e-Neuroforum 2016 · 7:23–30
DOI 10.1007/s13295-016-0022-6
Published online: 10 May 2016

Jan Wenzel · Markus Schwaninger

Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Universität zu Lübeck, Lübeck, Deutschland

The blood–brain barrier and its regulation by NF-κB

Introduction

The blood–brain barrier protects the brain from pathogens, blood components, and the immune system. This protection is required to prevent a disturbance of neuronal activity and to maintain essential brain functions, also in the case of infections and other disorders. The blood–brain barrier is not a static constituent of the brain but is subject to a coordinated and dynamic regulation, influenced by several cell types and signaling pathways. One important player in this regulation is the transcription factor NF-κB which has many effects mediated by several of its target genes. In this review, we will give an overview of the blood–brain barrier and its regulation by NF-κB signaling pathways.

The blood–brain barrier and its cellular components

Preliminary evidence of the existence of a barrier between blood and the brain were found by Paul Ehrlich in 1885 [1]. He described the distribution of a dye, which was injected into the blood, in almost all organs except the brain. Following experiments characterized this phenomenon as the blood–brain barrier which occurs during embryonal development and becomes tighter until birth. The barrier restricts diffusion in both directions, from blood to brain as well as from brain into blood. One main effect of this barrier is that the passage of pathogens, like viruses and bacteria, is limited. But also the infiltration of immune cells into brain tissue is inhibited and strictly regulated, as blood–derived immune cells move along the vessel walls but do not frequently penetrate into the parenchyma. Still, some leukocytes, mainly T lymphocytes, do patrol the CNS and search for infective agents. Similarly, serum proteins, which could damage neurons, are not able to extravasate into the brain. On the other hand, nutrients have to be transported across the blood–brain barrier to provide energy substrates for the cells in the parenchyma. As active neurons consume a lot of energy, there is a need for a coordinated transport system to efficiently deliver substrates to the cells in the brain. Additionally, the blood–brain barrier is essential for maintaining tissue homeostasis of water and electrolytes. Overall, the blood–brain barrier and its cellular components fulfill a multitude of pivotal tasks to preserve normal function of the brain.

There are different cell types involved in the formation of the barrier between the periphery and brain. All these cell types interact and influence each other to build up the blood–brain barrier and to keep it tight. The main structure of the blood–brain barrier is the vasculature, but other structures are also involved (e.g., the epithelial cell layer in the choroid plexus or the tanycytes in the hypothalamus). In this review we will mainly focus on the blood–brain barrier of the vessels, but most of the principles are similar between the different structures of the blood–brain barrier. The vessels in the brain have special molecular and cellular characteristics which differ from those in the periphery (**Fig. 1**). In principle, one can discriminate between blood–supplying arteries and arterioles, and veins and venules, which transport the blood back to the heart. Capillaries, which are the smallest vessels with a diameter of 3–10 µm, are located in between (**Fig. 1b**). Below, we will describe the cellular and noncellular components of the vascular blood–brain barrier in detail.

Endothelial cells. Endothelial cells form the inner layer of blood vessels (**Fig. 1c**) and are the most important cells regarding the physical sealing of the blood–brain barrier. They are connected to each other by so-called tight junctions (**Fig. 1d**). The main molecular components of the tight junctions are proteins of the claudin family (mainly claudin-3 and -5) as well as proteins of the MARVEL family (e.g., occludin or tricellulin), but also JAM-1 (junctional adhesion molecule-1) and intracellular anchor molecules like ZO (zona occludens)-1,-2, and -3. These factors build up connections between endothelial cells, which are much tighter than those in vessels of peripheral organs. In addition, endothelial cells are in direct contact with the blood and regulate the transfer of substrates which have to be transported into the brain but are not able to diffuse across the blood–brain barrier. They express receptors and transport systems, which are special to brain endothelial cells and thereby lead to sufficient supply for neurons. Similarly, they regulate the removal of different forms of waste, for example, through the P-glycoprotein transporter. Beside the maintenance of the integrity and the transport of molecules, endothelial cells express special surface proteins, which account for

