

Receptor-Mediated Transport of Lactoferrin into the Cerebrospinal Fluid via Plasma in Young Calves

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ABSTRACT. Milk, especially colostrum, contains different kinds of macromolecules abundantly, such as immunoglobulin G (IgG), lactoferrin (Lf), transferrin (Tf), and growth factors. These are essential for the development and maintenance of health, which greatly depends on the absorption and transportation of macromolecules to the target organs. To evaluate the macromolecular transport, and concentrations in plasma and cerebrospinal fluid (CSF), colostrum was fed to newborn calves followed by milk and milk replacer, and maintained up to the 4th week under farm conditions. Plasma and CSF were collected at different times, and were analyzed for Lf, Tf, IgG and iron concentrations. Lf, Tf and IgG concentrations were steeply increased in plasma and CSF after colostrum feeding, and fluctuating patterns were observed during the experiments. Furthermore, intraduodenal administration of bovine Lf alone in young calf experiments revealed that the Lf concentration reached a peak at 4 hr, and was 7 and 4 times higher than preadministration in plasma and CSF, respectively. To explore the transport mechanism of Lf into CSF in young calves, epithelial membranes of the choroid plexus were prepared and a binding assay for Lf receptors (Lf-R) was carried out with ¹²⁵I-Lf. The saturation kinetics revealed that the B_{max} of epithelial membranes was 26.15 nmol/mg protein with a K_d of 0.11 μM, which also showed that Lf-R is saturable and specific. Scatchard plot transformation showed the presence of a single type of Lf-R in the choroid plexus. These results suggest that Lf is transported into the CSF through receptor mediated transcytosis in young calves, and that Lf may play an important role(s) in brain function.

KEY WORDS: choroid plexus, immunoglobulin, lactoferrin, transcytosis, transferrin.

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Milk contains different kind of bioactive macromolecules which modify the growth and development of newborn. The bioactive substances include hormones, immunoglobulin G (IgG), growth factors, lactoferrin (Lf), transferrin (Tf), and so on. Lf, like Tf, is an 80 kDa nonheme iron-binding, single chain, multifunctional avid glycoprotein consisting of two lobes, each of which binds one ferric ion. Virtually all body fluids contain Lf and it is particularly abundant in colostrum from several species, such as humans, cattle, rhesus monkeys, mice and sows [25]. Nevertheless, endogenous plasma Lf mainly originates in the polymorphonuclear leukocytes in adults. A number of diverse biological roles have been proposed for Lf, such as anti-inflammatory, immunomodulatory, antimicrobial, and anticarcinogenic activity [31]. The physiological activities depend upon the intake, absorption and transportation of macromolecules. During the neonatal period of most mammals, macromolecules such as colostrum IgG pass intact across the intestinal epithelium into the systemic circulation [1]. The passage of macromolecules from plasma into the brain is limited by the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). These barriers to protein appear to be well formed in fetal and newborn animals, since tight junctions have been observed between cerebral endothelial cells and in the choroid plexus. Nevertheless, ultrastructural studies indicate that transendothelial vesicular transportation (pinocytosis) is a primary cellular mechanism by which

plasma proteins constitute and macromolecules pass through the cerebrovascular endothelium [35, 38]. It is also reported that increased BBB permeability to macromolecules occurs under a variety of experimental conditions such as acute hypertension [37], ischemia [36], and administration of histamine [14] and cyclic nucleotide [19]. Recently, Harada *et al.* [17] reported that bovine colostrum Lf and homologous IgG are transported into CSF via plasma in newborn piglets. In addition, Talukder *et al.* [33] observed that bovine colostrum Lf, Tf, IgG, and epidermal growth factor (EGF) are transferred into the CSF via plasma in newborn calves. Therefore, it can be predicted that these bioactive colostrum components cross the BBB or BCSFB to initiate or induce some physiological function(s) in the newborn brain. In addition, IgG is not absorbed after the "Gut closure" in calves. Furthermore, calves are born with a simple stomach which gradually turns into a compound stomach during their weaning period. The dramatic developmental changes are associated with digestive physiology. Harada *et al.* [16] found the presence of enterohepatic circulation of Lf in newborn piglets but, the absorption and transportation of macromolecules, especially Lf after the "Gut-closure" and in young calves has not yet been investigated.

The present study was addressed to explore the absorption, transportation, and trend of colostrum Lf in comparison with Tf, IgG and iron in plasma and CSF of newborns up to the 4th week under farm conditions with special emphasis on the mechanism of transportation of Lf into CSF in young calves.

