Receptor-mediated Transcytosis of Lactoferrin through the Blood-Brain Barrier*

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Lactoferrin (Lf) is an iron-binding protein involved in host defense against infection and severe inflammation; it accumulates in the brain during neurodegenerative disorders. Before determining Lf function in brain tissue, we investigated its origin and demonstrate here that it crosses the blood-brain barrier. An in vitro model of the blood-brain barrier was used to examine the mechanism of Lf transport to the brain. We report that differentiated bovine brain capillary endothelial cells exhibited specific high (KD = 37.5 nM; n = 90,000/cell) and low (KD = 2 μM; n = 900,000 sites/cell) affinity binding sites. Only the latter were present on nondifferentiated cells. The surface-bound Lf was internalized only by the differentiated cell population leading to the conclusion that Lf receptors were acquired during cell differentiation. A specific unidirectional transport then occurred via a receptor-mediated process with no apparent intracellular degradation. We further report that iron may cross the bovine brain capillary endothelial cells as a complex with Lf. Finally, we show that the low density lipoprotein receptor-related protein might be involved in this process because its specific antagonist, the receptor-associated protein, inhibits 70% of Lf transport.

Lactoferrin (Lf) is a mammalian cationic iron-binding glycoprotein belonging to the transferrin (Tf) family. Despite some striking differences, mainly in the glycans moieties, there are marked sequence and conformational homologies among Lfs from different species, as well as similar general functions (for review, see Ref. 2). Many physiological roles have been ascribed to Lf, particularly in the host defense against infection and severe inflammation (for review, see Ref. 3). This broad spectrum of biological functions relies on the interaction of Lf and its distribution in the vicinity of the inflammatory foci led us to investigate whether Lf may cross the blood-brain barrier (BBB).

In situ synthesis of Lf occurs in brain, and Lf transcripts were found in human (26) and mouse brain tissues (27). Moreover, we have shown that Lf expression was up-regulated in mouse brain tissues treated with the neurotoxic agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, which is used as a model for Parkinson’s disease (27). Nevertheless, this up-regulation was slight and might not explain the large increase of Lf observed in the case of neurodegenerative disorders (22, 25). In the mesencephalon, Lf is concentrated mainly in the dopaminergic neurons; in the case of Parkinson’s disease (25), the surviving neurons accumulate higher concentrations of Lf. The origin and function of Lf within either the normal or pathological brain are as yet uncharted.

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The BBB is formed from specialized endothelial cells (ECs) that are sealed together by continuous complex tight junctions to form a polarized barrier that restricts the free exchange of most solutes between plasma and the extracellular fluid of the brain. Furthermore, even though brain capillary ECs contain no direct transendothelial passageways such as fenestrations...
or channels, specific transport mechanisms located in the cerebral ECs ensure that the central nervous system receives an adequate supply of nutrients. Such receptors have already been identified for proteins (28–30) and lipoproteins (31).

We have used an in vitro model of the BBB which imitates the in vivo situation by means of the co-culture of bovine brain capillary ECs (BBCECs) on one side of a porous filter and astrocytes plated at the bottom of six-well dish (32, 33) to provide direct evidence that after binding to BBCECs, Lf crosses the endothelial monolayer from the apical to the abluminal surface. Lf transcytosis is receptor-mediated, and our results indicate that LRP might be involved in this transcellular transport across the brain endothelium.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—BBCECs isolated and characterized as described previously (32, 33) were cloned, allowing us to obtain a pure EC population uncontaminated by pericytes. The cells were cultured in the presence of Dulbecco’s modified Eagle’s medium supplemented with 15% (v/v) heat-inactivated calf serum (HyClone Laboratories), 2 mM glutamine, 50 μg/ml gentamycin, and 1 ng/ml basic fibroblast growth factor, added every other day. Nondifferentiated cells were obtained by growing BBCECs in the absence of astrocytes.

Primary cultures of mixed astrocytes were prepared from newborn rat cerebral cortex. After removing the meninges, the brain tissue was forced gently through a nylon sieve (34). Astrocytes were plated on six-well dishes (Nunc; Nunc A/S) at a concentration of 1.2 × 10⁵ cells/ml in 2 ml of Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (HyClone Laboratories). The medium was changed twice a week. 3 weeks after the seeding was completed, the purity of the astrocyte population was checked (33). Stabilized cultures contained more than 95% astrocytes that were glial fibrillary acidic protein-positive and were used for co-culture.

