2 Blood–Brain Barrier and Blood–CSF Barrier Function

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The restriction of free exchange of molecules and cells between the blood and the perivascular extracellular spaces is referred to as a “barrier.” Passage rates may vary greatly because of the different morphological structures, but all molecules, including the largest proteins, and entire cells can pass these structures, even the blood–brain barrier with its particularly tight intercellular junctions.

Blood–Brain Barrier

Structure. The blood–brain barrier is a morphologically defined structure. Its special components include capillaries, the basal membrane, and a perivascular layer of astroglial cells. Unlike other capillaries, some brain capillaries have an endothelium with cells connected by tight junctions. The tight junctions around brain capillary cells form a three-dimensional maze, which is not as impenetrable as their morphology in two-dimensional tissue sections suggests. In addition, the capillary structures in brain are not uniform but also contain fenestrated capillaries. All these morphological features are sufficient to explain the passage of large particles by passive diffusion from blood to brain and CSF. However, it is neither morphologically nor functionally justified to refer to any of these passageways as “pores.”

Permeability. The permeability and selectivity of the blood–brain barrier for proteins is determined by the diffusion of the macromolecules, which is dependent on molecular size. Properly speaking, what we are speaking of is a particular barrier function for proteins, as distinct from other facilitated or active transfer processes for other molecules—for in fact a variety of different barrier functions exist based on the different conditions of passage for different classes of substances, e.g., amino acids (Kruse et al., 1985), sugars, and vitamins (Reiber et al., 1993).

The Blood–CSF Barrier Function

Definition

It is the blood–CSF barrier function which is the really important function in CSF analysis. Empirically, it is described by the ratio of protein concentrations in venous blood and (lumbar) CSF. Unlike the blood–brain barrier, it includes dynamic aspects (e.g., CSF flow) that cannot be described in morphological terms (Reiber, 1994a).

A blood-derived protein, such as albumin, reaches the CSF via diffusion from local blood vessels directly into the ventricles, the cisterns, and the cerebral and spinal subarachnoid spaces. Thus, its concentration increases steadily along the pathway of CSF flow, and hence also (secondarily—Reiber, 2003) depends on the flow rate (Fig. 2.1).

The multiplicity of morphological structures restricting the diffusion of molecules between blood and CSF, and the additional functional effects of CSF flow rate on CSF protein concentrations, have given rise to the term blood–CSF barrier function.

![Fig. 2.1 Contributions of diffusion and CSF flow to blood–CSF barrier function. Molecules in serum at a concentration \(C_s\) diffuse through the tissue along the diffusion pathway \(x\) into the CSF of the subarachnoid space (concentration in CSF: \(C_c\)). The molecular flux \(J\) depends on the local concentration gradient \(\Delta c/\Delta x\) or \(dc/dx\) and the diffusion coefficient \(D\) (Fick’s First Law). When the CSF flow rate \(r\) decreases (and thus also the volume turnover), the molecular flux, which remains steady, enters a smaller volume, and thus the concentration of proteins in the CSF rises. As the concentrations \(C_s\) change, the local gradient \(dc/dx\) changes as well (Fick’s Second Law). This is why the bulk CSF flow rate \(F\) has a nonlinear influence on blood-derived proteins in CSF—that is, on the blood–CSF barrier function. The flow rate of a molecule in the CSF is \(r = F/A\), with \(A\) being the variable cross-section of the subarachnoid space volume fraction, \(\Delta s = A \cdot \Delta y\). \(J\) has a reciprocal relation to the flow rate of a molecule in CSF, \(r\).]
Fig. 2.2a, b Dynamics of blood-derived proteins between blood and CSF before (curve C) and after (curves D and E) pathological reduction of the CSF flow rate. Comparison of the nonlinear model (a) with the linear model (b) of the blood/CSF protein concentration gradient. The serum concentration ($C_s$) of an individual protein has been normalized, its maximum value being $C_s = 1$. Corresponding CSF concentrations are presented as dimension-free CSF/serum quotients ($Q_C$, $Q_D$, $Q_E$) with values ranging between 0 and 1. The idealized diffusion barrier is illustrated as the effective diffusion distance ($x$) (Reiber, 1994a). As the CSF flow rate drops (from C to E), the protein concentration rises (e.g., IgM). The calculated overall ratio between blood and CSF concentrations is the same in a and b, but in a the local concentration gradient ($dc/dx$) at the border with the subarachnoid space (at $x$) increases in curves C to E, due to the increasing mean penetration depth (Reiber, 1994a), whereas in the linear model (b) it decreases. The molecular flux $J$ (Fig. 2.1) increases nonlinearly from C to E (a), whereas it would decrease linearly from C to E in model b. Only the nonlinear model can give a quantitative explanation of the empirically observed nonlinear increase in CSF protein concentration as CSF flow rate drops and $Q_{ab}$ increases (Figs. 2.4 and 2.5).

