A dysfunctional BBB is a critical pathological event leading to the anomalous presence of Igs and excessive amounts of Aβ42 into the brain. In 2001, I was trying to imagine how a clinician could assess the integrity of the BBB in subjects, perhaps by some imaging modality using a tracer to see if it penetrates from potential leaky vessels into the brain.

I strongly feel that if a person has a dysfunctional BBB, it is logical to propose that there may also be an association with other cardiovascular disease factors such as hypertension, stroke, diabetes, and high cholesterol. Interestingly, these are all risk factors of AD. But how can you determine or assess the integrity of the BBB? Also it’s not clear if dysfunctional BBB occurs in stages to first leak smaller proteins such as Aβ42 into the brain, and then larger proteins such as Igs. If true, then I wonder if the initial pathological events in the AD brain are related to excessively uncontrolled entry of Aβ42 into neurons that die and release factors perhaps through the reactive inflammatory cells that make the BBB even more porous that allows the larger immunoglobulins to wreak their havoc.

Since vascular leakage is a normal physiological response to histamines, viruses, and infections, I wondered if they might also play a role in BBB health. For example, it is possible that a viral or bacterial infection could trigger the BBB to be more permeable to allow antibodies into the brain that would cause a subject with dementia or MCI to deteriorate further. It has been well reported that certain biochemical and physiological changes such as increased levels of inflammatory cytokines, hypoxia-inducible factor-1α, and matrix metalloproteinase-9 in neurological conditions could cause BBB disruption and hence vascular leakage. Is the pathology of the BBB from the inside-out (meaning factors in the brain cause BBB leakage), or from the outside-in (meaning factors in the vasculature cause BBB leakage)?

Interestingly, the need to regulate the entry of vascular components into the brain also exists in other areas of the body, such as in the retina where...
there is a blood–retina barrier (BRB). In 2004, I asked my optometrist how much detail you could really see when examining my retina after pupil dilatation. She said she could identify tiny hemorrhages, or tiny breaks in the retina vasculature. I shared my thought that perhaps the retina is the “window” into the brain, or more specifically, “the window of AD” and if the retina had issues, one can propose that the BBB may also have issues.

**BRB, A VASCULAR HARBINGER**

I began to do extensive literature searches on the association of BBB and BRB, retina pathology and AD, and glaucoma and AD. To my surprise, there were earlier data suggesting that the BRB can be dysfunctional in eye pathologies\(^2,3\) and that there is an association with vascular diseases, which is again a risk factor for AD.\(^4\) In fact, many reports support the notion that AD may actually be more a vascular disease than a neurodegenerative one.\(^5\) De la Torre\(^5\) provided a compelling argument while surveying published literature that revealed no evidence that amyloid deposition is neurotoxic in human beings or that it results in neurodegenerative changes involving synaptic, metabolic, or neuronal loss in human or transgenic mouse brains. By contrast, the data supporting AD as a primary vascular disorder are more convincing, which come from epidemiological, neuroimaging, pathological, and clinical studies.\(^5\)

It was concluded in the paper that endothelial dysfunction is an early event in AD patients,\(^6\) further supporting my model.

Endothelial damage may actually be the primary event on BBB and BRB dysfunction, suggesting that the primary pathological event may occur from outside the brain. Endothelial damage is also a primary event in diabetic retinopathy, as BRB breakdown precedes pathological retinopathy in diabetes.\(^7-9\) It has been similarly suggested through animal transgenic AD mouse models that vascular pathologies “precede” the presence of plaques and cognitive impairments.\(^10\) In addition to the detection of amyloid in the cerebrovasculature, which is particularly present in the leptomeningeal and cortical arteries, resulting in cerebral amyloid angiopathy, it was also determined that amyloid is targeted to the vasculature in a mouse model of hereditary cerebral hemorrhage with amyloidosis.\(^10,11\) Also, the Tg-SwD1 mice (transgenic mouse expressing neuronal Aβ precursor protein harboring the Swedish and Dutch/Iowa mutations) display early onset and robust accumulation of Aβ in the
brain with a high association with isolated cerebral microvessels that are highly associated with occasional signs of microhemorrhage.\textsuperscript{12}