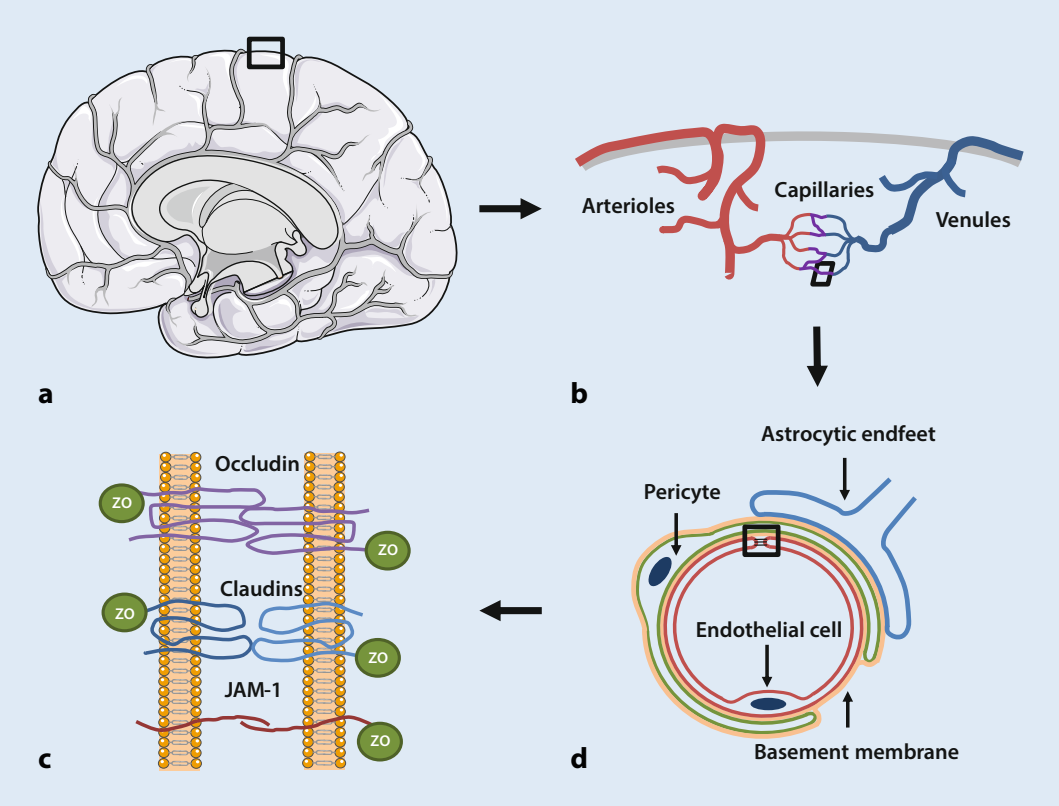

Fig. 1 ▲ The organization of the blood–brain barrier. **a** The whole brain is densely vascularized to provide a sufficient supply to neurons. **b** The vasculature is composed of surface arteries, which penetrate the brain as arterioles and branch out as capillaries, the smallest type of vessels. Capillaries are replaced by venules which end as larger veins that transport the blood back to the heart. **c** The blood–brain barrier is mainly represented by capillaries, which consist of endothelial cells, the basement membrane, and a surrounding layer of pericytes and astrocytic endfeet. **d** One special feature of brain endothelial cells are the so-called tight junctions. Components of the tight junctions are occludin, factors of the claudin family, junctional adhesion molecule-1 (*JAM-1*), as well as adaptor proteins of the zona occludens (*ZO*) family

the communication with cells of the immune system.

Basement membrane. The basement membrane is an extracellular structure, which surrounds the endothelial cell layer and consists of several types of molecules (**Fig. 1c**). The components of the basement membrane, some of which are not found in peripheral basement membranes, are expressed by endothelial cells, but also by pericytes. One main protein is type IV collagen, but also laminins play an important role for the stabilization of the basement membrane. Laminin α4 and α5 are secreted by endothelial cells, whereas laminin α2 is also expressed by pericytes or smooth muscle cells [2]. Together, those molecules form a net-like structure, which stabilizes the vessels but also interacts with neighboring cells. Additionally, the composition of the basement membrane influences the infiltration of immune cells. Dysfunction of the basement membrane, especially of collagen IV, leads to bleeding in the brain. Therefore, the basement membrane is an important noncellular part of the blood–brain barrier, which regulates the stability, intercellular communication, and immune cell trafficking.

Pericytes. Pericytes are embedded in the vascular basement membrane and surround the endothelial cell layer (**Fig. 1c**). In larger vessels, the pericyte layer is replaced by smooth muscle cells, which are able to change the vessel diameter actively. The pericyte to endothelial cell ratio is higher in brain vessels than in peripheral vessels and close communication between pericytes and endothelial cells is needed for normal development of the brain vasculature and the blood–brain barrier [3]. Transforming growth factor β (TGFβ) is an important factor that is secreted by pericytes and endothelial cells and binds to receptors on both cell types. Another factor is platelet-derived growth factor B (PDGFB) which is expressed in endothelial cells and acts on pericytes. By these factors and also others, pericytes are able to affect the function of the blood–brain barrier. Disturbed communication between pericytes and endothelial cells leads to an interruption of vessel development and an increased permeability of the barrier [3], for example if the TGFβ receptor is not expressed or the PDGFB release is decreased. TGFβ can lead to increased expression of tight junction proteins like claudin-5 or occludin. Beside their direct effects on the barrier function, pericytes also have a regulatory impact on the infiltration of immune cells. Whether pericytes can also change the vessel diameter actively is controversial.

e-Neuroforum 2016 · 7:23–30
DOI 10.1007/s13295-016-0022-6

J. Wenzel · M. Schwaninger

The blood–brain barrier and its regulation by NF-κB

Abstract

The brain is protected by a tight barrier between the blood and parenchyma. This so-called blood–brain barrier protects the brain from invading pathogens, infiltrating immune cells, and the extravasation of serum proteins. Beside pericytes and astrocytes mainly endothelial cells form this barrier.

Inflammation leads to an increase in the permeability of the blood–brain barrier. NF-κB is activated during inflammation and is a key regulator of inflammatory processes. In brain endothelial cells NF-κB protects the blood–brain barrier. Loss of the NF-κB activating protein NEMO in brain endothelial cells leads to endothelial cell death, increased permeability, and epilepsy in mice as well as in humans with the hereditary disease *incontinentia pigmenti*. Therefore, inflammatory mediators are able to disturb but also to protect the blood–brain barrier.