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MATERIALS AND METHODS

Experimental animals: A total of four Holstein-Friesian newborn calves were used to investigate Lf, Tf and IgG transportation into CSF via plasma after colostrum administration and maintained to study the trends up to the weaning period under farm conditions. In all the cases, gestation length was normal and parturition routine. In addition, 4 Japanese black young calves (3–5 months old) were used to explore the Lf absorption and transportation mechanism into CSF via plasma. All the animals were treated in a humane manner that complied with the Guiding Principles for the Care and Use of Animals in the field of Physiological Sciences of the Physiological Society of Japan and these experiments were approved by the Animal Research Committee of Tottori University.

Chemicals: Chemicals were purchased from the following sources: bovine Lf, sucrose, D-mannitol, Tris (hydroxymethyl) aminomethane, and bovine serum albumin (BSA) from Wako Pure Chemicals (Tokyo, Japan). Iodogen and bovine Tf from Sigma Chemical (St. Louis, MO, U.S.A.). Goat anti-bovine Lf from Bethyl Laboratories, Inc. (Montgomery, TX, U.S.A.). Rabbit anti-bovine IgG from Yagai Research Center (Yamagata, Japan) and goat anti-rabbit IgG-peroxidase from EY Laboratories, Inc. (San Mateo, CA, U.S.A.). Peroxidase rabbit anti-bovine IgG (H+L) from Zymed Laboratories, Inc. (San Francisco, CA). Disposable Sephadex G-25 PD-10 column, and ^{125}I (carrier free) from Amersham Pharmacia Biotech (U.K.). Membrane filters from Advantec MFS, Inc. (Japan).

Colostrum: The udder and perianal regions were washed with lukewarm water containing antiseptic, and the teats were means of a cleaned before milking. Approximately 7.0 to 9.0 kg of colostrum was collected by a milking machine in a clean bucket and stored in a sealed container at 4°C in a refrigerator.

Collection of blood and cerebrospinal fluid under farm conditions: The calves were anesthetized with intravenous (i.v.) administration of Xylazine-HCl (0.2 mg/kg). After anesthesia, 5 ml of blood and 1 ml of CSF were collected before and 12 hr after colostrum (30 ml/kg) feeding at 2 and 4 days (d), and 1, 2, 3 and 4 weeks (w). Blood was collected in a heparinized vacutainer and centrifuged at $6,500 \times g$ at 4°C for 15 min, and plasma was separated into different sample tubes. CSF was collected from the cisterna magna by inserting a needle (23 G, 0.65×60 mm; Terumo, Belgium) through the occipito-atlantal junction. Sampling was performed before and 12 hr after colostrum suckling at 2 and 4 d, and 1, 2, 3 and 4 w. Milk and milk replacers were given routinely after colostrum. Plasma and CSF samples were stored at -80°C until analyzed.

Collection of blood and CSF from young calves after intraduodenal Lf administration: A total of 4 Japanese black (3–5 month old) young calves were used for this purpose. Three calves were used for treatment and the remaining one as a reference. The calves were fasted overnight and water was given *ad libitum*. Laparotomy was performed at

the right flank under Xylazine-HCl (0.2 mg/kg) and Procaine-HCl (2%) anesthesia to infuse Lf (0.5 g/kg, 30 ml/kg) intraduodenally (i.d.) by means of a polyethylene tube. The reference calf received only saline. This Lf (Wako Pure Chemicals, Japan) contained 0.005% iron and is considered as an apo-Lf. Each time 5 ml (approx.) of blood was collected from the jugular vein in a heparinized vacutainer, and plasma was separated as in previous experiments. Subsequently, 1 ml of CSF was collected from the subarachnoid space by inserting a needle (23 G, 0.65×60 mm; Terumo, Belgium) through the lumbo-sacral junction. Blood was collected before and 0.5, 1, 2, 4, 8, 12 and 24 hr after; whereas CSF was collected before and 4, 8, 12 and 24 hr after Lf (i.d.) administration. All the samples were stored at -80°C until analyzed. On completion of the experiment, the calves were euthanatized after the placement of a cannula into the external carotid artery to drain out blood under deep Xylazine and Ketamine anesthesia.