Establishing the in vitro model of BBB was performed using a co-culture of BBCECs and astrocytes. Prior to cell culture, plate inserts (Millicell-CM 0.4 μm; 30-mm diameter; Millipore Corp.) were coated on the upper side with rat tail collagen (35). They were then set in the six-multidish containing the astrocytes prepared as described above, and BBCECs were plated at a concentration of 4 × 10⁵ cells/ml on the upper side of the filters in 1.5 ml of coculture medium. This experimental medium was changed every other day. Under these conditions, the iodinated protein was stored at 4 °C and used the same day.

Electrophoretic Characterization of bLf After Transcytosis—Transcytosis experiments were performed as follows. One insert covered with BBCECs was set into a six-well dish with 2 ml of Ringer-HEPES-bTf and left in contact with the cells for 45 min at 37 °C. After washing off the unbound bLf, filters covered with cells were cut in two and the permeability coefficient (PF, in cm/min) calculated as described previously (33).

**Preparation and Labeling of Bovine Lf (bLf)—bLf was purified from bovine colostrum by ion-exchange chromatography as partially saturated bLf (36).**

Fluorescent labeling of bLf on the glycan moiety was carried out by coupling 5-((2-carboxyhydrazino)methyl)-amino fluorescein (Hyf) on alkaline groups resulting from the mild periodate oxidation of N-acetylatedamino acid residues (7).

Iodination of bLf was performed using the iodogen-GEN reagent (Pierce). bLf (500 μg) was dissolved in phosphate-buffered saline and incubated with 0.2 mCi of Na125I (carrier-free, 100 mCi/ml, Amersham Pharmacia Biotech) for 15 min at 4 °C. After washing off the unbound 125I-bLf, filters covered with cells were cut in two and the permeability coefficient (PF, in cm/min) calculated as described previously (33).

**Preparation and Labeling of Bovine Lf (bLf)—bLf** was further iodinated with 0.05 mCi of Na125I for 500 μCi of 59Fe-bLf, giving a specific activity of 0.1 μCi/μg protein for 125I and 4 μCi/μg protein for 59Fe.

**Binding, Endocytosis, Internalization, Kinetics, and Transcytosis**—All of these studies were performed only with BBCECs to avoid any interference from the astrocyte population. Moreover, to eliminate endogenous bLf, BBCECs were always incubated before each experiment for 2 h in medium without serum. Because Tf has been described as an effective blocking component, able to prevent high levels of nonspecific Lf binding (37), all buffers contained 0.2% bTf (Sigma). Each point was done in triplicate, and the data are represented as the means ± S.E. Nonspecific controls were carried out with a 100-fold excess of unlabeled protein.

Equilibrium binding was performed on differentiated and nondifferentiated BBCECs in Ringer-HEPES-bTf for 2 h at 4 °C, with a 125I-bLf concentration ranging from 0.2 to 200 μg/ml (2.5–2500 nM). The cells were washed carefully, and cell-associated radioactivity was determined by removing the membrane of the culture insert and counting it in a gamma counter. The results were analyzed using the Enzfitter nonlinear regression data analysis program (Elsevier-BIOSOFT, Cambridge, U. K.). Scatchard plots and kinetic analyses were performed using the same software.

Determination of the luminal uptake of Hyf-bLf was performed before fixation with 4% paraformaldehyde. The luminal compartment of the differentiated BBCECs was exposed to Hyf-bLf (50 μg/ml; 625 nm) in Ringer-HEPES-bTf and left in contact with the cells for 45 min at 37 °C. Fluorescence microscopy experiments were carried out as already described (28).

The time course of internalization of 125I-bLf by differentiated BBCECs was measured using 125I-bLf (30 μg/ml; 375 nCi) which was presented to the luminal surface for 1 h at 4 °C prior to the experiment. After washing off the unbound bLf, filters covered with cells were incubated in prewarmed medium at 37 °C. At various times, filters were removed, and all subsequent steps were performed at 4 °C. The medium compartments were collected, and cells were treated with 0.1% Pronase E (Merck) in phosphate-buffered saline for 30 min at 4 °C. Surface-bound, internalized, released, and transported 125I-bLf was counted in the Pronase-sensitive eluate, the cell extract, and the trichloroacetic acid-washable fractions and incubation media of the upper and lower compartments, respectively.

