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Blood-brain barrier damage, but not parenchymal white blood cells, is a hallmark of seizure activity

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Abstract

It has long been held that chronic seizures cause blood-brain barrier (BBB) damage. Recent studies have also demonstrated that BBB damage triggers seizures. We have used the BBB osmotic disruption procedure (BBBD) to examine the correlation between BBB opening, pattern of white blood cells (WBCs) entry into the brain and seizure occurrence. These findings were compared to results from resected epileptic brain tissue from temporal lobe epilepsy (TLE) patients.

We confirmed that a successful BBB osmotic opening (BBBD) leads to the occurrence of acute epileptiform discharges. Electroencephalography (EEG) and time-joint frequency analysis reveal EEG slowing followed by an increase in the 10-20 Hz frequency range. Using green fluorescent protein (GFP)-labeled WBCs (GFP-WBCs) suspended in Evans Blue we found that, at time of BBB-induced epileptiform discharges, WBCs populated the perivascular space of a leaky BBB. Similar results were obtained at time of pilocarpine seizure. No frank WBCs extravasation in the brain parenchyma was observed.

In TLE brain specimens, CD45-positive leukocytes were detected only in the vascular and perivascular spaces while albumin and IgG extravasates were parenchymal. The pattern was similar to those observed in rats.

Our data suggest that neither acute-induced nor chronic seizures correlate with WBC brain parenchymal migration while albumin and IgG brain leakage is a hallmark of acute and chronic seizures.

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Keywords

seizures; white blood cell; albumin extravasation; temporal lobe epilepsy (TLE); electroencephalograms (EEG); cerebrovasculature

Introduction

Seizures and epilepsy affect a significant portion of pediatric and adult populations. The mechanisms underlying their pathogenesis are not entirely clear [1]. Beside the extensively studied neuronal mechanisms of seizures, recent studies have demonstrated that blood-brain barrier (BBB) damage may be an important etiological factor in the development of seizures [2-6]. However, the temporal and causal positioning of cerebrovascular damage and seizures in chronic epilepsy is still debated.

The BBB separates brain parenchyma from the blood. Its main function is to regulate brain homeostasis [7;8]. Reports on mechanisms of BBB damage leading to the development of seizures or epileptiform discharges suggest that serum constituents such as albumin or potassium might have participated in their initiation [5;9]. Electron microscopic studies of brain distribution of human serum albumin in spiking regions demonstrated extravasation into the neuropil and in the basal lamina of the blood vessels [10;11]. The latter space was defined as extravascular or perivascular. It was also proposed that serum albumin causes delayed seizures by binding to specific glial receptors while extracellular potassium increases and glutamate imbalance induce synaptic facilitation [5;9].

While loss of BBB function can promote seizure, less clear is whether white blood cells (WBCs) extravasate into the brain parenchyma possibly contributing towards the pathogenesis of seizures. Thus, even though the interaction between neutrophils and endothelial cells in the brain may be responsible for the generation of seizures [12], the presence and the possible significance of leukocytes in the brain parenchyma are still debated.

We used carotid artery injection of hypertonic mannitol to achieve disruption of BBB in rats. We have analyzed the pattern of serum protein and WBC extravasation using Evans Blue and GFP-transfected WBCs. In temporal lobe epilepsy we used serum albumin and immunoglobulin-G to detect BBB damage while the leukocyte marker CD45 was used to identify WBCs. We quantified the amount of albumin and the number of WBCs present intravascularly, perivascularly and in the brain parenchyma. Results relative to pattern of WBC extravasation at time of pilocarpine-induced status epileptics (SE) are also included.