Analysis of samples: Lf and Tf concentrations in plasma and CSF were assayed quantitatively by double-antibody enzyme-linked immunosorbent assays previously described by Talukder *et al.* [33]. IgG concentrations in plasma and CSF were quantitatively measured according to the method described by Talukder *et al.* [33]. The iron concentration in plasma and CSF was quantified with an iron-measuring kit (Nitroso-PSAP; Wako Pure Chemicals, Japan).

Collection of choroid plexus and membrane preparation: CSF is mainly produced by the choroid plexus in the ventricles of the brain. A total of 3 Japanese black young calves (3–5 month old) were used for this purpose and euthanatized under deep Xylazine and Ketamine-HCl anesthesia. The brain was removed from the skull, and the choroid plexus was collected as soon as possible. Endothelial membranes of the choroid plexus were prepared by the method of Elisabethsky *et al.* [8]. Briefly, the choroid plexus was homogenized (20:1 vol/weight) in 0.32 M sucrose containing 10 mM Tris/HCl buffer (pH 7.4) and 1 mM MgCl_2 . All steps were carried out on ice or at 4°C. The homogenate was centrifuged twice at $1,000 \times g$ for 15 min and the final pellet was discarded. The two supernatants were pooled and centrifuged at $30,000 \times g$ for 60 min. The supernatant was discarded and the resulting pellet was lysed (20:1 vol/weight) for 30 min in 10 mM Tris/HCl buffer (lysing buffer, pH 7.4). The lysed pellet was washed three times with 10 mM Tris/HEPES buffer (pH 7.4; 20:1 vol/weight) by centrifuging at $30,000 \times g$ for 30 min. The supernatant was discarded and the final pellet was passed through a 27 G needle to make vesicles and stored at -80°C for the receptor binding assay.

Iodination of Lf: Labeling of Lf with ^{125}I was performed in the presence of iodogen as a catalyst, by the method of Fraker and Speck [12] and described by Talukder *et al.* [34].

Binding assay: Prior to the binding assay, the protein concentration in the prepared endothelial membranes was determined by the method of Lowry *et al.* [23] with BSA as a protein standard. Assays were performed in duplicate by incubating ^{125}I -labeled Lf (5 to 60 μM) with 20 μg of membrane protein (1 mg/ml) in a final volume of 300 μl of incu-

bation medium. The incubation medium contained 40 mM tris (hydroxymethyl) aminomethane-*N*-2-hydroxyethylpiperazine -*N'*-2-ethanesulfonic acid (Tris-HEPES) buffer, 0.1 M D-mannitol, 0.1 M NaCl, and 2 mM D-glucose (pH 7.4) as described previously by Davidson and Lonnerdal (5). The incubation was carried out in a water bath at 37°C and the reaction was terminated by adding of 1 ml of ice-cold saline. This solution was immediately vacuum-filtered through a pre-wetted 0.2 μ m, hydrophilic membrane (Advantec MFS, Inc., Japan) and rinsed three times with 1 ml of ice-cold saline. The filters were counted in an automatic gamma counter (WALLAC, 1480 WizardTM, Finland) to determine the amount of ¹²⁵I associated with the membrane. Nonspecific binding of Lf to the membrane was determined by adding a 1000-fold excess of Lf to the incubation medium in the same series.

Statistical analysis: All data are expressed as the mean \pm S.E. and were compared by unpaired t-test. P values of $P < 0.05$ were considered statistically significant.

RESULTS

Absorption and trend of parameters under farm conditions

Absorption of colostral proteins in plasma and CSF: The total protein concentration in the preadministration state was 46.9 and 0.36 mg/ml in plasma and CSF, respectively. The plasma protein concentration was increased 1.4-fold at 2 d, and 5-fold in CSF at 12 hr, ($P < 0.05$) as shown in Fig. 1A. The plasma protein level gradually declined while almost a steady state existed in CSF for up to 4 w.

Changes in IgG concentrations in plasma and CSF: IgG is a major macromolecule present in colostrum which is transported into CSF, as previously reported elsewhere [17, 33], though its trend up to weaning was not known, when remarkable changes occur in the gastrointestinal tract. Therefore, it may reflect the immunological impact to prevent disease(s) during the weaning period. As shown in Fig. 1B, the plasma IgG level was 20.6 μ g/ml in the preadministration state, which steeply increased 209- and 188-fold ($P < 0.01$) at 12 hr and 2 d postfeeding, respectively. There were no significant changes at 2, 3 or 4 w. The prefeeding CSF contained 0.08 μ g/ml IgG, and attained the peak value of 60.8 μ g/ml at 12 hr after colostrum feeding, declined to the lowest at 2 w and gradually increased to 56.8 μ g/ml at 4 w.