Keywords

Blood–brain barrier · NF-κB · NEMO · Endothelial cells · Inflammation

Astrocytes. Astrocytes were originally described as cells which support neurons by building up a stabilizing structure and electrically isolate them. Today it is known that astrocytes have many additional functions in the brain. They provide energy substrates to neurons, are able to regulate blood flow, and can affect inflammation. The so-called astrocytic endfeet are an important part of the blood–brain barrier (**Fig. 1c**). Endfeet enclose almost all vessels in the brain and enable astrocytes to interact with other components of the blood–brain barrier directly. There are special proteins located at the endfeet, by which astrocytes control water and electrolyte homeostasis and thereby affect the functions of neighboring cells. Prominent examples are aquaporin 4 (AQ4), which is a main regulator of water influx into the parenchyma, and potassium inwardly rectifying channel 4.1 (K_{ir}4.1), which contributes to ion homeostasis. Astrocytes express factors which influence the barrier properties of endothelial cells in a negative or in a positive way; examples are TGFβ, vascular endothelial growth factor (VEGF), fibroblast growth factor, or angiopoietin 1. The interaction between endothelial cells and astrocytes is essential for the formation and the maintenance of the blood–brain barrier.

Inflammation and its impact on the blood–brain barrier

Many diseases are accompanied by an increased permeability of the blood–brain barrier. With different etiologies the disturbance of the blood–brain barrier seems often to be a secondary effect. Thus, a long-lasting disturbance of the blood–brain barrier is found in diverse conditions such as neurodegenerative disorders, diabetes, or psychiatric diseases. In addition to chronic changes of the brain vasculature, acute pathological factors also regulate the function of the barrier. Acute inflammation opens the blood–brain barrier and reseals it at a later stage. In this section, we will describe the changes which occur at the blood–brain barrier during inflammation.

Opening of the blood–brain barrier. Usually, the blood–brain barrier provides sufficient protection for the brain against pathogens and immune cells. However, when there is an immune reaction in the brain, the blood–brain barrier opens. Many factors are involved in this process, coming from different cell types and affecting all parts of the barrier. During acute inflammation, tight junctions in endothelial cells are disorganized and tight junction proteins are expressed at a lower level, leading to increased permeability. In addition, the expression of adhesion proteins in endothelial cells increases, which allow for enhanced interaction with immune cells. After stimulation, pericytes release nitric oxide, but also interleukins and VEGF, all of which are factors which lead to increased permeability of the blood–brain barrier. VEGF induces a decrease in claudin and occludin expression in endothelial cells. Additionally, pericytes serve as a structure along which immune cells migrate from the blood into the parenchyma [4]. During this process pericytes actively enlarge gaps, thereby facilitating the infiltration of immune cells. One important player in this mechanism is tumor necrosis factor (TNF).

Infiltration of immune cells. During an inflammatory reaction in the CNS, immune cells like neutrophils start to infiltrate the brain. Once in the brain, immune cells secrete proinflammatory factors which may interfere with neuronal function. The process of infiltration can be subdivided into distinct steps and takes place mainly in postcapillary venules of the brain (**Fig. 2**). First of all, circulating blood cells loosely contact the endothelial cells, through endothelial selectins and their ligands (rolling). This first contact can induce activation of the involved cells, which consequently secrete several factors leading to the next steps of infiltration. Surface proteins of immune and endothelial cells, like integrins, VCAM-1 or ICAM-1, interact to form a more adhesive binding (arrest). Subsequently, the immune cells start to migrate on the endothelial cell layer, often against the direction of blood flow (crawling). They move until they find a suitable site to cross the barrier to reach the parenchyma (infiltration). There are two ways to traverse the endothelial lining, either at a site where two endothelial cells are connected or directly through one endothelial cell body. Neither leads to a disruption of tight junctions. After crossing the endothelial cell layer immune cells secrete enzymes which degrade the basement membrane, so-called matrix metalloproteinases (MMPs). The infiltration and opening of the blood–brain barrier can induce damage to neurons leading to impaired CNS function, particularly in chronic conditions.

Resealing of the blood–brain barrier. Not much is known about the termination of an inflammatory reaction. Once believed to be a passive process this termination is now considered to be an active program, during which factors are secreted that actively induce the end of the inflammation. This so-called resolution starts in a later phase of an immune reaction and is induced

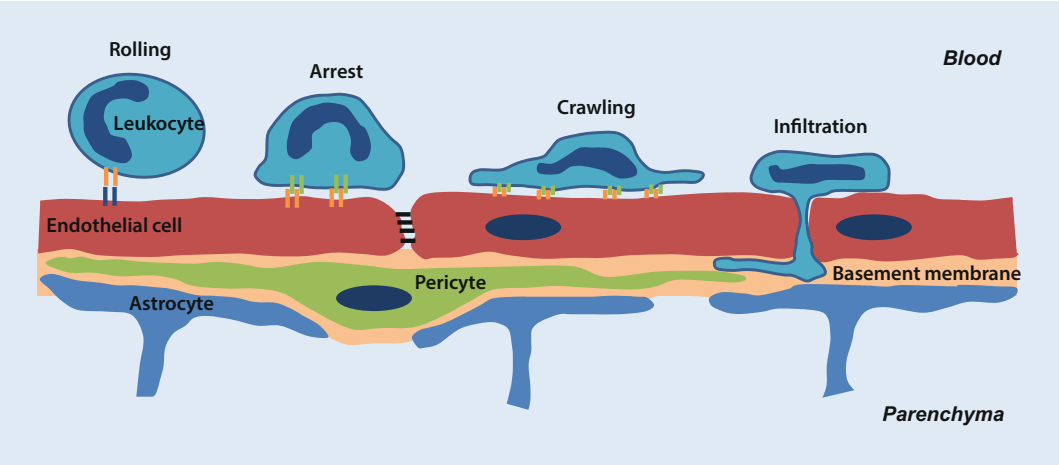

Fig. 2 ▲ Infiltration of immune cells during an inflammatory reaction in the brain. The process of infiltration is subdivided into several steps. First, the blood circulating leukocytes contact endothelial cells loosely (rolling). This could lead to an activation of the cells and a more adhesive binding (arrest). The immune cells start to migrate on the endothelial cell layer (crawling), before they move across this layer towards the parenchyma (infiltration)