Results

BBBD-induced EEG abnormalities in rats

We recorded EEG before, during and after hypertonic mannitol injection into the internal carotid artery (Figure 1A). BBB opening was evaluated by gross anatomy (Figure 1A) or fluorescent microscopy (Figure 2). BBB was successfully disrupted in 20% of the rats and only under this circumstance acute EEG changes were detected (Figure 1C). In particular, we recorded a brief EEG slowing (*arrowheads* in Figure 1B) followed by a gradual increase in activity. Examples of EEG changes are depicted in Figure 1B1. Time-joint frequency analysis was performed to visualize changes which were not immediately apparent in the time domain. Both frequency and amplitude were increased after BBBD (Figure 1B1-D). A similar pattern of frequency and amplitude increase was observed after BBB opening of in human subjects (see Supplemental Figure 1). This is in agreement with our previous results

[13;14]. Consistent with the hypothesis linking BBBD to acute seizures, in both cases (rodent or human) epileptiform discharges occurred only when the BBB was successfully disrupted by intrarterial mannitol.

Pattern of WBCs and serum proteins brain extravasation during seizures in rats

As shown in Figure 2A-B a successful BBBD procedure caused abundant extravasation of FITC-albumin or Evans Blue in the injected hemisphere compared to the contralateral brain. Note that FITC-albumin and Evans Blue signals in the contralateral hemisphere were mostly confined inside the blood vessels. Sham-operated rat displayed negligible amount of protein leakage across the BBB vessels of both hemispheres (see examples: sham in Figure 2B2 and intact vessel in Figure 5A2).

In order to determine the pattern of WBC brain extravasation at time of BBBD-induced EEG abnormalities, we injected a bolus containing GFP-transfected autologous WBCs (see methods for details). Figure 2B1 shows that in spite of extensive leakage of serum protein (Evans Blue, *red fluorescence*), the majority of GFP-WBCs remained inside the vessels or were distributed in the immediate perivascular space. A quantification of these results is provided in Figure 4. Note that, during pilocarpine-acute SE, BBB damage (Evans Blue leakage) was not accompanied by significant WBC brain extravasation as evaluated by CD45 staining (Supplemental Figure 2). CD45 positive cells were detected in the subdural space (Supplemental Figure 2B).

To locate GFP-WBCs in the brain and to determine their relationship to brain vessels, we first identified and quantified the amount of leaky and non-leaky vessels in temporal, frontal and thalamic regions of the rats. We found, on average, 2 leaky vessels and 6.5 intact vessels in an area of 1 mm³ in these three regions (Figure 4A). We next quantified the number of GFP-WBCs in the same regions. As quantified in Figure 4B, in leaky vessels majority of GFP-WBCs were located in the perivascular space. Virtually no GFP-WBC was detected in the brain parenchyma in all three regions tested. In correspondence of intact vessels (contralateral hemisphere to BBBD procedure and sham operated rats), the majority of WBCs were contained in the intravascular space (Figure 4C).

Pattern of WBCs brain and serum proteins extravasation in TLE patients

We have studied BBB damage in brain specimens obtained from TLE patients undergoing resection surgery (Table 1). Brain specimens were obtained from the operating room and care was taken (*e.g.*, proper oxygenation and fixation procedures) to avoid damage (also see Methods and Discussion). As shown in Figure 3A1-A9, IgG- and albumin-targeting antibodies showed the presence of extravasates around blood vessels and in the brain parenchyma. Note that the pattern of IgG (3A1-A3) or albumin (3A4-A9) extravasation in epileptic human brain was similar to the one observed in rats after BBBD (Figure 2) or pilocarpine-seizures (Supplemental Figure 2). In addition to spotty leakage, human TLE brain displayed large extravasation areas (delimited by a *dotted line* A3 and A5).

In the brain of TLE patients, extravasation of leukocytes was assessed by detection of CD45 immunoreactivity (Figure 3B). The number of CD45 positive cells was measured in correspondence of leaky vessels in those brain regions displaying albumin extravasation (*e.g.*, adjacent slices). As in rat brains challenged by acute seizures, we found in TLE several cells accumulated in the perivascular space of small or large caliber vessels (Figure 3B). The number of cells present in the parenchyma was low (Figure 4D).