Changes in Lf concentrations in plasma and CSF: The Lf concentration in plasma and CSF in the nonfeeding state was 238.0 and 9.8 ng/ml, respectively. After colostrum feeding, the Lf concentration abruptly increased, reaching a peak value of 1,696 and 74.0 ng/ml ($P < 0.01$) at 12 hr in plasma and CSF, respectively (Fig. 2). The plasma Lf concentrations gradually declined to the lowest at 2 w. On the other hand, CSF Lf gradually decreased to 36.0 ng/ml at 1 w, but was significantly increased at 3 and 4 w, in spite of the absence of bovine Lf in the milk replacer.

Changes in Tf concentrations in plasma and CSF: Transferrin is also an iron-binding protein with a molecular mass of 80 kDa, and Lf belongs to the Tf family. Therefore, the

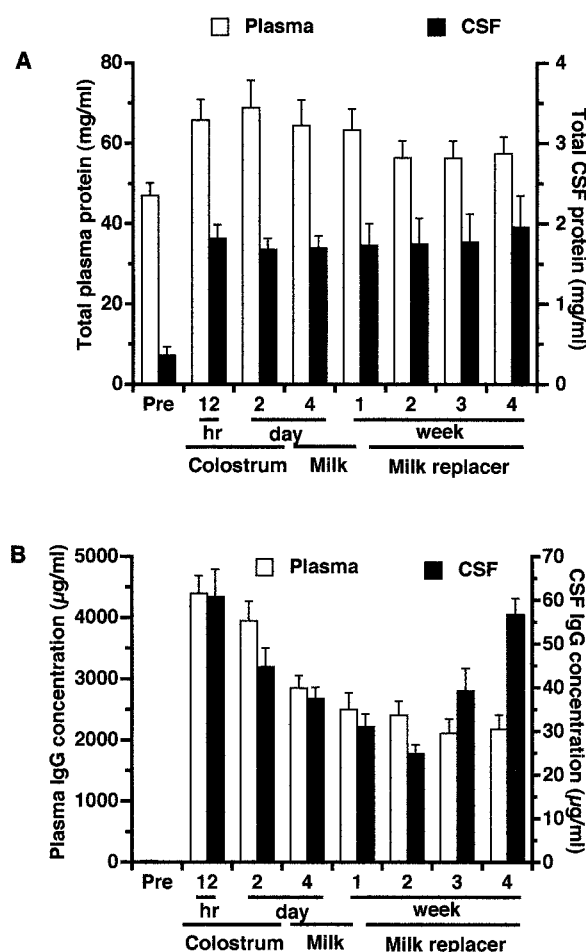


Fig. 1. A. Relationship between total protein in plasma and cerebrospinal fluid (CSF) at pre and post suckling of colostrum (30 ml/kg) in newborn calves, followed by milk and milk replacer (given routinely) up to the weaning period (mean \pm S.E., $n=4$). B. The immunoglobulin G (IgG) concentration in plasma and cerebrospinal fluid (CSF) at pre and post feeding of colostrum (30 ml/kg) in newborn calves, followed by milk and milk replacer (given routinely) up to the weaning period (mean \pm S.E., $n=4$).

transportation of Tf was investigated and quantified to compare it with Lf. In the nonsuckling state, the Tf concentration was 127.0 and 0.35 μ g/ml which were dramatically increased 6- and 200-fold ($P < 0.01$) in plasma and CSF, respectively (Fig. 3A). The plasma Tf level declined to 359.2 μ g/ml at 4 d and remained without greater changes up to 4 w. On the other hand, the CSF Tf level declined to the lowest (44.5 μ g/ml) at the same time as in plasma, but gradually increased to 73.9 and 71.9 μ g/ml at 3 and 4 w, respectively.