by proinflammatory mediators themselves. In many chronic diseases, like Alzheimer's disease, diabetes or multiple sclerosis, important factors of the resolution process are dysregulated. Also the blood–brain barrier reseals again at a later stage of inflammation. In stroke, for example, there is an initial opening of the barrier which resolves; this is followed by an even higher increase in permeability again after approximately two days. After several more days the blood–brain barrier tightens again and the inflammatory reaction decreases. Which factors and cells are involved in this time course is largely unknown.

NF-κB as important mediator of inflammatory reactions

NF-κB (nuclear factor-κB) was initially described in 1986 as transcription factor, which regulates the expression of the immunoglobulin κ light chain in mature B cells and plasma cells [5]. In the following years, many target genes were discovered, by which NF-κB controls essential functions of the immune system. NF-κB subunits were identified in almost all cells and can be activated by proinflammatory stimuli, but also by other factors like ultraviolet or γ radiation. The NF-κB protein complex is located in the cytosol and is transported into the nucleus after activation (◘ **Fig. 3**). Once there, it can bind to specific DNA sequences to change the expression of the respective genes. Until now many NF-κB target genes are known, which are mainly involved in inflammation, cell division, and the regulation of cell survival. Because of the huge number of effects of NF-κB, it is necessary to control its activity at different levels, for example by target genes, which in turn affect NF-κB again like A20. The activity of NF-κB and its target genes strongly depend on the cell type and the type of stimulus.

NF-κB is a complex which is composed of two either identical (homodimers) or different subunits (heterodimers). NF-κB subunits are characterized by homologies in their amino acid sequence and are assigned to one protein family, which consists of five members: p65 (RelA), RelB, c-Rel, p105/p50 (NF-κB1), and p100/p52 (NF-κB2). Not all of the theoretical combinations are really detectable. The most common variant is a heterodimer of p65 and p50, which can be found in almost all cell types. As already mentioned, the inactive NF-κB dimer is kept in the cytosol of the cells. It is retained by IκB proteins, which mask a nuclear transition signal at the NF-κB complex and do not dissociate until they are phosphorylated. So far, seven different IκB proteins have been discovered. After phosphorylation by the IκB kinase (IKK) complex, IκB is tagged with ubiquitin to direct this factor to the proteasome, where it is degraded. There are two different pathways leading to the degradation of IκB and to the translocation of NF-κB into the nucleus: the classical and the alternative signaling pathways (◘ **Fig. 3**).

Classical signaling pathway. In the classical NF-κB signaling pathway, the IKK complex consists of the two enzymatic subunits IKK1 (IKKα) and IKK2 (IKKβ) and the regulatory subunit NEMO (NF-κB essential modifier, IKKγ). IKK phosphorylates IκB proteins (e. g., IκBα) and induces its degradation in the proteasome. In the classical pathway, IκBα binds the NF-κB proteins p65 and p50 and the activation of IKK is triggered by extracellular proinflammatory stimuli like TNF or interleukin 1β as well as for microbial factors that are released during infections. The stimuli bind to specific receptors and induce the activation of IKK through several steps. One of these steps is mediated by TGFβ-activated kinase 1 (TAK1). TAK1 phosphorylates IKK2, leading to an activation of the IKK complex and subsequently to a translocation of NF-κB into the nucleus (◘ **Fig. 3**).

Alternative signaling pathway. In the alternative pathway, IKK consists of an IKK1 homodimer and is regulated by NF-κB-inducing kinase (NIK). NEMO and IKK2 do not contribute to the activation of NF-κB in this pathway. Under normal conditions NIK is continuously degraded, but after stimulation NIK is

