in the vascular endothelial cells that form the BBB. Some of these adhesion molecules, like the cadherins are involved in the formation of intercellular tight junction complexes between the brain microvessel endothelial cells. Other adhesion molecules form the selectins that are involved in the initial binding of the leukocytes to the endothelial cells. And still others in the integrin subclass have a role in the secondary adhesion process and subsequent migration of the various leukocytes across the endothelial cells. Of the three general classes of adhesion molecules, it is alterations in the selectin and integrin classes of adhesion molecules that most affect penetration of leukocytes across the BBB.

Early in the study of the cell adhesion molecules, it was suspected that the apparent selectivity with regard to leukocyte penetration of the BBB was due to the expression of BBB-specific cell adhesion molecules. However, more likely, the selective permeability of the BBB to the leukocytes is related, in part, to the level of expression of the various adhesion molecules during normal and inflammatory conditions. Immunohistochemical studies of frozen brain tissue show minimal to non-detectable levels of expression of various integrin adhesion molecules, including ICAM-1, VCAM-1 and PE-CAM-1 in the BBB (Williams et al, 1996; Washington et al, 1994; Engelhardt et al, 1994; Rossler et al, 1992). However, during activation of immune responses in the CNS, as seen in such conditions as MS or EAE, there is a significant up-regulation of the expression of ICAM-1, VCAM-1 and PECAM-1 in the brain microvessels (Williams et al, 1996; Washington et al, 1994; Engelhardt et al, 1994; Bo et al, 1996).

Studies with cultured BMVEC also indicate low levels of expression of ICAM-1 and VCAM-1 under normal unstimulated conditions (Lou et al, 1996; Nottet et al, 1996; Gan et al, 1999). Furthermore, there was a significant up-regulation of ICAM-1 and VCAM-1 expression in the cultured brain microvessel endothelial cells following exposure to pro-inflammatory agents or activated T cells (Lou et al, 1996; Nottet et al, 1996; Gan et al, 1999). These studies suggest that the increased penetration of monocytes and T cells into the central nervous system during activated or inflammatory conditions may be the result of increased expression of integrin adhesion molecules.

Alterations in the expression of the selectin group of adhesion molecules in the BBB during normal and inflammatory conditions is less conclusive. Studies by Egelhardt and colleagues (1997) in mice under both normal conditions and following the induction of EAE failed to detect the expression of mRNA for either E- or P-selectin in the brain microvessel endothelial cells. Furthermore, treatment of the mice with anti E- or P-selectin antibodies had no appreciable effect on the migration of activated T cells across the BBB (Engelhardt et al, 1997). This suggested that E- and P-selectin were neither constitutively expressed under normal conditions in the BBB or induced during inflammatory conditions in which the BBB becomes permeable to the immune cells.

In contrast, E-selectin has been detected in cultured human brain microvessel endothelia cells (Lou et al, 1996; Nottet et al, 1996; Wong et al, 1999). Furthermore, exposure of human brain microvessel endothelial cells to activated lymphocytes resulted in an increased expression of E-selectin (Lou et al, 1996). Evidence for the potential involvement of selectin adhesion molecules in the penetration of lymphocytes in the BBB is the observation that treatment of stimulated human BMVEC monolayers with E-selectin antibodies significantly reduced lymphocyte migration across

the monolayer (Wong *et al*, 1999). In a different *in vitro* model, the adherence of monocytes to human brain tissue was reduced following treatment with E-selectin antibodies (Nottet *et al*, 1996). Combined treatment with both E-selectin and VCAM-1 antibodies reduced monocyte adherence to brain microvessels by greater than 70% indicating the potential involvement of both the selectin and integrin adhesion molecules in the adherence and translocation of monocytes across the BBB (Nottet *et al*, 1996).

In vitro studies using cultured human BMVEC also demonstrate reduced lymphocyte penetration following treatment with antibodies to ICAM-1, PECAM-1 or E-selectin (Wong *et al*, 1999). These studies highlighted the importance of an activated or 'primed' endothelial cell preparation, as lymphocyte adherence and penetration across unactivated human BMVEC monolayers was minimal. Interestingly, while adherence of T cells to TNF-activated BMVEC monolayers was dependent in part to VCAM-1, antibodies to VCAM-1 did not effect the migration of the lymphocytes across the monolayers (Wong *et al*, 1999).

Perhaps the most convincing evidence for the selectins involvement in the translocation of the immune cells across the BBB are the studies in selectin knock-out mice (Tang *et al*, 1996). In these studies, mice genetically deficient in P-selectin, or both P- and E-selectin were examined for their susceptibility to acute cytokine-induced meningitis. In these studies, the control mice treated with the cytokine developed severe meningeal inflammation that was characterized by significant increases in leukocyte accumulation in the central nervous system and enhanced BBB permeability (Tang *et al*, 1996). However, in the P-selectin knock-out mice, leukocyte infiltration in the CNS and BBB permeability were significantly inhibited. Furthermore, in the doubly deficient P- and E-selectin mice, there was a nearly complete inhibition of leukocyte migration and BBB permeability (Tang *et al*, 1996). These studies suggest that the selectins do have a role in the increased penetration of immune cells in the CNS observed following inflammatory events in the brain.