Changes in Fe concentrations in plasma and CSF: It is well known that Lf and Tf are iron-binding proteins, although with diversified functions, especially of Lf discovered day by day, and are not merely related to iron transportation. Therefore, we investigated the correlation between

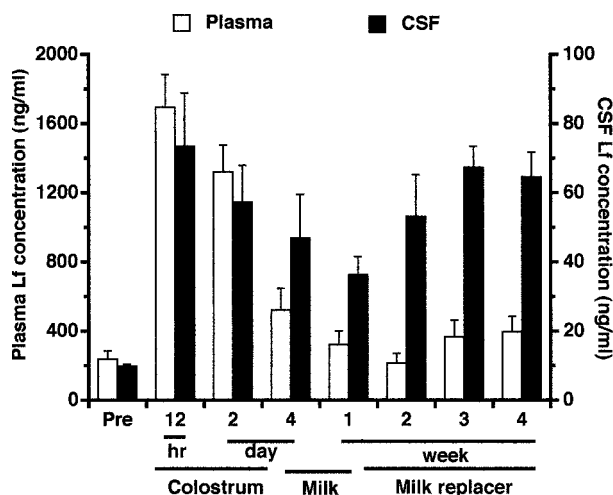


Fig. 2. The lactoferrin (Lf) concentration in the plasma and cerebrospinal fluid (CSF) at pre and post feeding of colostrum (30 ml/kg) in newborn calves, followed by milk and milk replacer (given routinely) up to the weaning period (mean \pm S.E., $n=4$).

the iron binding protein and iron status in the plasma and CSF. In the nonsuckling state, the plasma iron concentration was 99.2 $\mu\text{g/dl}$, reached a peak value of 144.8 $\mu\text{g/dl}$ at 2 d, and maintained nearly the same level up to 4 d ($P<0.05$). Thereafter, it declined up to 4 w with a value of 74.0 $\mu\text{g/dl}$ (Fig. 3B). The preadministration CSF was 7.1 $\mu\text{g/dl}$, which had increased 2-fold at 2 d after feeding ($P<0.05$), and followed the same decreasing trend as in plasma.

Experiments in young calves under laboratory conditions

Uptake of Lf from the gut lumen and transported into CSF: Newborn experiments under the farm conditions revealed that colostrum Lf was transferred into the plasma and CSF after suckling, and these findings are akin to previous reports on newborn piglets [17] and calves [33]. Furthermore, Fig. 2 shows that the Lf concentration gradually increased from the second to the fourth week, which was enigmatic, especially in CSF. This Lf increasing trend might be concerned with Lf in the milk replacer or endogenous synthesis. To clarify this point, we maintained efforts to measure the Lf concentrations in the milk replacer, but we could not detect the presence of bovine Lf in milk replacer. Therefore, it could be concluded that this Lf increasing trend in weaned calf CSF was due to that endogenously synthesized by the brain itself or transferred from the plasma through the choroid plexus. To clarify these possibilities, and whether transfer of Lf into CSF occurs in young calves, we investigated the ability of Lf to be transported by the choroid plexus in young calves. Bovine Lf (0.5 g/kg) was administered intraduodenally also to examine whether absorption of Lf occurs in the intestines after the "Gut closure". As shown in Fig. 4, the Lf concentration gradually increased and attained the peak at 4 hr in both fluids, but there were no marked changes in the reference calf. The Lf concentration was 1,968.3 and 756.3 ng/ml in plasma and