So, can you evaluate BRB integrity through a routine eye exam? Apparently so, as there are also data on detailed eye examinations that can reveal BRB dysfunction, which can lead to albumin in the vitreous humor of the eye.\textsuperscript{13} For example, all people with diabetes (both type 1 and type 2) are at risk of diabetic retinopathy, which is the reason for a comprehensive eye exam at least once a year for diabetic patients. Between 40\% and 45\% of Americans diagnosed with diabetes have some stage of diabetic retinopathy that often has no early warning signs, and if left unchecked by an eye care professional, results in small hemorrhages over time (Figure 14.1). Based on the Optomap\textsuperscript{®} website (http://www.optos.com/en-US/Professionals/Image-library/Color-fundus-images/Diabetic-retinopathy/), their scanning system can present images of leaking blood vessels, pale, fatty deposits on the retina, which are themselves signs of leaking blood vessels. Perhaps this kind of technology could be used to assess the integrity of the retina blood vessels in patients with AD and MCI diagnoses to validate this hypothesis.

Fig. 14.1. Optomap\textsuperscript{®} 100\° retinal image of a patient with diabetic retinopathy. This image demonstrates the optic nerve and macula in a patient’s right eye. Small hemorrhages (arrows) can be visualized around the macula (http://www.optos.com/en-US/Professionals/Image-library/Color-fundus-images/Diabetic-retinopathy/).
Beyond the current standard fundus photography, a promising non-invasive method of optical coherence tomography was presented as a way to quantify retinal thickness. Microaneurysm counts, assessment of length and diameter of retinal vessels, and computerized quantification of all pathological elements may also be useful as diagnostic tools and/or efficacy end points. Other nonimaging studies that attempt to assess the permeability of the BRB and BBB have been proposed and include detection of sucrose and albumin. For example, sucrose was used to determine the permeability of the BRB and BBB simultaneously using an intravenous injection in a rat.

There is also a positive correlation between retinal pathology and AD. AD patients often exhibit poor vision and others show visual signs of impairment. These clinical manifestations are also supported by regional neuron loss and glial changes in the ganglion cell layer of the retina; however, no explanation was given to the causes of these losses of neurons. I suspect the α7 receptor and Aβ42 may be involved. It was of interest that, despite extensive neuronal loss, no neurofibrillary tangles (NFT) were observed in the retina as shown also for the visual cortex, suggesting that neuronal loss could occur without NFT formation. It was interesting to note that the neuronal loss in the AD eye was different from the neuronal loss from patients with glaucoma. Although there is a decrease in the neurons of the eye during normal aging, the decrease of neurons in the AD eye is well outside the normal range and was not correlated with age, in contrast to the normal.

It is my belief that pathological data obtained by high-resolution analysis of the BRB could indirectly assess the integrity of the BBB, and BBB dysfunction can predict AD, that is, I see the retina as a harbinger of AD through the integrity of the BBB. This is only half of the story as I believe there is at least a two-level paradigm for the causes of AD that starts with the BBB dysfunction leading to the unregulated, and certainly unwelcomed, presence of vascular components such as amyloid and autoantibodies. Blood–brain dysfunction is also reported in Binswanger’s disease (BD), where serum-derived immunoglobulins have been observed in the brain, although it was unclear if BBB dysfunction was a primary or secondary event. Furthermore, BD is very much a dementia disease and has at least two of the following criteria: hypertension (75%) or known systematic vascular disease, evidence of cerebrovascular diseases (60%), and/or subcortical brain dysfunction.
Autoimmunity is also common in retina pathologies. For example, serum autoantibodies to optic nerve head glycosaminoglycans have been reported in patients with glaucoma. Furthermore, evidence suggests that autoimmune damage to the optic nerve in glaucoma may occur directly by autoantibodies to heat shock protein 27 or indirectly by way of a “mimicked” autoimmune response to a sensitizing antigen to heat shock protein 60 or rhodopsin, which in turn injures retinal ganglion cells. The presence of autoantibodies to neuron-specific enolase was also detected in glaucoma patients that cause retinal dysfunction in vivo. Although it was not mentioned in this article, it is believed that the only way antibodies can pathologically affect the retina is through a BRB dysfunction. However, they similarly claim that antibody avidity, affinity, and specificity, as well as antibody class, will determine the extent of the pathology.

