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The impact of interferon-β treatment on the blood–brain barrier

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Changes in the blood–brain barrier (BBB) are crucial to the pathogenesis of multiple sclerosis (MS). There are currently few established treatments for MS, and interferon-β (IFN-β) therapy is one of the most promising – proposed to act as an immunomodulator of the cytokine network reducing inflammatory damage. However, there is increasing evidence that direct effects on the BBB could also be relevant. This review surveys the evidence that IFN-β stabilizes the BBB, and that this process itself might be the key target. Understanding IFN-β-derived changes at the BBB will not only provide new insights in the pathogenesis of MS but will also be helpful to develop new, more-specific drugs for MS treatment.

The blood–brain barrier in the pathogenesis of multiple sclerosis

Multiple sclerosis (MS) is the most common neurological disease affecting young adults in Europe and North America [1]. Worldwide ~1 million people are afflicted with this chronic inflammatory disease [2]. The etiology of MS is, up to now, unknown. Whereas previously MS was considered to be a T helper 1 (Th1)-mediated autoimmune disease directed against proteins within the myelin sheath of the central nervous system (CNS), there is now growing evidence of pathogenetic heterogeneity in MS [2]. Lassmann *et al.* [1] have described four different histologically defined MS subtypes.

Independently of the different pathogenetic concepts, the recruitment of mononuclear cells, from peripheral blood across endothelial cells of the blood-brain barrier (BBB), is a hallmark in the pathogenesis of inflammatory MS lesions (Box 1) [3]. In this process, the cell adhesion and transendothelial migration of peripheral blood mononuclear cells is regulated by adhesion molecules (AM) such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), as well as their ligands: very late antigen-4 (VLA-4), α4β1-integrin, leukocyte function antigen-1 (LFA-1), CD11a/CD18 and αMβ2-integrin (Mac-1, CD11b/CD18) [4]. Importance of AM for the formation of inflammatory MS lesions was demonstrated in a placebo-controlled clinical trial, where natalizumab, a humanized monoclonal blocking antibody against human α4-integrin, was shown to be effective in the treatment of relapsing–remitting MS (RRMS) patients, leading to fewer inflammatory brain lesions and a reduced number of relapses [5]. However, studies investigating the influence of anti-VCAM-1 and anti-α4-integrin antibodies on the migration of immune cells across murine and human brain endothelial cells (BECs) are controversial [6]. Therefore, several authors believe that natalizumab impacts the movement of leukocytes within the extracellular matrix surrounding the BBB, and not leukocyte migration across BECs.

Furthermore, breakdown of the barrier function of the BBB and of postcapillary venules are initial steps in the pathogenesis of the typical inflammatory demyelinating lesions in MS [7]. This can be shown by histological investigation of brain specimens, as taken by stereotactic biopsies containing acute MS lesions [8]. In recent years, magnetic resonance imaging (MRI) has been introduced as a routine tool in the diagnosis and treatment of MS. In particular, the application of the MRI contrast agent Gadolinium-DTPA (Gd), which is administered intravenously, allows one to draw the following conclusion about BBB function in individual MS patients: Gd-enhancement indicates an acute BBB dysfunction and, therefore, acute lesions in brains of MS patients can be determined by Gd-enhancement [9]. Moreover, an increase in the cerebrospinal fluid (CSF):serum albumin ratio also indicates that the BBB is dysfunctional [10]. However, an increased

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BOX 1

Pathology and clinical courses of multiple sclerosis

Multiple sclerosis (MS) is a chronic autoimmune disease in which inflammatory white-matter lesions lead to neurological symptoms – according to their localization within the central nervous system (CNS). These lesions reveal blood–brain barrier breakdown. Several subforms of MS are known, the relapsing–remitting course of MS (RRMS) is the most common subform, afflicting ~70% of MS patients. Patients with RRMS suffer from acute relapses (also called exacerbations) that are defined as the occurrence of neurological symptoms attributed to MS. These relapses normally last up to six weeks and patients then recover completely, or at least partially. Relapses are followed by phases of remission without any new neurological symptoms. After several years most of the patients with RRMS experience a different course of the disease, the secondary progressive form of MS (SP-MS), when patients suffer from a more silent, continuous deterioration of neurological symptoms that can also be superposed by relapses.

CSF:serum albumin ratio is not a specific indicator for acute MS relapse, as it is also seen in other inflammatory CNS diseases.

Several different mediators of BBB disruption have been implicated in playing a pathogenetic role in MS; however, the mechanism for BBB breakdown in MS is incompletely understood. The current opinion is that BBB breakdown in MS is caused by direct effects of soluble inflammatory mediators, such as the cytokines interferon (IFN)-γ, tumor necrosis factor (TNF)-α and interleukin (IL)-1β [11], the metalloproteinases (MMPs) MMP-7 and MMP-9 [12], nitric oxide [13], or mast-cell-derived histamine [14]. Moreover, non-inflammatory indirect effects are also suggested to play a role, such as changes in the microenvironment of the BECs derived by dysfunction or loss of glial cells on the CNS side of the BBB [15].