Quantification of BBB damage

We quantified the amount of albumin inside and outside the vessels in BBBD rat brain and TLE human brain samples. As shown in Figure 5A for rats and Figure 5B for TLE brain specimens, FITC fluorescent signals spread outside leaky vessels indicating the diffusion of albumin into the brain parenchyma through an impaired BBB. There was a statistical difference between the fluorescent units measured in brain parenchyma surrounding the leaky and non-leaky vessels in both rats and humans. No fluorescent signal was detected in contralateral hemisphere of BBBD procedure and sham operated rats (intact BBB, Figure 5A).

Discussion

The main finding of the work presented herein is that significant extravasation of WBCs into brain parenchymal was not observed during acute epileptiform discharges or SE (BBBD and pilocarpine) and in TLE specimens. Albumin or IgG leakage is a hallmark of acute and chronic seizures.

Our results have shown a similarity between vascular (*e.g.*, BBB damage) and cellular (*e.g.*, pattern of WBC extravasation) events in the presence of spontaneous (TLE) or provoked (BBBD, pilocarpine) epileptiform discharges or seizures. In addition, the similarity of rat and human data (Figure 1 and Supplemental Figure 1) validates the use of BBBD in rats as a means of studying the generation of iatrogenic seizures. A corollary to this study was obtained by comparing EEG data from patients undergoing osmotic BBB disruption and rats undergoing the same procedure (Supplemental Figure 1, see also our previous studies [13;14];[15;16]. A slowing and silencing of the EEG (Figure 1 and Supplemental Figure 1, *arrowhead*) was evident. The mechanism of mannitol-induced EEG slowing is currently unknown.

Relevance of BBB and WBCs to etiology of seizures

Several lines of research support BBB damage as an etiologic mechanism of seizures [3-5;17]. Recent studies have demonstrated that peripheral inflammatory processes perturbing the interaction between BBB and WBCs are seizure inducers [12;18;19]. A few recent reports have investigated the presence of WBCs in the brain parenchyma of epileptic human brain tissue [12;20-23]. However, all the evidence accumulated do not point to a chief presence of WBCs in the brain parenchyma of epileptics. In addition to post-mortem or resected brain tissue, we have also evaluated the presence of WBC extravasation in the settings of acute iatrogenic seizures. In our experiments with GFP-labeled autologous WBCs we have seen a minimal WBC presence in the brain parenchyma after acute seizures. In human TLE brain, we used an antibody against CD45 (a leukocyte marker). We found a similarity with what we found after acute, induced seizures, *i.e.*, minimal numbers of cells homing in the brain parenchyma (Figure 4, Supplemental Figure 2 and Supplemental Table 2). This is also in agreement with the fact that WBC extravasation under *sterile* conditions is an uncommon event [24;25]. Supplemental Table 2 suggests that, despite of apparent species and timing related differences, a common thread of WBCs blood-to-brain distribution can be postulated. Thus, the common denominator of human and animal studies herein summarized is the scarce parenchymal WBCs presence in contrast to a perivascular accumulation [23].

This analysis is however not devoid of pitfalls. One issue at heart is whether or not extravasation of cells into the brain is a requirement for seizures. In our experiments, even when brain samples were processed after epileptiform discharges development, the lack of parenchymal WBCs is consistent with the hypothesis that extravasation was not necessary

for the development of such EEG abnormalities. The possibility exist that the time window chosen is too short to allow WBCs into the brain. However, this does not relate to BBBD seizure development since epileptiform discharges were already recorded at time of sampling. Moreover it is plausible that the event of brain extravasation is extremely brief. In other words, it might be that cells extravasate and then return into the blood stream within a few minutes between EEG abnormalities and sacrifice. The latter may be not likely since the process of trans-mural movement of cells require time, and molecular triggers [21]. However, further studies are needed to rule out this possibility.

Moreover, we did not evaluate the patterns of BBB interaction and brain entry using markers for sub-population of WBCs. However, the lack of general CD45 immunoreactivity in the brain parenchyma does not support further investigation since this antigen is expressed by virtually all WBCs. Further studies (e.g., electron microscopy) will better clarify the exact positioning of WBCs at the BBB during seizures.