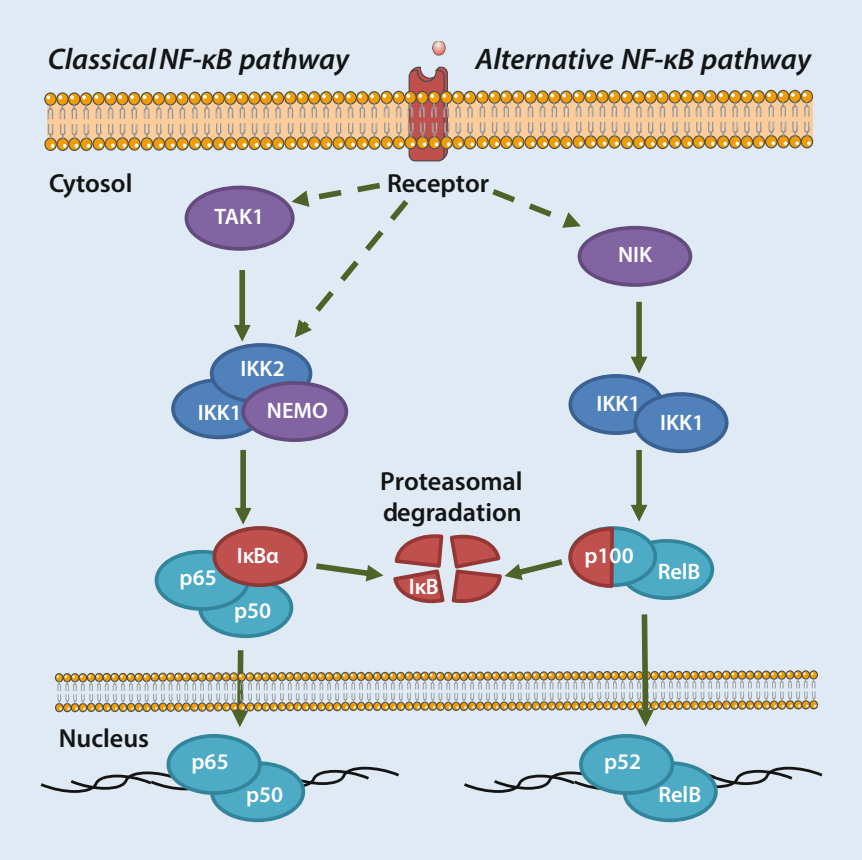

Fig. 3 ▲ NF-κB signaling pathways. There are two different NF-κB signaling pathways. During the classical pathway the IκB kinase (*IKK*) complex is activated by different stimuli. The stimulation could be mediated by TGFβ-activated kinase 1 (*TAK1*). The IKK complex consists of the enzymatic subunits IKK1 and IKK2 as well as of the regulatory subunit NF-κB essential modifier (*NEMO*). IKK phosphorylates IκB proteins, which are degraded in the proteasome. The degradation of those inhibitory factors leads to a translocation of NF-κB into the nucleus, where it acts as transcription factor on several target genes. In the classical pathway NF-κB is often composed of p50 and p65. An activation of the alternative pathway is mediated by the NF-κB-inducing kinase (*NIK*), which also activates an IKK complex. Different from the classical pathway the IKK complex consists of two IKK1 proteins. In the alternative pathway IKK activation leads to a cleavage of p100, resulting in the degradation of the IκB sequence and the release of the NF-κB subunit p52. Together with RelB p52 forms the active NF-κB complex, which translocates into the nucleus and again acts as transcription factor

stabilized and activates IKK, which consequently binds to the inhibiting protein IκB. The IκB protein of the alternative pathway is p100. Similar to the classical pathway, p100 is phosphorylated and cleaved in the proteasome. But in contrast to the classical pathway this cleavage is not complete and the resulting cleavage product p52 acts as an active NF-κB subunit (◘ **Fig. 3**). P52 mostly binds to RelB, which leads to an active NF-κB heterodimer which translocates into the nucleus and induces the expression of specific target genes. Activation of the alternative pathway is described for several receptors and stimuli, which partially also activate the classical pathway. Thus, both signaling pathways can operate in parallel and also interact with each other. For example, the classical pathway through p65 could be blocked by p100, whereas inhibition of the classical pathway by a loss of NEMO leads to increased activity of NIK and the alternative pathway.

Inflammation. In the case of an acute inflammatory reaction, NF-κB is activated in almost all cells. The effects of activation are strongly dependent on the cell type. In cells of the immune system, proinflammatory mediators, such as surface proteins or signal molecules, are expressed and facilitate adhesion or guide other immune cells to the site of inflammation. In other cell types, NF-κB activation regulates cell survival and differentiation. How NF-κB activation is terminated in inflammation is poorly understood. NF-κB is able to inhibit its own activation at different levels. Thus, IKK1 has inhibitory properties at later time points after activation. But also the expression of NF-κB target genes, such as IκB proteins or A20, leads to an inhibition of NF-κB activity. When A20 is lost, NF-κB is continuously activated leading to severe immune reactions.

In summary, NF-κB is a key player of the activation of the immune system and its activity is balanced by complex mechanisms. The importance of NF-κB signaling is reflected by the consequences of a dysregulation in the pathway. Several mutations were found in humans, which affect factors involved in the NF-κB signaling pathways. These mutations all lead to a dysfunctional immune system or changes in cell survival [6]. In addition, dysregulation of NF-κB signaling pathways can be found in many chronic immune and neoplastic diseases.

The role of NF-κB in brain endothelial cells

Also in the brain, inflammatory mechanisms are important, especially when tissue is damaged and the blood–brain barrier is open, like in stroke. As important regulator of the immune system NF-κB is activated in different cell types when the blood supply is reduced. Depending on the cell type, NF-κB induces different reactions. In neurons, NF-κB activation leads to neuronal damage and cell death. These effects, which were induced by ischemia, are dependent on the NF-κB subunits p50 and p65 [7]. The activation of NF-κB in neurons after stroke is mediated by the classical pathway, as an activation of IKK2 leads to increased neuronal damage [8]. Inhibition of the IKK complex, especially of IKK2 reduces tissue damage after stroke. But also in cell types other than neurons, NF-κB plays a prominent role. NF-κB activation was detected in many components of the blood–brain barrier, like astrocytes and endothelial cells, after inflammation. As described above, an inflammatory re-