Transcytosis experiments were performed as follows. One insert covered with BBCECs was set into a six-well dish with 2 ml of Ringer-HEPES-bTf added to each well at 37 °C. 125I-bLf (30 μg/ml), 59Fe-bLf (50 μg/ml), and 125I-59Fe double-labeled bLf (50 μg/ml) were added to the upper side of the filter covered with cells. At various times, the inserts were transferred to a 2-ml well of Ringer-HEPES-bTf and left in contact with the cells for 45 min at 37 °C. After incubation, intact bLf was assessed using trichloroacetic acid precipitation of lower media, and protein degradation was assessed with AgNO₃ precipitation. For 125I-bLf studies, all results were expressed as 125I-bLf equivalent flux (ng/cm²), which represents trichloroacetic acid-precipitable radioactivity recovered in the lower compartments. The Fe equivalent flux (μg/cm²) corresponds to the total iron radioactivity found in the lower compartment.

The influence of temperature on the transport of 125I-bLf (30 μg/ml) was studied with the monolayers kept at 4 °C. In parallel, the paracellular passage of sucrose (0.1 μCi of [14C]sucrose) was assayed at 4 °C and 37 °C in the same conditions.

To demonstrate whether the transendothelial transport was directional, 125I-bLf (30 μg/ml) was added to the lower compartment of the wells, and the transcytosis experiment was conducted as above.

**Effect of RAP on Lf Transport**—The effect of RAP on the transendothelial transport of 125I-bLf across BBCECs was determined by adding 100 nM recombinant RAP to the upper side of the filter covered with cells for 1 h at 37 °C before experiments. Recombinant RAP was prepared in Escherichia coli as a fusion protein that contains the entire sequence of the human 59Fe-binding component fused to glutathione S-transferase (38). All experiments were then performed with 125I-bLf as described above.

Electrophoretic Characterization of bLf After Transcytosis—After 2 h of transcytosis, the apical and basolateral compartment solutions were collected and analyzed by 10% SDS-polyacrylamide gel electrophoresis. After electrophoresis, the gel was dried and exposed for autoradiography 2 h at ~80 °C (Kodak X-Omat AR film).
sites/cell and a low affinity binding site with a \( K_d \) of about 1,900 nM and 890,000 sites/cell. Both sites were specific for bLf. bLf binding to nondifferentiated BBCECs was also investigated (Table I). Scatchard analysis revealed that the affinity of bLf for these cells (\( K_d = 2, 100 \text{nM} \)) and the number of bLf binding sites (920,000/cell) were comparable to the low affinity binding site parameters found on the differentiated cells. These results suggest that only differentiated BBCECs are able to express high affinity binding sites for bLf.

**Endocytosis of bLf in BBCEC Monolayers**—To determine whether bLf was internalized from the luminal surface of the BBCECs, Hyf-bLf was first used as a probe. Staining visualized with a fluorescent microscope is shown in Fig. 2A. Hyf-bLf was found as small, individual vesicles throughout the differentiated cells. This uptake was completely inhibited at 4 °C (Fig. 2B). The endocytosis process was then monitored using radiolabeled bLf. Cells were first incubated at 4 °C for 1 h until the binding site occupancy reached a steady state. After washing off the unbound ligand, cells were incubated at 37 °C and the distribution of surface-bound, internalized, released, and transported radioactivities was counted at various times. As shown in Fig. 3, the Pronase-resistant fraction increased rapidly and reached a maximum of 18% of the initially bound \( ^{125}\text{I}-\text{bLf} \), 3 min after the start of the incubation. The Pronase-resistant fraction decreased slowly thereafter, as \( ^{125}\text{I}-\text{bLf} \) started to appear in the lower compartment a few minutes later, showing that transcytosis occurs. During the experiment, the Pronase-sensitive fraction decreased, and \( ^{125}\text{I}-\text{bLf} \) appeared in the upper compartment, suggesting a marked dissociation from the cell surface or a release of \( ^{125}\text{I}-\text{bLf} \) by the cells.