A positive relationship between diabetes, aging, dementia, and stroke has also been reported. Diabetes was presented as a probable risk for Alzheimer’s disease mainly through the cerebrovascular disease causes, thereby strengthening the association of AD, diabetes, and the BBB. It is also interesting that successful diabetes treatment produces significant cognitive improvement.

It is unclear whether small retina changes may be associated with any abnormal clinical features, but work could be “focused” to see if there are subtle changes in the retina vasculature and if they correlate to systematic vascular health and to changes in the BBB function that could lead to cognitive impairment. I feel that it will be invaluable data to collect at perhaps minimal costs, and the rewards could be astronomical. Also, I would explore the frequency of subtle retinal issues in MCI patients as another possible prognostic marker for developing AD.

**IN VIVO BBB SUPPORT**

With the publications I had up to this point I was disappointed that more interest was not generated. I designed another study that could validate my findings. In a series of experiments, mice were injected with fluorescent Aβ42 through the tail vein, and a few other mice were injected with the pertussis toxin, which is a known toxin used to erode the BBB. The goal was to unequivocally show that Aβ42 can get into the brain through a BBB leak, and will get into the neurons over
time. In only an hour, the fluorescently labeled Aβ42 crossed the BBB of pertussis toxin-treated mice and entered the brain tissue as vascular-associated diffuse plaques and also into populations of neurons (Figure 14.2).36

Fig. 14.2. Blood-borne green fluorescent Aβ42 and Aβ40 cross the blood–brain barrier of PT-treated mice and enter into the brain tissue and bind selectively to neurons (but not glial cells) in the cerebral cortex, subcortex, hippocampus, and cerebellum. In all images, free-standing arrows designate neurons. (A–C) Within 1 hour postinjection, green fluorescent Aβ40 leaks from local vessels (red dotted line) and binds to the surfaces of neurons. Neurons positioned outside of the leak zone show little or no labeling (yellow arrow). (D–F) Neurons with bound green fluorescent Aβ42 are abundant in the indicated brain regions, and comparison of green fluorescent and blue-DAPI (nuclei) image pairs of the same section reveals a preferential binding of Aβ42 on the large neurons occupying the region enclosed by the dentate gyrus. “+” added for orientation. (G) At 48 hours postinjection, labeled neurons are still abundant. (H, I) Some of the larger pyramidal neurons show small bright, green fluorescent Aβ42-positive granules in the basal portion of the perinuclear cytoplasm. (J, K) Basal level of autofluorescence in the cerebral cortex demonstrated in a mouse treated with only pertussis toxin. (L) Mouse given saline in lieu of PT and subsequently treated with green fluorescent Aβ42 demonstrates confinement of fluorescence in the context of an intact BBB. GC, granular cell; PT, pertussis toxin. Scale bar equals 20 μm (I), 30 μm (H), 60 μm (A, C, L, J, K), 100 μm (B, D, L), and 150 μm (E–G). (Courtesy of Brain Res. 2007;1142:223–236.)
This was proof that \(A\beta42\) can penetrate the brain through a dysfunctional BBB and once in the brain, the \(A\beta42\) will internalize in neurons; therefore, although the neurons are dying from the “inside-out,” the true general way to describe the pathological events in the brain is from the outside of the brain. The follow-up study would be a continuation to show that over time these fluorescently labeled \(A\beta42\) neurons die to form dense-core, inflammatory plaque. Finally, for additional absolute certainty, the study should track the behavior of these mice in validated learning models such as the water maze test with well-executed controls.

REFERENCES