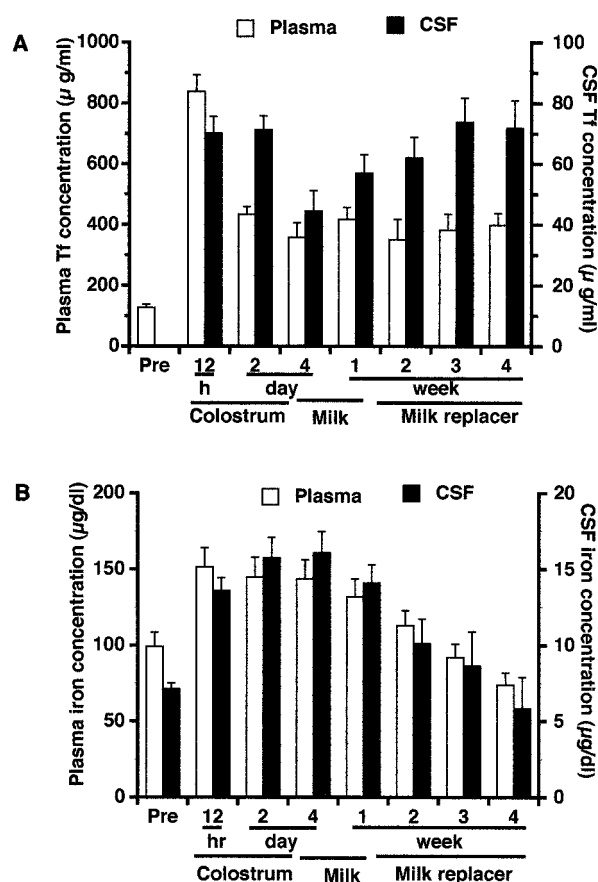


Fig. 3. A. Transferrin concentrations in the plasma and cerebrospinal fluid (CSF) at pre and post feeding of colostrum (30 ml/kg) in newborn calves, followed by milk and milk replacer (given routinely) up to the weaning period (mean \pm S.E., $n=4$). B. Iron concentration in the plasma and cerebrospinal fluid (CSF) at pre and post feeding of colostrum (30 ml/kg) in newborn calves, followed by milk and milk replacer (given routinely) up to the weaning period (mean \pm S.E., $n=4$).

CSF, respectively ($P<0.05$), which is 7 and 4 times higher than the preadministration concentration in the treatment group. These results, indeed, clearly revealed that Lf is absorbed into the blood from the gut lumen and transferred into CSF through the choroid plexus in young calves.

Binding of Lf to the epithelial membrane of the choroid plexus: The macromolecules are transported by receptor-mediated transcytosis or nonspecific transcytosis [29]. To explore the transportation phenomenon, a binding assay for Lf-R was considered. In addition, intestinal mucosal brush border membrane vesicles (BBMV) contained Lf-R in different species, such as piglets [13], humans [20] and adult cows [34]. Therefore, an Lf-binding assay of the choroid plexus was performed. Saturation kinetics and Scatchard plot transformation are shown in Figs. 5 and 6, respectively. A binding assay for Lf-R demonstrated that Lf binds to the epithelial membrane, which is saturable at 60 μM and specific. Saturation kinetics showed B_{max} 26.15 nmol/mg pro-

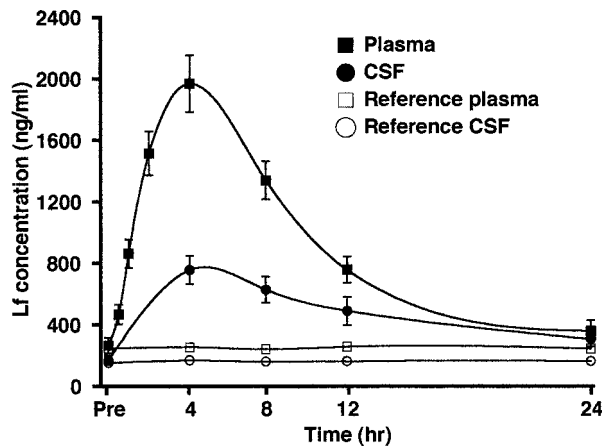


Fig. 4. Lactoferrin (Lf) concentration in the plasma and cerebrospinal fluid (CSF) at pre and post administration of Lf (0.5 g/kg, 10%, i.d.) in 3–5 month old calves (mean \pm S.E., $n=3$). Reference calf received only saline (i.d.).

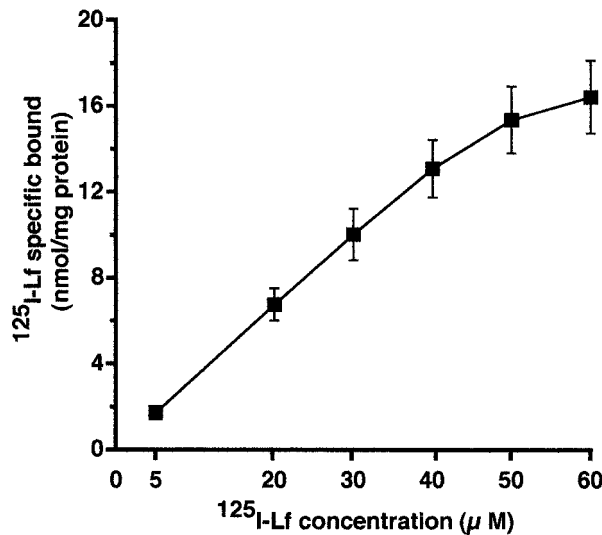


Fig. 5. Saturation kinetics for the specific binding of ^{125}I labeled lactoferrin to the epithelial membrane of the choroid plexus. Different concentrations of ligand were incubated with 20 μg of membrane in the presence or absence of 1,000-fold excess unlabeled Lf at 37°C in a water bath for 15 min (for details please see Materials and Methods). Each point represents the mean \pm S.E. for 3 individual experiments, each of which was assayed in duplicate.

tein with a K_d of 0.11 μM . In addition, Scatchard plot transformation revealed the presence of a single type of Lf-R in the choroid plexus.