**Apical to Basolateral Transport of bLf Across BBCEC Monolayers**—\( ^{125}\text{I}-\text{bLf} \) was added to the luminal chamber of the culture, and its progressive transfer across the cell monolayer was followed for 90 min. A range of different bLf concentrations (10–100 μg/ml) was assayed, and no evidence of saturation was detected at concentrations lower than 100 μg/ml (data not shown). Fig. 4 shows the total, nonspecific and calculated specific bLf flux values as a function of time with a luminal concentration of 30 μg/ml bLf. Our results demonstrate that differentiated BBCECs are not a barrier for the passage of bLf. The transport of labeled bLf from the luminal to the abluminal compartment was reduced severely by an excess of unlabeled bLf, suggesting that bLf transport from the apical to the basal side of the cells was effected by specific receptor-mediated transport. The rate of transcytosis was evaluated, and about three to four bLf molecules were transported via one high affinity binding site in 1 h, suggesting that one bLf molecule crosses the BBCEC monolayer in 15–20 min. The possible toxicity of a high concentration of bLf (3 mg/ml) for the integrity of the BBCEC monolayer was assessed by calculating the permeability to sucrose. No leakiness in the barrier function occurred (\( P_f = (0.65 \pm 0.02) \times 10^{-3} \) and \( 0.67 \pm 0.03) \times 10^{-3} \) cm/min for the control and in the presence of 3 mg/ml of bLf, respectively).

The effect of temperature on bLf transport from the apical to

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**Table I**

| Cells                  | \( K_d \) | Sites/cell |
|-----------------------|-----------|------------|
| Differentiated BBCECs | Site 1    | 37.5 ± 7   |
|                       | Site 2    | 1,900 ± 160|
| Nondifferentiated BBCECs | 2,100 ± 310 |
|                       |           | 920,000 ± 65,000 |

**Fig. 1.** Scatchard analysis of the binding of \( ^{125}\text{I}-\text{bLf} \) to differentiated BBCECs. bLf binding was performed on BBCECs at 4 °C, and increased concentrations of \( ^{125}\text{I}-\text{bLf} \) were added at the luminal side of the cells. Low (panel A) and high (panel B) concentration ranges of bLf were used. Specific binding was obtained after subtraction of the nonspecific binding in the presence of a 100-fold excess of unlabeled bLf from the total counts. The specific bound \( ^{125}\text{I}-\text{bLf} \) was analyzed by the Scatchard procedure. B/F, bound/free ratio.
incubated at 37 °C (Fig. 2A) or 4 °C (Fig. 2B) for 45 min with 50 μg/ml Hyf-bLf. After washing, the cells were fixed and processed for fluorescence microscopy as described under “Experimental Procedures.” Bar = 20 μm.

Fig. 2. Hyf-Labeled bLf endocytosis in BBCECs. BBCECs were incubated at 37 °C (panel A) or 4 °C (panel B) for 45 min with 50 μg/ml Hyf-bLf. After washing off the unbound ligand, cells were incubated with 125I-bLf at 37 °C. At the indicated times, filters were removed, and the amounts of surface-bound, internalized, released, and transcytosed radioactivity were counted. Ordinates represent the percentage of the total radioactivity initially bound to BBCECs at 4 °C, which was segregated within the cells (●) and released into the lower incubation medium (■) or into the upper incubation medium (▲) or associated with membranes (◆). The data are expressed as ng of 125I-bLf transported/cm², which refers to the surface area of the cells. Each point is a mean of three different filters.

Abluminal to luminal transport of bLf was assayed by added 125I-bLf to the lower chamber of the culture. No specific transport of bLf was detected across the cell monolayer in these conditions (data not shown), showing that the transport of bLf across BBCECs is unidirectional.

Effect of RAP on the Transendothelial Transport of 125I-bLf Across BBCECs—To determine whether LRP might be involved in the receptor-mediated transport of bLf, transcytosis experiments were performed in the presence of RAP. RAP is known to interact with LRP and block the binding of any kind of LRP ligand. The fusion molecule with glutathione S-transferase was used because it has been shown that LRP does not bind glutathione S-transferase (15, 39). The integrity of the monolayer was evaluated, and no increase in the permeability of sucrose in the presence of recombinant RAP was detected (P_e = (0.52 ± 0.02) × 10⁻³ and (0.55 ± 0.03) × 10⁻³ cm/min for the control and in the presence of 100 nM RAP, respectively). As shown in Fig. 6, recombinant RAP decreases the rate of the passage of bLf through the BBCEC monolayer. A decrease of 70% of the initial transcytosis was observed, suggesting the involvement of LRP in this intracellular traffic.