While it is apparent that adhesion molecules have a major role in the margination and diapedesis of immune cells across the BMVEC, little is know about the second messenger systems that may be activated to facilitate the penetration of the immune cells across the BBB. In this regard, the studies of Adamson *et al* (1999) have shed light on cellular events which occur as a result of binding of lymphocytes to ICAM expressed in the BMVEC. By cross-linking the ICAM-1 expressed on an immortalized rat brain endothelial cell line with IgG directed at anti-ICAM-1 antibodies, Adamson and colleagues were able to demonstrate a clustering of ICAM-1 and an increase in actin stress fiber formation in the BMVEC. The stress fiber formation was associated with the activation of the small guanosine triphosphate-binding protein, Rho, suggesting that the Rho signalling pathway was involved in ICAM-1 mediated lymphocyte migration in the BBB. Further evidence for activation of Rho in the BMVEC for penetration of the immune cells is the experiments performed in the presence of the Rho inhibitor, C3 transferase. While activated T lymphocytes were still able to adhere to the BMVEC monolayers, treatment with C3 transferase significantly reduced the migration of the lymphocytes across the monolayer, the formation actin stress fibers, and the accumulation of Rho in the cells (Adamson *et al*, 1999). These studies indicate that the adhesion molecules, such as ICAM-1, not only bind to the lymphocytes as they travel through the bloodstream, but also activate the endothelial cell through various signalling pathways to facilitate migration of the lymphocytes across the BBB.

Role of the blood-brain barrier as an immune response modifier

It is clear that the brain endothelial cells are affected by a number of different pro-inflammatory mediators such as cytokines, prostaglandins, and bradykinin. In many cases, these pro-inflammatory agents have an important role in initiating the changes in the permeability or adhesion properties of the endothelial cells that allow the immune cells to infiltrate the CNS. However, in addition to their effects on BBB permeability, activation of the brain microvessel endothelial cells by various pro-inflammatory agents may serve other purposes important to the immune response.

Evidence for the BBB to act as an immune response modifier is illustrated in the studies of Quan *et al* (1997) and Herkenham *et al* (1998) in which the temporal expression of intracellular markers of cellular activation was examined following peripheral administration of either lipopolysaccharide or interleukin-1, respectively. In both these studies, there was a time dependent response to the pro-inflammatory agents, with increased expression of c-*fos* and inhibitory factor kappaβalpha first observed (within 30–60 min) in the barrier cells of the choriod plexus and blood-brain barrier, and circumventricular organs that are not protected by the BBB (Quan *et al*, 1997; Herkenham *et al*, 1998). Interestingly, following the initial burst in activity observed in the brain microvasculature, the microvessel endothelial cells returned to their quiescent state, and a delayed increase in activity was observed in the cells within the CNS that were adjacent to the BBB. The temporal cellular activation pattern observed, prompted the authors to suggest that peripheral immune response modifiers

may activate cells within the CNS by secondary mediators released from the cells of the BBB and blood-cerebral spinal fluid barrier.

In considering the identify of the secondary immune response modulators released from the brain microvessel endothelial cells there are a multitude of potential candidates. The vascular endothelium expresses several different cytokine and cytokine receptors. The demonstration that exposure of human brain microvessel endothelial cell monolayers to stimulated T lymphocytes results in the secretion of interleukin 6 and interleukin 8 from the endothelial cells supports the contention that cytokines released from the BBB may act as secondary immune response modulators (Lou *et al*, 1996). The cytokines released from the brain endothelial cells may act to modulate activity of themselves or other nearby cells such as the astrocytes and glial cells. Other potential immune modulators released by the endothelial cells of the BBB are arachidonic acid metabolites such as prostaglandins and thromboxanes. Support for the possible involvement of the prostaglandins is the studies by Quan and coworkers (1998) that demonstrated an induction in cyclooxygenase 2, one of the isoenzymes involved in prostaglandin production, in brain microvessel endothelial cells several hours after exposure to bacterial LPS or cytokines. A similar induction of cyclooxygenase 2 with a resulting increased release of prostaglandin E, has been observed in our laboratory in primary cultured bovine brain microvessel endothelial cell monolayers following treatment with tumor necrosis factor (manuscript in preparation).

The concept of the BBB acting as a relay point for either amplifying or dampening immune responses in the brain is an area that is underdeveloped. Most attention has focused on the role of the BBB in allowing the penetration of immune cells into the CNS. While immune cell trafficking across the BBB is an important event, the BMVEC that form the BBB also have the ability, through increased permeability, altered release, or cell-to-cell junctional communication, to control the immuno-responsiveness of the brain.

Summary

The BMVEC forming the BBB have an important role in mediating immune responses in the central nervous system. Given the anatomical location of the brain microvessel endothelial cells, they represent a point of passage for immune cells from the systemic blood into the extracellular environment of the brain. While normal trafficking of the immune cells across the BBB is limited, under the appropriate conditions, the brain microvessel endothelial cells can allow the selective entry of leukocytes into the central nervous system. Characterization of the stimuli required to produce the change in BBB permeability to the cells of the immune system and the cellular factors involved in such changes are areas of intense examination. Both *in vivo* and *in vitro* studies suggest the increased passage of immune cells across the BBB during inflammatory conditions involve changes in the expression of adhesion molecules in the endothelial cells that form the BBB. The presence of various chemokines may also contribute to the permeability of the BBB to select leukocytes observed during inflammatory conditions.

The brain microvessel endothelial cells also act as an immune response modifier, releasing agents that in turn act upon cells within the central nervous system. One important target of these agents released from the brain microvessel endothelial cells is the microglia. The importance of this can not be overlooked as modification of the activity of the microglial cells, the resident monocytes of the central nervous system, would be expected to play an important role in immune responses in the brain. In this regard, the release of endogenous immune response modifiers (i.e. cytokines, prostaglandins, etc) by the endothelial cells in the BBB may act to either intensify or inhibit the propagation of the immune response in the brain.

Acknowledgements

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