DISCUSSION

The present study provides evidence of receptor-mediated transcytosis of Lf into the CSF of young calves. We have also found that a single type of Lf-R is present in the

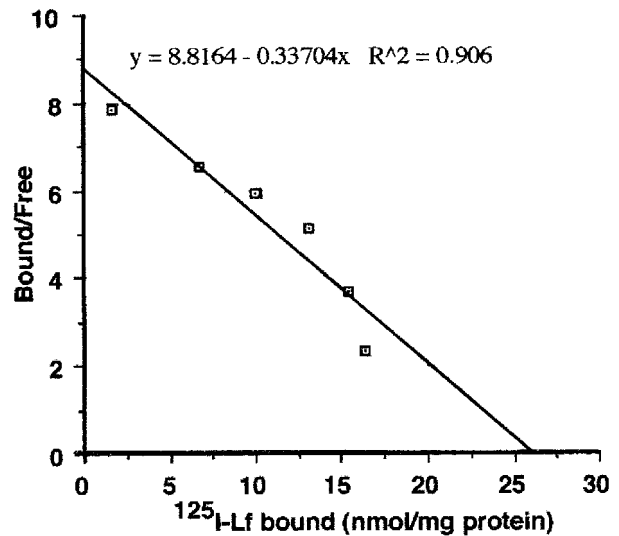


Fig. 6. Scatchard plot analysis of ^{125}I labeled lactoferrin (Lf) binding to the epithelial membrane of the choroid plexus. Each point represents the mean for 3 individual experiments, each of which was assayed in duplicate.

choroid plexus through which Lf is transferred into the CSF. In addition, Lf is absorbed in the gut and transferred into the CSF via plasma in a time dependent manner in young calves as in newborns.

The intestines of neonatal pigs absorb the homologous and heterologous proteins [28], such as bovine IgG [21], bovine Lf [17] and nonprotein macromolecules [24]. The effective transportation of ingested proteins is facilitated by a decreased proteolytic degradation of proteins due to the presence of protease inhibitor in colostrum [39], and Lf and Tf are remarkably resistant to proteolytic degradation [3]. In addition, Lf, Tf, IgG, and EGF-like proteins are also transferred into CSF via plasma in newborn calves after colostrum administration [33]. In this experiment, we also found that colostrum Lf, Tf and IgG are transferred into CSF via plasma under farm conditions. The transfer of macromolecules across the intestinal epithelium represents an important transportation mode that facilitates the uptake of different kinds of protein molecules. Macromolecules are transported from the gut to the systemic circulation either by specific receptor-mediated transcytosis or nonspecific transcytosis [29]. Nevertheless, IgG is not absorbed after the "Gut closure" in the intestines, but Lf-R are present in the intestines of different species, such as mice [18], piglets [13], monkeys [5], humans [20] and cows [34]. Together these studies suggest that Lf absorption in the gut involves receptor-mediated transcytosis. Our findings showed that colostrum macromolecules are absorbed from the gut into the systemic circulation, raising the possibility of physiological function(s) such as immunomodulation. Furthermore, the macromolecular concentration fluctuated up to 4 w, which draws attention to important physiological challenge(s) to prevent disease(s) when milk and milk replacer are given

routinely.

In the present study, total protein concentrations had increased 5-fold in CSF at 12 hr after colostrum suckling, and maintained the same level without significant changes. Moreover, Lf, Tf and IgG concentrations in CSF were increased 7.5-, 200-, and 202-fold at 12 hr after colostrum feeding, and gradually declined up to 1 w, 4 d and 2 w, respectively. The BBB is relatively impermeable and protects the brain from factors that could impair neuronal function [2]. It is also reported that the greater penetration of proteins from the blood to the CSF in immature fetal brain tissues occurs through the transcellular passage via a system of tubulocisternal endoplasmic reticulum in the epithelial cells of the choroid plexus [26]. Furthermore, it is demonstrated in *in vitro* experiments that bovine Lf crosses the BBB and is internalized by lipoprotein receptor-related protein. Lf is transported unidirectionally from the luminal to the abluminal side of the cells, and is not degraded during passage through the intraendothelial pathway [10]. We observed that the Lf concentration in plasma remained stable without marked changes from 4 d to 4 w, but gradually increased in the CSF. This increased amount of Lf might be due to the unidirectional transcytosis from plasma to CSF or be endogenously synthesized by the microglial cells due to increasing demands of brain function(s). In addition, we found Lf-R with a K_d of $0.11 \mu\text{M}$ in the epithelial cells of choroid plexus in young calves, although we could not detect Lf-R subtypes using high concentrations of ligand up to $200 \mu\text{M}$ (data not shown). Spik *et al.* [32] characterized two kinds of lactoferrin receptors on different target cells, and Fillbeen *et al.* [10] observed low and high affinity binding sites of Lf-R with a K_d of $2 \mu\text{M}$ and 37 nM in bovine brain endothelial cell lines. This discrepancy might be due to the differences in experimental conditions, receptor expression in the endothelial cell culture system, and so on. These findings, however, suggest that Lf is transferred into the CSF through receptor mediated transcytosis.