Characterization of bLf After Transcytosis—To determine whether bLf undergoes degradation within BBCECs during transcytosis, 125I-bLf recovery was followed by counting the trichloroacetic acid precipitable, and 125I-bLf degradation was followed by measuring the AgNO₃ precipitate in the upper and lower compartments. Controls were carried out with filters coated with collagen and without cells as above. The results showed that 125I-bLf degradation was no more than 2 ± 0.5% either in the upper or in the lower compartment. Moreover, Fig. 7 shows that 125I-bLf was recovered as an intact protein after its transport through the BBCEC monolayers. These results suggest that bLf was transported across BBCECs via a specialized pathway that does not lead to degradation.

Transendothelial Iron Transport Studies Using 59Fe-bLf and 125I-59Fe-Double-labeled bLf—To determine whether iron-bound Lf might reach brain tissues, iron transport was studied across BBCECs. Transcytosis experiments were repeated with 59Fe-bLf. Fig. 8 represents specific transcytosis of 59Fe through BBCECs. This transport was reduced by an excess of unlabeled...
suggesting that 59Fe crosses the monolayer associated with bLf. To elucidate this point, experiments were then carried out with 125I-59Fe-double labeled bLf, and the data are summarized in Table II. 59Fe-bLf equivalent flux was calculated from 59Fe flux because one molecule of bLf binds two molecules of iron. The 59Fe-bLf equivalent flux (0.044 pmol cm\(^{-2}\) h\(^{-1}\)), which is equal to one-half of the 59Fe flux (0.088 pmol cm\(^{-2}\) h\(^{-1}\)), was very close to that of the 125I-bLf equivalent flux observed (0.046 pmol cm\(^{-2}\) h\(^{-1}\)). These results suggest that iron found in the lower compartment crossed the BBCEC monolayers bound to bLf.

**DISCUSSION**

The purpose of the present study was to determine whether Lf was able to cross the BBB, which would explain its presence in brain tissues. We have demonstrated that Lf binding to BBCECs was followed by transcytosis of the protein from the luminal to the abluminal surface of brain capillary ECs in the absence of any demonstrable degradation. Using RAP as an antagonist of LRP, we have been able to show that this receptor might be involved in the intracellular traffic of Lf.

![Fig. 5. Effect of a reduction in temperature from 37°C (●) to 4°C (□) on the transport of [14C]sucrose (panel A) and 125I-bLf (panel B) across BBCECs. Results are expressed as a percentage of sucrose recovered in the lower compartments. For bLf, data are expressed as an equivalent bLf flux (ng/cm\(^2\)), and values are means of triplicate inserts.](image)

We have used an in vitro BBB model consisting of a coculture of brain capillary EC and astrocytes. When cultured on permeable supports, these cells form a well polarized monolayer that mimics the in vivo situation (28, 31, 33) and displays remarkable phenotypic similarities to the in vivo BBB with respect to the expression of specific markers and characteristics such as tight junctions, electrical resistance, low paracellular permeability, specific enzymatic activities, and carrier-mediated transport (40, 41).

Our data are equally consistent with the existence of low affinity binding sites on both types of BBCEC cells with a K\(_d\) of 2 µM and approximately 9 × 10^5 binding sites/cell. As already shown, Lf binds to various specific ligands at the cell surface.

![Fig. 6. Effect of RAP on the transport of 125I-bLf across BBCEC monolayers. After incubation for 1 h at 37°C with (●) or without (□) RAP (4 µg/ml), bLf (30 µg/ml 125I-bLf, 3 mg/ml unlabeled bLf) was added to the luminal side of the cells. The data are expressed as ng of 125I-bLf transported/cm\(^2\). Each point is a mean of three different filters.](image)

![Fig. 7. Electrophoretic characterization of 125I-bLf after transcytosis across BBCEC monolayers. bLf (30 µg/ml 125I-bLf) was added to the luminal side of cells for 2 h at 37°C. Lane 1, medium of the luminal compartment after transcytosis experiment; lane 2, medium of the abluminal compartment after transcytosis experiment; lane 3, 125I-bLf before its addition to the luminal compartment. The arrow indicates the dye front.](image)
and both proteinases and proteinase-inhibitor complexes (for bLf (50). The functional involvement of LRP in bLf uptake by BBCECs was tested by inhibition experiments in the presence of RAP, the LRP universal antagonist (15). RAP is an endoplasmic reticulum resident protein (46) that functions intracellularly as a molecular chaperone for LRP and maintains LRP in an inactive, non-ligand binding state along the secretory pathway (47). We demonstrated that Lf transcytosis was mediated by LRP because 70% of the Lf traffic was inhibited by RAP. The distribution of LRP on ECs was reported previously (48, 49). It has also been studied in the central nervous system and was found associated with neurons (50, 51), weakly on some glial cells, and discontinuously along the membranes of the capillaries (51). LRP was also associated with neurodegenerative disorders such as Alzheimer’s disease (52) and was found in increased concentrations in some neurons. LRP might therefore be responsible for Lf accumulation in some specific brain areas.