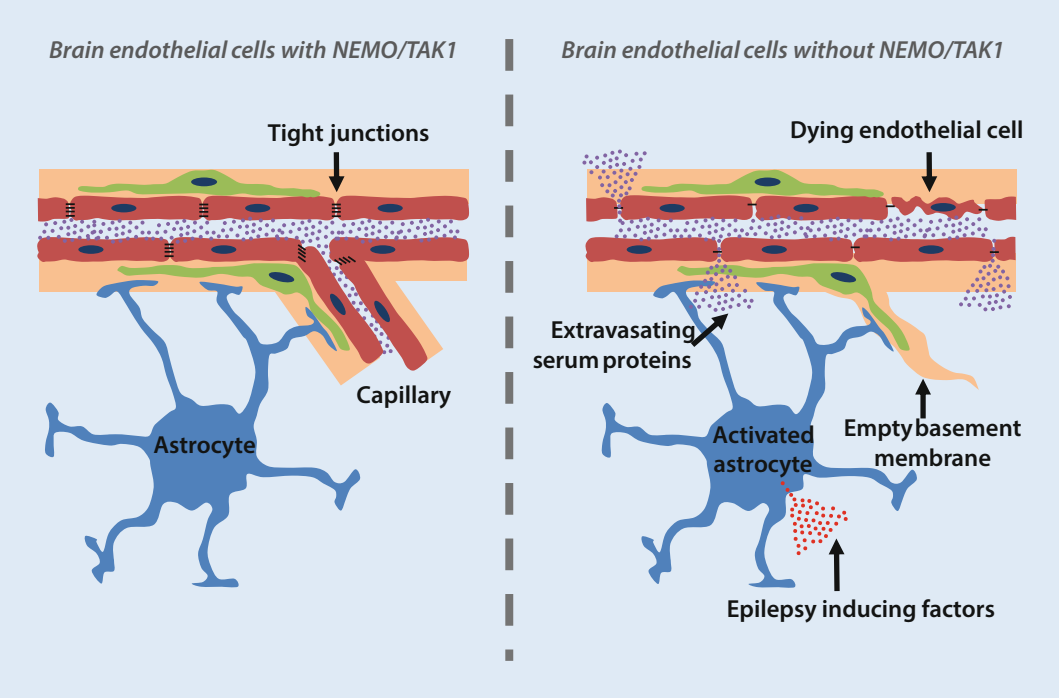

Fig. 4 ▲ The effect of endothelial NEMO and TAK1 on the blood–brain barrier. On the left side, the blood–brain barrier with its cellular components is illustrated. On the right side, the effects of a loss of NEMO or TAK1 in brain endothelial cells are presented. Deletion of those factors leads to the death of some endothelial cells, resulting in empty basement membrane tubes instead of capillaries. Additionally, tight junctions are disrupted due to reduced occludin. This opening of the blood–brain barrier leads to extravasation of serum proteins, which can activate astrocytes. Activated astrocytes can release factors which induce a change in neurons and cause epileptic seizures

action is often accompanied by increased permeability of the blood–brain barrier. Whether NF-κB is involved in the opening of the barrier or just activated in parallel is not completely understood. NF-κB activation in pericytes leads to secretion of MMPs and thereby to degradation of the basement membrane which results in an opening of the blood–brain barrier [9]. In endothelial cells strong NF-κB activation can induce a disruption of tight junctions [10], leading to increased permeability of the endothelial cell layer. NF-κB also plays an important role in astrocytes. If NF-κB in astrocytes is continuously activated by deletion of IκBα, inflammation occurs in the brain, which then could lead to an opening of the blood–brain barrier.

On the other hand, NF-κB is not only activated by inflammatory stimuli but has also basal activity in many cell types in the brain. The basal activity seems to be needed for cell survival and function. NF-κB proteins in epithelial cells of the intestine were shown to protect the intestinal barrier [11], and in neurons NF-κB has effects on plasticity and memory formation. For the maintenance of the blood–brain barrier, endothelial cells are of great importance, as already described. Until recently, it was not known how the basal activity of NF-κB affects those cells. To examine the classical NF-κB signaling pathway in brain endothelial cells, a mouse model was generated, which lacks the regulatory subunit of IKK, NEMO, in brain endothelial cells [12].

Vessel morphology. To investigate the role of NEMO, the brain vasculature was examined in brain endothelial cell-specific NEMO knockout mice. Mice without NEMO in brain endothelial cells showed decreased vessel density in the brain, which could be explained by an increase in endothelial cell death (◘ **Fig. 4**). At the sites of cell loss, only the basement membrane remained. Reduced vessel density and increased endothelial cell death were found in all areas of the brain, which led to lower perfusion in the whole brain. Survival of endothelial cells was not only dependent on NEMO but also on the upstream kinase TAK1 and the proinflammatory mediator TNF. Mice with a deletion of TAK1 or NEMO in brain endothelial cells showed the same phenotype, reduced vessel density and empty basement membrane tubes. An involvement of TNF was indicated by the effect of neutralizing antibodies against TNF, which led to decreased endothelial cell death. Interestingly, endothelial cell death and the reduced vessel density were independent of the enzymatic subunit IKK2 and the NF-κB protein p65. These findings suggest a function of NEMO and TAK1 in cell survival which appears to be independent of the classical NF-κB pathway and requires other factors.