The blood–brain barrier is defined in morphological terms, whereas the blood–CSF barrier is defined in terms of function: after all, CSF protein concentrations are measured a long way away from the actual “barrier”—after a long CSF pathway with constant opportunities for exchange—and then related to the serum concentration in venous blood.

**Biophysical Model of Blood–CSF Barrier Function**

**Comparison with earlier models.** The new paradigm of blood–CSF barrier function and dysfunction was derived from the laws of diffusion (Reiber, 1994a, 1994b). It replaces many linear and nonlinear empirical data fits that were not supported by any physiological or biophysical theory (for references see Reiber, 1994a). The essential difference of the new paradigm from earlier models, which assumed that the overall concentration gradient between blood and CSF was linear (Fig. 2.2b), is that it assumes a nonlinear concentration gradient between blood and CSF, with a steady state between molecular diffusion and CSF flow rate (Figs. 2.1, 2.2a). In this molecular flux/CSF flow model, proteins diffusing from the blood through the tissue into the CSF are eliminated by bulk flow with the passing CSF, and these two processes create a dynamic equilibrium—a steady state. Without CSF flow, the protein concentration in CSF would gradually approach the serum concentration (as it does in a corpse shortly after death, when CSF flow ceases).

**Protein concentrations in CSF.** The sigmoid change in tissue concentration along the diffusion barrier from the blood into the CSF (Fig. 2.2a) is determined by:

- Diffusion.
- CSF flow rate.

The rate of protein diffusion into the CSF space—the molecular flux $J$ (in Fig. 2.1)—depends on:

- The size of the protein molecules (Table 3.1).
- The local concentration gradient at the border between endothelium and subarachnoid space.

It is important for the mathematical treatment that the local concentration gradient $(dc/dx)$ (Fig. 2.2a) at the endothelial surface(s) facing the subarachnoid space is used (Reiber, 1994a), rather than the (linear) overall concentration gradient between blood and CSF (Fig. 2.2b).

The crucial point of the theory—particularly for the evaluation of pathological processes—is that the slope of the local concentration gradient is affected by the CSF flow rate, and in a nonlinear way.
With this discovery it became possible to derive a hyperbolic function (Fig. 2.3) for the description of the relationship between CSF concentrations of molecules of different sizes—for example, between $Q_{IgG}$ and $Q_{IgA}$ (Figs. 2.4, 2.5).

Figure 2.4 shows an example of hyperbolic functions for a fraction of the empirical data collected from 4300 patients (Reiber, 1994a) as a basis for the establishing of the reference values in the CSF/serum quotient diagrams (Reiber and Peter, 2001). This diffusion/CSF flow theory requires no assumptions to be made about the morphology of the structures participating in the blood–CSF barrier function, since the same diffusion conditions apply for all molecules whose ratio during passage is being considered. The molecular-size-dependent "selectivity" for the passage of blood proteins into the CSF space (Table 3.1) is fully explained by the molecular-size-dependent diffusion coefficient.

Impact of the CSF Flow Rate

Decreasing CSF flow rate. A pathologically reduced CSF flow rate has the following consequences (Reiber, 1994a):

- The protein concentration in the CSF increases as a primary consequence, because the volume turnover is reduced but the molecular flux $J$ remains constant (Figs. 2.1, 2.2a). $Q_c$ thus becomes $Q_b$ (Fig. 2.2a).
- As the result of the higher concentration in the CSF, the mean concentration in the tissue also rises (curve C becomes curve D, Fig. 2.2a).
- This in turn alters the local concentration gradient ($dc/dx$) in a nonlinear fashion, thus increasing the molecular flux $J$ in a nonlinear fashion (Fick’s Second Law).
- This facilitates the entrance of molecules into the subarachnoid space (for $Q_{IgA} < 0.5$) and causes a further (secondary) increase in protein concentration in the CSF until a new steady state is reached (curve E, Fig. 2.2a).

Thus, this process contains a positive feedback mechanism—like autocatalysis—which enhances the effect of a process once begun. This nonlinearity is what distinguishes this model from earlier, linear models.