Another concern regards the surgical *manoeuvre and* intra-operative monitoring operations (e.g., subdural grids) that could induce pro-inflammatory process and BBB leakages. Moreover, pre-surgical condition of the patients (e.g., pre-existing inflammation). Post-resection steps (e.g., modality of fixation) could also affect tissue integrity and the results. While appropriate tissue handling was performed (see also Methods), these issues are, in our opinion, not crucial to this particular study because we did not find any evidence of inflammation in the tissue other than a few cells that appear to be lodged in the perivascular space.

Relevance for a BBB mechanism of seizures

In the past few years, the relationship between BBB damage and the development of seizures has been a topic of extensive research. It is known that traumatic brain injury, ischemic or vascular inflammatory events cause the breach of BBB integrity, and that seizures commonly result [6;26]. It has been proposed that in correspondence to BBB damage, blood constituents such as potassium and serum albumin extravasate into the brain parenchyma [3;9;17]. Interestingly, angiogenesis and formation of leaky vessels were reported in drug resistant epilepsy [27]. Our results confirm these studies [27].

It has been shown that traumatic brain injury leads to altered potassium concentrations in the brain, which may contribute to seizure development. Interestingly, the interstitial potassium concentration in the brain is lower than it is in the serum [28;29]. It is thus plausible that the opening of BBB leads to a rapid increase in extracellular potassium levels. This rapid increase in potassium could serve as a trigger for the generation of acute seizures [3;17;28;29].

It was recently reported that extravasated albumin is taken up by astrocytes via a TGF- β receptor-mediated pathway, which in turn down-regulates inward-rectifying potassium (Kir 4.1) channels in astrocytes [30]. The down-regulation of potassium channels results in impaired potassium buffering capacity and elevated extracellular potassium. In these studies, albumin-induced epileptic events were observed a few days after albumin exposure. In our study, epileptiform discharges were recorded immediately after BBB opening. The fact that in our experiments no delay time was required may be due to the extent of BBB opening, e.g. hemispheric in our experiments vs. local in the experiments by Friedman's group [9]. Nevertheless, both studies confirm that BBB opening is associated with altered EEG activity and both suggest potassium as an ultimate trigger for seizures.

From CNS to non-CNS mechanisms of seizures and epilepsy

Non-CNS mechanisms of seizures are gaining acceptance. A link between activation of peripherally circulating lymphocytes and the development of seizures was recently established [18;19;31]. Peripheral inflammation induced BBB leakage leads to a decreased seizure threshold [18;19;21]. The BBB, therefore, plays an important role in preventing unwanted electrical activity in human brain. The similarities in the pattern of albumin and WBC extravasations in rats with acute seizures and chronic epileptic patients suggest the captivating hypothesis that a similar pathogenesis may be responsible for both disease processes.

In summary, we have shown the lack of significant leukocyte brain parenchymal distribution in animal models of seizure and in TLE. Our findings suggest that WBC extravasation into the parenchyma may not be a prerequisite for acute or chronic seizures.

Experimental Procedure

Rodents

Rats were housed in a controlled environment ($21 \pm 1^\circ\text{C}$; humidity 60%; lights on 8AM–8PM; food and water available *ad libitum*). Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with related laws and policies (Guide for the Care and Use of Laboratory Animals, U. S. National Research Council, 1996) and were approved by the Institutional Animal Care and Usage Committee.

EEG recordings

Approximately half of the rats were implanted for EEG recordings. Recording were performed continuously during surgery and injections (*e.g.*, under general anesthesia) and up to 1 hours after completion of surgical procedures. Digital EEG recording (*e.g.*, cortical electrode) was performed using a Pinnacle Technologies Model 8206 EEG-Video recording apparatus consisting of a recording workstation and a review system connected to a 21-inch monitor. EEG data were sampled at a rate of 200 Hz. Spectrum and spike amplitude analysis was performed after the experiments. Origin FFT software is used in conjunction to the acquisition system for data analysis. For Time-joint-frequency analysis and preparation of spectral figures, we used Diadem (National Instruments, Austin, TX).