Blood–brain barrier function. Additionally, mice without NEMO or TAK1 in brain endothelial cells had a disturbed blood–brain barrier [12]. Molecules of different sizes leaked from the blood into the brain and also serum proteins were dramatically increased in the parenchyma (◘ **Fig. 4**). The increased permeability was accompanied by re-

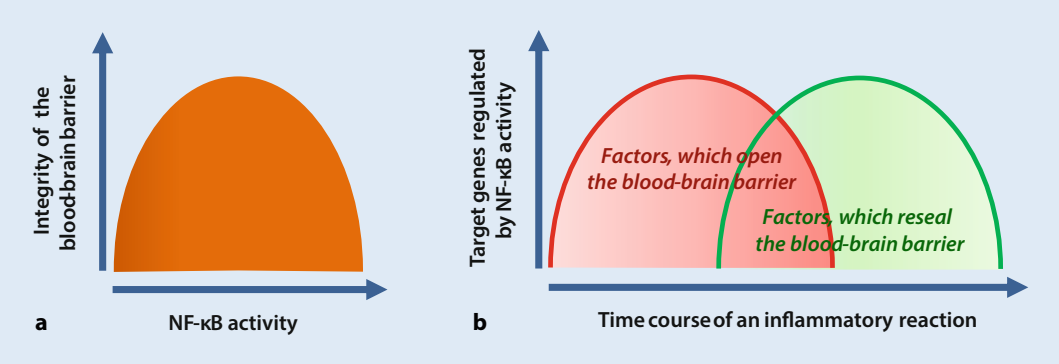

Fig. 5 ▲ The role of NF-κB in the regulation of the blood–brain barrier integrity. Effects of NF-κB are described, which could lead to an opening as well as to a tightening of the blood–brain barrier. This seemingly contradiction could be explained by two different hypothesis. **a** A basal activity of NF-κB is necessary to maintain the blood–brain barrier integrity, but excessive activation of NF-κB signaling pathways leads to an opening of the blood–brain barrier, for example during inflammation. **b** Activation of NF-κB during an inflammatory reaction leads to the expression of proinflammatory factors, which can induce an opening of the blood–brain barrier at the beginning of the reaction, but also to the expression of factors which lead to a re-sealing of the barrier at a later time point

duced amounts of the tight junction protein occludin, whereas other tight junction factors like claudin-5 and ZO-1 were not changed. Blood–brain barrier disruption occurred independently of endothelial cell death as it was present in many capillaries and not restricted to the sites of cell death. In addition, blood–brain barrier function was regulated by a different signaling pathway than endothelial cell death, as p65 and IKK2 were involved in maintaining the barrier but not cell viability. The involvement of IKK2 was investigated by overexpression of a constitutively active variant of IKK2 in brain endothelial cells, which led to a rescue of the classical NF-κB pathway in those mice. The IKK2 overexpression led to a tightening of the barrier and normal amounts of occludin in the tight junctions of endothelial cells, which did not have TAK1. In support of the role of the classical pathway in barrier control, deletion of p65 in brain endothelial cells induced a blood–brain barrier disruption, but no endothelial cell death. Hence, the blood–brain barrier is protected by the classical NF-κB signaling pathway, whereas endothelial cell survival is maintained by other effects of NEMO and TAK1.

Functional consequences. A tight blood–brain barrier and a normal vessel structure are fundamental for the function of the whole brain. Therefore, the cellular effects of a NEMO loss in brain endothelial cells had dramatic consequences on different brain functions and animal health. Extravasated serum proteins are able to interact with astrocytes, leading to activation of those cells and increased expression of inflammatory proteins. As astrocytes not only communicate with vessels but also surround synapses of neurons, a change of astrocytic function can impact neuronal activity. The close interaction of different cell types within the neurovascular unit induced a massive disturbance of neuronal function in mice without NEMO in brain endothelial cells. These mice suffered from spontaneous epileptic seizures that were lethal in some animals [12]. Different types of seizures were recorded, and seizures occurred in almost all animals. Not only epileptic seizures could be detected but also behavioral changes. Several behavioral tests showed that the mice had an increased anxiety-like behavior and were less interested in other mice.

Thus, the disruption of only one signaling pathway in brain endothelial cells induced dramatic changes in the brain, leading to epileptic seizures, behavioral changes, and the death of some animals. Altogether, the phenotype of mice without NEMO in brain endothelial cells reflects the neuronal symptoms of a disease called incontinentia pigmenti. Incontinentia pigmenti is caused by mutations in the *Nemo* gene, which lead to skin changes and often to epileptic seizures and other neurological symptoms. Until recently, it was unknown why the patients experience epileptic seizures, because a loss of NEMO in astrocytes or neurons does not induce seizures or behavioral changes. However, it has been shown that a dysfunction of brain endothelial cells is the most likely cause of the neurological symptoms of incontinentia pigmenti patients. Using this finding, one could potentially develop new treatment strategies for incontinentia pigmenti patients because endothelial cells are easier to target by pharmacological drugs than other cell types in the brain.

Summary and implications

The blood–brain barrier forms an essential interface between the blood and parenchyma with a lot of functions exceeding those of a simple physical barrier. There are different cell types involved in the formation of the blood–brain barrier and all can influence its permeability. During infections and other diseases the blood–brain barrier opens, but usually reseals again after some time. In many chronic diseases of the CNS the blood–brain barrier function is disturbed. An important factor in inflammatory reactions is NF-κB, which is activated in different cell types of the blood–brain barrier and might be

involved in its opening. NF-κB is not only activated during inflammatory reactions but also has basal activity. In brain endothelial cells the basal activity of the classical NF-κB signaling pathway is necessary to maintain the blood–brain barrier. Additionally, the upstream proteins NEMO and TAK1 are needed for endothelial cell survival. Whether loss of NEMO in brain endothelial cells leads to a different response to acute inflammation is unclear. The functions of NEMO in brain endothelial cells explain neurological symptoms like epileptic seizures in patients who suffer from a mutation in the *Nemo* gene. On the one hand NF-κB is needed to maintain essential functions of the blood–brain barrier, but on the other hand it mediates important steps during an inflammatory reaction, which in turn could lead to blood–brain barrier disruption. One theoretical concept, which could explain these findings, can be described by a bell-shaped relationship between NF-κB activity and blood–brain barrier integrity. Too little activity leads to dysfunction of the blood–brain barrier, whereas a strong activation of NF-κB could also lead to a higher permeability (**Fig. 5a**). To keep this balance, a complex regulation of NF-κB activity is necessary, which needs to be further explored. An alternative hypothesis would be that acute activation of NF-κB leads to the opening of the barrier which would be resealed after some time, again mediated by NF-κB (**Fig. 5b**). In that case, NF-κB would be a main regulator of the resolution process that terminates inflammation and acts via anti-inflammatory proteins. Altogether, NF-κB is a key regulator of the blood–brain barrier, which is balanced by a complex signaling pathway and could play an important role during the development of neuronal diseases.