Lf endocytosis (10, 13) and transcytosis (16, 17) have been reported previously, and partial or complete degradation of Lf always occurs. Lf released during neutrophil activation has a rapid turnover, and its clearance by the liver keeps its level in the plasma very low. Lf internalized by Jurkat cells accumulates in the endosomal compartment, and then both intact and degraded Lf is released, suggesting that part of the Lf is recycled (10). Lf is also endocytosed by the intestinal epithelial cells HT-29. In this case, transcytosis of 10% of Lf occurred, whereas 90% of the Lf followed a major degradative pathway (16). In the present study, we report that Lf is taken up and transported through the BBCECs without any degradation. The nondegradation of Lf during the transcytosis indicates that the transcytotic pathway in BBCECs is different from the classical Lf receptor pathways described above. The existence of a receptor-mediated process that bypasses lysosomes seems to be a feature of ECs; the absence of degradation of proteins such as insulin (29), albumin (30), Tf (28), and lipoproteins such as LDL (31) through ECs was reported previously. Microscopy studies also confirmed that no accumulation of LDL was observed in lysosomes (31). The precise transcellular pathway for the passage of blood-borne molecules across the BBB is not yet elucidated. One of the characteristics of brain capillaries ECs is the paucity of clathrin-coated pits, and previous studies strongly support the involvement of caveolae in endocytosis (53, 54). These structures seem responsible for the uptake and transcellular transport of albumin (55) and human Tf (28). They have recently been implicated in the traffic of LDL through the brain microvascular endothelia leading to LDL accumulation in early endosomes and in multivesicular body structures (31). To determine whether Lf transport through BBCECs is caveolae-dependent, electron microscopy investigations are currently under way.

Alterations in iron metabolism occur in neurodegenerative diseases which lead to excessive iron deposits (56–58). The causes and consequences of such deposits in the brain are unknown, as is the nature of the responsible iron complex. Conflicting results have been reported on the implication of the proteins responsible for maintaining brain iron homeostasis, but no significant variation in the levels of expression of Tf, transferrin receptor, and ferritin have been found in pathological brain tissues (59, 60), whereas changes in the distribution, the amount (22–25), and the level of expression (27) of Lf were observed. In the case of inflammatory responses, high plasma levels of Lf are available, and larger amounts of Lf may cross the BBB and accumulate in the inflammatory foci. This process might explain why a strong labeling of Lf was observed on cerebral microvessels located in the vicinity of the neurodegenerative lesions (22). On the other hand, in response to local...
tissue injury or inflammation, additional transport pathways for large molecules may be opened and existing pathways modified or made less restrictive. It was shown recently that the intracellular traffic kinetics of Tf and LDL are disturbed in BBCECs in the presence of tumor necrosis factor α (40).

The most striking characteristic of Lf is its high affinity for iron. In this study, we have been able to show that Lf crosses the BBB as both the iron-saturated and native forms. Under physiological conditions, Lf functions as a major specialized iron scavenger and acts as an antioxidant (61) rather than an iron donor. Sequestration of free iron by Lf may inhibit the iron-catalyzed formation of hydroxyl radicals, and the presence of Lf at sites where oxidative stress occurs may limit cell damage. Nevertheless, iron-saturated Lf may act as a prooxidant agent and contribute to cell injury (62). The precise role of Lf in the brain is not yet known, but we can hypothesize that in response to oxidative stress in brain tissue, Lf could have a beneficial effect in neurodegenerative disorders by capturing iron in higher concentrations in some specific brain regions and act in this way as a natural scavenger of reactive oxidative species. Alternatively, its in situ synthesis at abnormal levels, its release from necrosing neurons, or its possible increased uptake and transcytosis by BBB ECs during the inflammatory process may exacerbate and amplify the lesions, leading to a cytotoxic effect resulting in an increase in neuronal death. Our current approach toward testing these conflicting hypotheses is to investigate Lf intracellular traffic in BBCECs in the presence of inflammatory mediators.

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