BBBD, WBCs and Evan's Blues injection

Rats (male Sprague-Dawley 350–450 g) were anesthetized with ketamine and xylazine. We have used a total of 15 rats. After anesthesia, a 2–3 cm vertical incision was made from the suprasternal notch to below the chin. After exposure of the sternomastoid muscle, the common carotid artery was exposed and was separated from the caudal portion of the vagus. After exposure of the bifurcation of internal and external carotid arteries, a silk tie (3-0, Ethicon, 24", Ethicon, Sommersville, NJ) was looped around the external branch of the external carotid artery (ECA), while a micro-clamp was positioned ready at the common carotid artery (CCA). Figure 1A shows the appearance of the surgical field. In temporal sequences the following steps were then performed within 2–3 min: (1) clamp the CCA; (2) ligate the distal ECA, leaving approximately 5 mm stump proximally; (3) insert the polyethylene catheter (PE-2P) into the external carotid artery retrogradely toward the bifurcation with the common carotid; (4) tighten the silk tie around the catheter; (5) injection of a hyperosmolar mannitol solution (1.4 Molar bolus in saline, 0.1 ml/s, 2 ml total); (6) remove the catheter and ligate the proximal stump of the ECA; and (7) release the clamp at the CCA.

A solution containing GFP-WBCs and Evans Blue (see below for exact composition) was injected immediately after mannitol *via* ICA catheter. Animal were sacrificed within one hour after injections. No sign of pain or discomfort altered respiratory patterns or other behavioral changes consistent with procedure-induced damage were observed.

Preparation of GFP-WBCs

White blood cells were collected from a donor rat. Briefly, blood mononuclear cells were obtained from the abdominal aorta of Sprague-Dawley rats anesthetized with 1% isoflurane. Mononuclear cell suspension was prepared by density gradient centrifugation (histopaque-1077) (Sigma-Aldrich, ST. Louis, MO). Cell viability was confirmed by Trypan blue prior to culture. The cells were counted and $3-4 \times 10^9$ cells were grown in DMEM for 24 hours before being treated with an Adeno-GFP viral vector (Dr. Marc Penn Laboratory, Cleveland Clinic). The GFP sequence was cloned into this vector under control of cytomegalovirus (CMV) promoter. Cells were treated with Adeno-GFP vector for 24 hours and then allowed to grow for an additional 24 hours. GFP expression was confirmed by fluorescent microscopy. Cells were collected and suspended in a 2% solution of Evans Blue in sterile phosphate buffer solution (PBS) for injection into the internal carotid artery (ICA) after mannitol injection as described below. This will allow concomitant determination of BBB leakage (Evans blue) and presence of cell extravasates (GFP).

Pilocarpine model of SE

Rats (male Sprague-Dawley 250-300g) were injected with methylscopolamine (0.5 mg/kg, i.p., Sigma-Aldrich) and, 30 minutes after, with pilocarpine (340 mg/Kg, Sigma-Aldrich). Development of seizure and status epilepticus was evaluated by behavioral (Racine's scale) and EEG assessment. Rats were sacrificed few hours after SE.

Rat immunohistochemistry

Rats were sacrificed by isoflurane overdose. Brains were immersion fixed for 48 hours in 4% paraformaldehyde pH 7.4, and then cryoprotected overnight in a 20% sucrose solution, frozen in isopentane, and stored at -80°C . Coronal sections were cut on a cryostat. Sections were then washed with saline and mounted in a Mowiol-based mounting medium containing 0.1% para-phenylenediamine hydrochloride and DAPI staining for nuclei. Sections were examined for Evans blue and GFP-WBCs using a Leica confocal laser-scanning microscope. CD45 staining in pilocarpine rats was performed as described below for TLE specimens.