Hyperbolic function. If we now relate the concentration of two molecules of different sizes in CSF (e.g., $Q_{IgG}/Q_{IgA}$), their ratio to one another changes as CSF flow diminishes (and the protein concentration in the CSF increases) in accordance with the following function (Reiber, 1994a):

$$Q_{IgC} = \frac{1}{\text{erfc} \left( \frac{z}{\sqrt{\frac{D_{IgC}}{D_{IgA}}}} \right)} \cdot Q_{IgA}$$

This function has been recognized as a hyperbolic function (Reiber, 1994a) in which the ratio of $Q_{IgC}/Q_{IgA}$ depends entirely on the ratio of the diffusion coefficients ($D_{IgC}/D_{IgA}$). This equation, which uses complicated trigonometric series (error function complement, erfc) for the diffusion pathway ($z$), can now also be described by a common hyperbolic function (Fig. 2.4):

$$Q_{IgC} = \frac{1}{a/b \sqrt{(Q_{IgA})^2 + b^2}} \cdot Q_{IgA}$$

This is the function introduced earlier on a purely empirical basis (Reiber and Felgenhauer, 1987). Parameters $a/b$, $b$, and $c$ (see Table 5.3) have now been improved by empirical fit of the data measured for IgG, IgA, and IgM in the CSF, using a larger study group (Reiber, 1994a). This concept is valid for all blood-derived proteins in CSF (Fig. 2.5).
Rostrocaudal Concentration Gradient

The continuous diffusion of serum molecules into the CSF from the blood vessels along the CSF flow paths, e.g., along the spine, creates a rostrocaudal concentration gradient in the lumbar subarachnoid space. This gradient explains the observation that the diffusion of molecules from the blood into lumbar CSF is faster than their diffusion into ventricular CSF. The gradient is again nonlinear (Reiber, 2003). The increasing CSF concentration down the spine also induces an increasing concentration in the tissue (Reiber, 1994) and consequently an increase in the local concentration gradient going down the lumbar CSF space, to be understood as locally different steady states—as in a standing wave in acoustics, with locally different amplitudes.

Fig. 2.4 CSF/serum quotients for IgG ($Q_{IgG}$) as a function of the albumin CSF/serum quotient ($Q_{Alb}$). The reference ranges of proteins in CSF that derive exclusively from blood have been determined from patients ($n=4154$) without a humoral immune reaction (normal controls and patients with various neurological diseases). The range of reference values is characterized by the hyperbolic functions for $Q_{Lim}$, $Q_{Mean}$, and $Q_{Low}$ shown in this figure, which represents a small fraction of the whole range with albumin quotients up to $Q_{Alb}=150 \times 10^{-3}$. The corresponding parameters of the hyperbolic functions are reported in Table 5.3, for IgA and IgM as well as for IgG. In the diagnostic routine for detection of intrathecal IgG synthesis we discriminate between CNS-derived and blood-derived fractions in the CSF by reference to $Q_{Lim}$. In the figure, the intrathecal fraction (IF, upper arrow) is shown for the example of a patient with intrathecal IgG synthesis (large dot, with $Q_{Alb}=10 \times 10^{-3}$ and $Q_{IgG}=12.2 \times 10^{-3}$). When comparing groups of patients (disease group vs. control group) for statistical evaluation, it is more relevant to refer to $Q_{Mean}$ as the mean of the control group (Reiber and Albaum, 2008). The quotient diagram, in particular the discrimination line $Q_{Lim}$, is valid for ventricular, cisternal, and lumbar CSF. Detection of a barrier dysfunction only requires the use of different age-related reference values for the albumin quotient (Chap. 21, “Proteins”).

![Fig. 2.4](image)

Fig. 2.5 Molecular-size-dependent changes in the mean CSF/serum quotients ($Q$) of serum proteins in CSF with diminishing CSF flow rate. The description of protein quotients as a function of the albumin quotient by means of a hyperbolic function is valid for IgG, IgA, and IgM, as well as for all other serum proteins in the CSF studied so far. Proteins with molecular sizes larger than that of albumin lie below the 45° line, and the larger the molecule, the less steep the slope of the line (see also Table 3.1). Transferrin (TT, 54 kDa), which is associated with retinol-binding protein (RBP, 21 kDa), passes the barrier at nearly the same molecular size as albumin (67 kDa). Consequently, their quotient ratio follows a 45° line.

![Fig. 2.5](image)
References


Neoplastic Meningitis in Malignant Non-Hodgkin Lymphoma and Leukemia

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Malignant Non-Hodgkin Lymphoma

Characteristics. Malignant non-Hodgkin lymphomas (NHL) are primary intracerebral lymphomas, also called primary central nervous system lymphomas (PCNSL). They now constitute 4–7% of all primary brain tumors, and the incidence is increasing. More than 98% of them are B-cell lymphomas, and according to the Revised European–American Lymphoma (REAL) classification, they are predominantly diffuse large-cell lymphomas. The presence of the Epstein-Barr virus (the EBV genome is detected in >95% of cases) and immunosuppression (in AIDS patients, or after organ transplantation) promote the occurrence of PCNSL. In immunocompetent patients, the age peak appears in the 6th and 7th decades of life (male:female ratio 3:2); in immunosuppressed patients it is considerably earlier. Clinically, PCNSL manifest with focal neurological deficits, signs of elevated intracranial pressure, and psychopathological abnormalities.