Corresponding address

Dr. rer. nat. J. Wenzel
Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Universität zu Lübeck
Ratzeburger Allee 160, 23562 Lübeck, Deutschland
jan.wenzel@pharma.uni-luebeck.de

Prof. Dr. med. M. Schwaninger
Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Universität zu Lübeck
Ratzeburger Allee 160, 23562 Lübeck, Deutschland
markus.schwaninger@pharma.uni-luebeck.de

Dr. rer. nat. J. Wenzel, born in 1982 in Dresden, studied Molecular Life Science at the University of Lübeck. In 2011 under the direction of Heinrich Terlau, he graduated with the thesis on a new pharmacological model system for the estimation of arrhythmic effects on the human heart. As a postdoctoral fellow for Markus Schwaninger, his research focused on the vascular system of the brain. Since 2015, he has been leading a research group at the Institute for Experimental and Clinical Pharmacology and Toxicology in Lübeck, studying the effects of endothelial cells on the various functions of the blood–brain barrier.

Prof. Dr. med. M. Schwaninger studied medicine in Freiburg followed by a postdoctoral position at the Pharmacological Institute in Freiburg. After specialization at the Neurological Clinic of the FU Berlin and a postdoctorate position at the Department of Molecular Pharmacology at the University of Göttingen, he became senior physician and later head of the Section Molecular Neuropharmacology at the Neurological Clinic of the University of Heidelberg. In 2007, he accepted a position as Professor of Pharmaceutical Pharmacology at the University of Heidelberg and in 2011 became Professor for Pharmacology and Toxicology at the University of Lübeck.

Compliance with ethical guidelines

Conflict of interest. J. Wenzel and M. Schwaninger state that there are no conflicts of interest.

The accompanying manuscript does not include studies on humans or animals.

References

1. Ehrlich P (1885) Das Sauerstoff-Bedürfniss des Organismus. Hirschwald, Berlin
2. Yousif LF, Di Russo J, Sorokin L (2013) Laminin isoforms in endothelial and perivascular basement membranes. Cell Adhes Migr 7:101–110
3. Daneman R, Zhou L, Kebede AA, Barres BA (2010) Pericytes are required for blood-brain barrier integrity during embryogenesis. Nature 468:562–566
4. Proebstl D, Voisin MB, Woodfin A, Whiteford J, D'Acquisto F, Jones GE, Rowe D, Nourshargh S (2012) Pericytes support neutrophil subendothelial cell crawling and breaching of venular walls in vivo. J Exp Med 209:1219–1234
5. Baltimore D (2011) Nf-kappab is 25. Nat Immunol 12:683–685
6. Senegas A, Gautheron J, Maurin AG, Courtois G (2015) Ikk-related genetic diseases: Probing nf-kappab functions in humans and other matters. Cell Mol Life Sci 72:1275–1287
7. Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M (1999) Nf-kappab is activated and promotes cell death in focal cerebral ischemia. Nat Med 5:554–559
8. Herrmann O, Baumann B, de Lorenzi R, Muhammad S, Zhang W, Kleesiek J, Malfertheiner M, Kohrmann M, Potrovita I, Maegele I, Beyer C, Burke JR, Hasan MT, Bujard H, Wirth T, Pasparakis M, Schwaninger M (2005) Ikk mediates ischemia-induced neuronal death. Nat Med 11:1322–1329
9. Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, Holtzman DM, Betsholtz C, Armulik A, Sallstrom J, Berk BC, Zlokovic BV (2012) Apolipoprotein e controls cerebrovascular integrity via cyclophilin a. Nature 485:512–516
10. Aveleira CA, Lin CM, Abcouwer SF, Ambrosio AF, Antonetti DA (2010) Tnf-alpha signals through pkczeta/nf-kappab to alter the tight junction complex and increase retinal endothelial cell permeability. Diabetes 59:2872–2882
11. Nenci A, Becker C, Wullaert A, Gareus R, van Loo G, Danese S, Huth M, Nikolaev A, Neufert C, Madison B, Gumucio D, Neurath MF, Pasparakis M (2007) Epithelial nemo links innate immunity to chronic intestinal inflammation. Nature 446:557–561
12. Ridder DA, Wenzel J, Müller K, Töllner K, Tong XK, Assmann JC, Stroobants S, Weber T, Niturad C, Fischer L, Lembrich B, Wolburg H, Grand'Maison M, Papadopoulos P, Korpos E, Truchetet F, Rades D, Sorokin L, Schmidt-Supprian M, Bedell BJ, Pasparakis M, Balschun D, D'Hooge R, Löscher W, Hamel E, Schwaninger M (2015) Brain endothelial tak1 and nemo safeguard the neurovascular unit. J Exp Med 212:1529–1549 (DA Ridder and J Wenzel contributed equally)