TLE subjects immunohistochemistry

All patients signed an informed consent form according to Institutional Review Board protocols at The Cleveland Clinic Foundation and the Declaration of Helsinki. Brain specimens were obtained from eight patients undergoing temporal lobectomy to relieve drug resistant seizures (see Table 1). Tissues were oxygenated from the operating room to the laboratory and maintained at 4°C throughout the procedure of transportation from the O.R. to laboratory manipulation such as fixation. Tissues were cut into smaller samples to allow the penetration of fixative into deep layers.

Immunostaining for identification of leukocytes (CD45), IgG and albumin was conducted using diaminobenzidine (DAB) or fluorescence. Sections were washed in phosphate buffer saline (PBS, 1X), then permeabilized with 0.3% TWEEN/PBS for 1 hour and rinsed once for 5 minutes in PBS. Blocking for endogenous peroxide was done with 0.3% hydrogen peroxide in methanol for 20 minutes. Sections were washed with PBS and then incubated for 1 hour in 5% goat serum. Overnight incubation was done in mouse monoclonal anti-human CD45, leukocyte common antigen (1S751) (1:100, Dako, North America, Inc. Real

Carpinteria, CA) on adjacent sections in a shaker at 4°C followed by three 5 minutes rinses with PBS and a 1 hour long incubation with a biotinylated anti-mouse IgG (1:600, Vector laboratories, Burlingame, CA) prepared in 0.4% Triton-X. IgG staining was also performed on consecutive brain sections using human IgG (1:2000; Jackson ImmunoResearch Laboratories Inc., West Grove, PA). This was followed again by rinses with PBS. Because biotin amplification with avidin enhances visualization of reaction products, sections were incubated for 1 hour with an avidin/biotin complex (Elite Vectastain ABC Kit; Vector Labs, Burlingame, CA). After 3 more rinses with PBS the antibody binding sites were visualized with DAB (Peroxidase Substrate Kit, Vector Labs Burlingame, CA). Slides were then dehydrated and mounted using Permount solution.

Immunofluorescence staining for Albumin in the human brain sections was performed as follows. The adjacent free floating sections were processed by blocking in 5% goat serum followed by primary antibody, mouse monoclonal anti-human Albumin (1:1000, Sigma, Saint Louis, MI), and secondary antibodies Fluorescein isothiocyanate (FITC)-conjugated affinipure donkey anti-mouse IgG (1:100, Jackson ImmunoResearch Laboratories Inc., West Grove, PA). Autofluorescence was blocked with Sudan black B and finally the sections were coverslipped on glass slides using Vectashield mounting medium with DAPI (Vector Labs, Burlingame, CA) and analyzed by fluorescent microscopy.

Quantitative measurement of albumin and leukocyte extravasation

Images were acquired using a Leica confocal laser-scanning microscope. Cells were counted using Phoretix while QCapture Software was used to quantify fluorescent signal correspondent to albumin brain extravasation. WBCs were categorized into intravascular, perivascular space or parenchymal based on anatomical location. Three fields/slices (n=5 slices each condition) were used. In particular, for rat BBBB we calculated the number of WBC / vessel using 20X images (*e.g.*, frontal, temporal cortices and thalamus). For human TLE we quantified the number of CD45 positive cells / field (20 X) using n=3 section/patient (Table 1).

Statistical analysis

Data are expressed as mean $\pm$ SEM (significance $p < 0.05$). Two-way ANOVA was used for comparisons of multiple observations. JMP statistical software was used for analysis and data comparison.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Description of BBBD-induced epileptiform discharges in rats

A) Experimental set-up and gross evaluation of BBB leakages. The internal carotid artery (ICA) was cannulated via external carotid artery (ECA). When BBBD is successful Evan's Blue extravasation was noticeable by visual inspection. **B-B1)** EEG traces and time-joint frequency analysis. Note the slowing of EEG activity (*arrow heads*) during mannitol injection and the progressive appearance of spikes in the post-BBBD. The electrographic changes are more evident when displayed as a time-joint frequency graph. Note the appearance of high amplitude signals (*red signal*) and the broad distribution of ictal frequencies post-BBBD. **C)** We confirmed that in rats, similar to human BBBD procedures (in 20% of the procedures BBB was opened and epileptiform discharges detected, [3]). **D)** Frequency spectrum highlights the appearance of 10-20 Hz spikes after successful BBBD (red traces).