The BBB restricts the passage of polar compounds and macromolecules from the blood into the brain interstitium [30]. Talukder *et al.* [33] demonstrated the transportation of colostrum Tf and IgG in newborn calves. It is reported that Tf receptor is abundantly expressed on blood vessels, large neurons in the cortex, striatum, hippocampus, oligodendrocytes, and astrocytes [4], and BBB transports Tf through receptor-mediated transcytosis in *in vitro* experiments [11]. Moreover, the epithelial cells of the choroid plexus are enriched in proteins such as iron carrier Tf, which can act as a trophic factor in the brain [9]. Protein and Tf concentrations in the fetus remained very high, but the plasma protein concentration steeply declined just after birth. We could not measure the fetal plasma Tf level to compare it with that post partum. Nevertheless, the plasma Tf concentration in this study could be compared with the findings in newborns [33]. But one of our intriguing findings is that the plasma Tf concentration remained almost stable from 2 d to 4 w, whereas it gradually increased in CSF from 4 d to 3 w (Fig. 3A), possibly to meet the requirements of brain tissues. This

leads us to believe that Tf also may have important physiological function(s) in the brain.

Lf is a multifunctional glycoprotein, belonging to the Tf family, and binds one atom of iron in each lobe. Recently, Hagiwara *et al.* [15] demonstrated that Lf facilitates iron absorption when there is a shortage of iron stored in the body in weaning and young (adult) mice. On the other hand, de Vet and van Gool [6] observed an inverse relationship between the Lf concentration in duodenal fluids and the iron status of adult mice, and concluded that Lf may act to inhibit iron absorption when iron stores are sufficient in the body. Our findings revealed that the iron concentration increased 1.5- and 1.9-fold in plasma and CSF after colostrum feeding at 12 hr and 4 d, respectively. Iron is essential for a wide variety of cellular processes including oxidative phosphorylation and DNA synthesis. Iron is also involved with heme, cytochromes, aconitase, ribonucleotide reductase, succinyl dehydrogenase and so on. Nevertheless, it is notable that the iron concentration gradually declined up to 4 w in both fluids (Fig. 3B) and did not show a fluctuating trend, unlike Lf or Tf (Figs. 2 and 3A). This raises the possibility that Lf alone may modulate brain function(s) besides regulating the iron stores in the body.

Duthille *et al.* [7] demonstrated that Lf promotes differentiation but not proliferation of Jurkat lymphoblastic cells, and acts by activating a transduction pathway. We observed that the plasma/CSF ratio of Lf concentrations (Fig. 2) in colostrum preadministration and at the peak (12 hr) in newborns was 24.3 and 23.0, respectively. On the other hand, the plasma/CSF ratio of Lf concentrations in young calves (Fig. 4) at the base level and peak (4 hr) after Lf administration was 1.5 and 2.6, respectively. The differences between the ratios of colostrum preadministration (newborn) and the base level (young) might be attributable to the transfer of endogenous plasma Lf into the CSF of young calves, as Lf was found to be associated with CNS during aging [22]. In addition, differences might be due to the well-developed transportation system including increased expression and greater affinity for Lf-R in the choroid epithelium of young calves than in newborns. Transport of Lf into CSF via plasma in young calves suggests that Lf will be transported into the CSF in adult cattle in the same way.

In conclusion, colostrum Lf, Tf and IgG were transported into the CSF via plasma and their concentrations were changed at different time points during the weaning period under farm conditions. In addition, intraduodenally administered Lf is transferred into the CSF of young calves probably through receptor-mediated transcytosis via plasma. These findings indeed suggest that Lf plays an important role in the regulation of brain function (s) irrespective of the age of the animal.

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