Diagnosis. The suspected diagnosis is established by CT and MRI and is supported by spectroscopy. Confirmation of the diagnosis requires detection of lymphoblasts. However, at first diagnosis of a PCNSL lymphoblasts are found in the CSF in less than 50% of patients (Balmaceda et al., 1995). If the cytomorphology is not unambiguous, immunocytochemistry and clonality analysis may help to strengthen the diagnosis (Storch-Hagenlocher et al., 2000). If doubts still remain regarding the CSF analysis, brain biopsy must be performed to confirm the diagnosis.

Leukemia

Leptomeningeal metastasis occurs particularly in acute leukemia, the lymphoblastic type more often (about 15% of adults) than the myelogenous type. Recurrences of acute lymphoblastic leukemia (ALL) are often primarily leptomeningeal. As with lymphomatous meningitis, a definite diagnosis requires CSF collection without blood contamination.

Lymphomatous Meningitis

Leptomeningeal metastases of a primary extraneural lymphoma have been described in 10–30% of cases. In by far the majority of cases the underlying disease is NHL, particularly highly malignant NHL and Burkitt’s lymphoma. CSF analysis, in addition to CT and MRI, plays an important role in confirming the diagnosis of leptomeningeal lymphomatous spread.

Cytology

The diagnosis of lymphomatous meningitis may create considerable problems at cytomorphologic assessment. It is often accompanied by an inflammatory reaction, and the transformed lymphocytes are often difficult to distinguish from neoplastic cells. However, a highly homogenous cell population associated with pathological neurochemical findings is regarded as indicating meningeal spread.

Cytomorphologically, the diagnosis of lymphomatous meningitis can be difficult because of a concomitant reactive inflammatory reaction.

Morphology. The cytomorphological criteria of malignancy apply (Figs. 16.6, 16.7). Occasionally, staghorn or cloverleaf nuclei are found.

Immunological diagnosis. For immunological typing of leukocytes, membrane-bound or intracellular antigens are detected in the cytospin preparation using specific monoclonal antibodies. The cellular antigens expressed are designated as clusters of differentiation (CD) and are numbered. They can thus be assigned to the B-cell line (e.g., CD 19, CD 20), the T-cell line (e.g., CD 4, CD 8), or the myeloid cell line of leukocyte differentiation (e.g., CD 13), and also to the stage of cell maturation (Fig. 16.8).

For preference, cytospin preparations are analyzed using the immunoenzyme technique. The primary monoclonal...
antibody binds to cells expressing the antigen for which it has specificity. Binding of this antibody is made visible using a second antibody linked to an enzyme (peroxidase, alkaline phosphatase) that catalyzes the cytochemical staining.

The direct immunofluorescence technique is used to study CSF cells in suspension. For this purpose, the specific primary antibody is coupled with a fluorescence dye. Using different fluorochromes, several antibodies can be used simultaneously, and the cells are then analyzed by fluorescence-activated cell sorting (FACS). However, this method is limited in its usefulness as the quantity of CSF available is often insufficient.

**Clonality analysis.** Molecular clonality studies can confirm a diagnosis of lymphomatous or leukemic meningitis when the cytology remains unclear. The nuclear DNA from CSF cells is used as the starting material. The CDR3 region of the immunoglobulin heavy chain (IgH) is selectively amplified by PCR. The highly variable CDR3 region is specific for every B-cell clone and is formed during B-cell maturation by rearrangement of various segments of the IgH genes (Fig. 16.9a). Further analysis of the PCR products by automated fluorescence analysis may detect a monoclonal neoplastic B-cell population (Fig. 16.9b).

Molecular analysis of CSF cells (clonality analysis) may confirm the diagnosis of lymphomatous and leukemic meningitis.

**Neurochemistry**

Elevated total protein and CSF lactate levels together with a low CSF glucose level are typical signs of meningeval infiltration. The specificity of soluble surface markers (e.g., sCD 25) is not sufficient. Intrathecal IgM production may indicate a lymphoma (Fig. 19.4 d).

**Serum analysis.** Serum tests are important to detect blood–CSF barrier dysfunction and intrathecal immunoglobulin production.

There are no specific diagnostic serum markers of lymphoma or leukemia. Serum albumin is often decreased. Elevated β2-microglobulin and/or LDH levels may indicate meningeval infiltration. β2-Microglobulin is detected in the serum by immunoassay: values above 4.0 mg/L are pathological.