Figure 2. Lack of WBCs brain extravasation at time of epileptiform discharges

A) Fluorescent micro-angiography was performed after BBBB procedures. Rat brain tissue was sectioned to locate FITC-albumin. In successful BBBB procedures a significant albumin accumulation in the parenchyma of the injected hemisphere was observed. Details of leaky spots are indicated by asterisks (A3-A4). In the contralateral hemisphere fluorescent signals were primarily intravascular (A1-A2). **B)** GFP-WBCs predominantly remained inside the blood vessels or homed into the perivascular space (B1). Evans Blue extravasation (red) was used to identify protein leakage and to visualize the cerebral microvasculature. Note that WBC extravasation was seen but that cells remained in the proximity of vessels. In the contralateral hemisphere or sham procedures, GFP-WBCs were segregated inside the blood vessels. Quantification is provided in Figures 4 and 5.

Figure 3. Lack of WBCs extravasation in brain in TLE subjects

A) Sections from TLE brain were stained using antibody against IgG and albumin. IgG positive signal was detected (*asterisks*) around leaky blood vessels (A1-A3). Abundant albumin positive signal (*green*) was seen in the same regions where IgG was detected (A4-A9). Examples of intact blood vessels are also shown (indicated by a diamond in A6). **B)** CD45 positive cells (WBCs) were commonly observed in the perivascular space (B1-B3) in regions characterized by BBB leakage. Extensive microglia staining was also seen (B4). *Arrow heads* point to perivascular CD45 positive cells. Scarce parenchymal WBCs was observed. Quantification is provided in Figures 4 and 5.

Figure 4. Quantification of GFP-WBC and CD45 positive cells

A) Overall number of leaky and non-leaky vessels in rat brain after successful BBBD. **B-C)** We determine the distribution of WBC within the two population of vessels (*e.g.*, leaky vs. intact). Note that in leaky vessels the number of WBCs in perivascular space was significantly higher compared to brain parenchyma and intravascular spaces. In intact vessel the majority of WBCs resided in the intravascular space. **D)** Chronic human TLE brain was characterized by lack of parenchymal homing of WBCs. Note the significant number of WBCs or CD45 present in the perivascular space. The * indicates $p < 0.05$.

Figure 5. Human TLE brain displays patterns of albumin distribution in leaky and non-leaky vessels quantitatively and qualitatively similar to rat BBBD

A) The amount of FITC-albumin present inside and outside vessels was quantified (see methods for details). Note that no fluorescent signal was detected or quantified outside intact BBB vessels, while a significant amount of fluorescence was quantified outside leaky vessels (A1-A2). Representative graph of fluorescent density line profile in leaky and non-leaky vessels is shown in A1. **B)** Note that similar to rat BBBD, in TLE a significant amount of albumin (detected using primary Ab) was present outside leaky vessels. Note that the signal sharply abated at the vascular wall in intact vessels where intravascular albumin was present (B1-B2). When leakage was observed, the signal progressively tapered reflecting extravasation. The * indicates $p < 0.05$.

Table 1

Summary information of the tissue donors used for IHC studies.

TLE patients	Age (yr)	Gender	Classification	Etiology
TLE 1	14	F	Right hemispheric seizures	Malformation of Cortical development
TLE 2	15	M	Left frontal lobe seizures	Cortical dysplasia
TLE 3	15	M	Right lateral temporal lobe seizures	Right hippocampal sclerosis
TLE 4	5	F	Right hemispheric epilepsy	Remote symptomatic epilepsy
TLE 5	39	M	Focal epilepsy	Unknown
TLE6	1	F	Hemispheric Epilepsy	Malformation of cortical development (right hemisphere)
TLE 7	43	F	Right mesial temporal lobe epilepsy	Mesial temporal sclerosis
TLE 8	37	M	Left mesial temporal lobe epilepsy	Mesial temporal sclerosis