Adhesion molecules as surrogate markers in multiple sclerosis

For several years, one goal in patient-orientated MS research has been to find surrogate markers for the diagnosis of MS, as well as for disease activity, disease severity and treatment efficacy. Many studies have been performed investigating immunological parameters in the CSF and blood of MS patients because it is, on the one hand, presumed that immunological changes precede clinical symptoms. On the other hand, determination of a laboratory parameter would be more objective than neurological symptoms presented or reported subjectively by an individual patient. Owing to the importance of AM in the pathogenesis of MS lesions, researchers focused on soluble AM in CSF and blood [4,16]. They found elevated levels of soluble VCAM-1, ICAM-1 and ICAM-3 in CSF and serum of MS patients compared with healthy individuals, and also in MS patients with active disease compared with MS patients in remission. When analyzing cell-surface bound forms of ICAM-1 and ICAM-3 on mononuclear cells a stronger correlation of clinically defined disease activity and disease severity was observed, as compared with the soluble forms of ICAM-1 and ICAM-3 [17,18].

Moreover, a significant correlation of BBB disruption, as indicated by Gd-enhancing MRI lesions with AM in CSF and peripheral blood, was shown [19]. Again, the correlation between immunological markers and morphological data was stronger for the cell-surface bound forms on CSF and peripheral blood mononuclear cells, as compared with the soluble forms of the investigated AM. Thus, it was shown that cell-surface bound AM, in particular as determined in CSF, could act in principal as valid surrogate markers for disease activity in MS. However, this is limited by the fact that obtaining CSF is an invasive procedure that cannot be used frequently.

The blood–brain barrier is built by highly specialized endothelium

The BBB is formed by highly specialized endothelial cells that allow control over the molecules that enter and leave the CNS [20]. The BECs, therefore, restrict the passage of molecules across the BBB. This is achieved by an extremely low pinocytotic activity of these microvascular BECs and a restriction of the paracellular diffusion of hydrophilic molecules by an elaborate network of complex P-face-associated tight junctions between the BECs. In freeze–fracture electron micrographs (Figure 1), the tight junctions of BECs at the BBB appear as a chain of fusion points on the outer plasma membrane leaflet of adjacent cells. Therefore, the morphology of BBB endothelial tight junctions resembles that of epithelial cell tight junctions, rather than that of endothelial cell tight junctions elsewhere in the body [21].

The differentiation of BECs into highly specialized endothelium starts during embryogenesis (Figure 2). During early embryogenesis BECs have a large diameter, are irregular in shape and contain diaphragmed fenestrae. They are relatively permeable to small hydrophilic substances, but probably not to macromolecules [22]. During differentiation, BECs lose their fenestrae and become

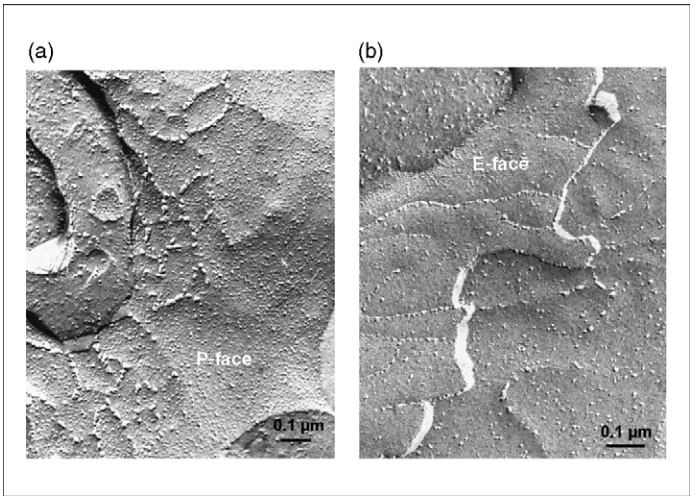

FIGURE 1

Freeze–fracture morphology of blood–brain barrier tight junctions. Brain endothelial cells restrict the passage of molecules across the blood–brain barrier (BBB), which is achieved by an extremely low pinocytotic activity of these microvascular BBB endothelial cells and a restriction of the paracellular diffusion of hydrophilic molecules by an elaborate network of complex P-face-associated tight junctions between the endothelial cells. In freeze–fracture electron micrographs the tight junctions of BBB *in vivo* endothelial cells appear as a chain of fusion points on the outer plasma membrane leaflet of adjacent cells, see P-face **(a)**. Therefore, the morphology of BBB endothelial tight junctions resembles that of epithelial cell tight junctions rather than endothelial cell tight junctions found elsewhere in the body. However, in contrast to the epithelium, the integrity of the BBB is strictly dependent on ambient factors provided by the central nervous system (CNS) microenvironment, in particular from astrocytes, which can be elegantly shown by *in vitro* experiments **(b)**.

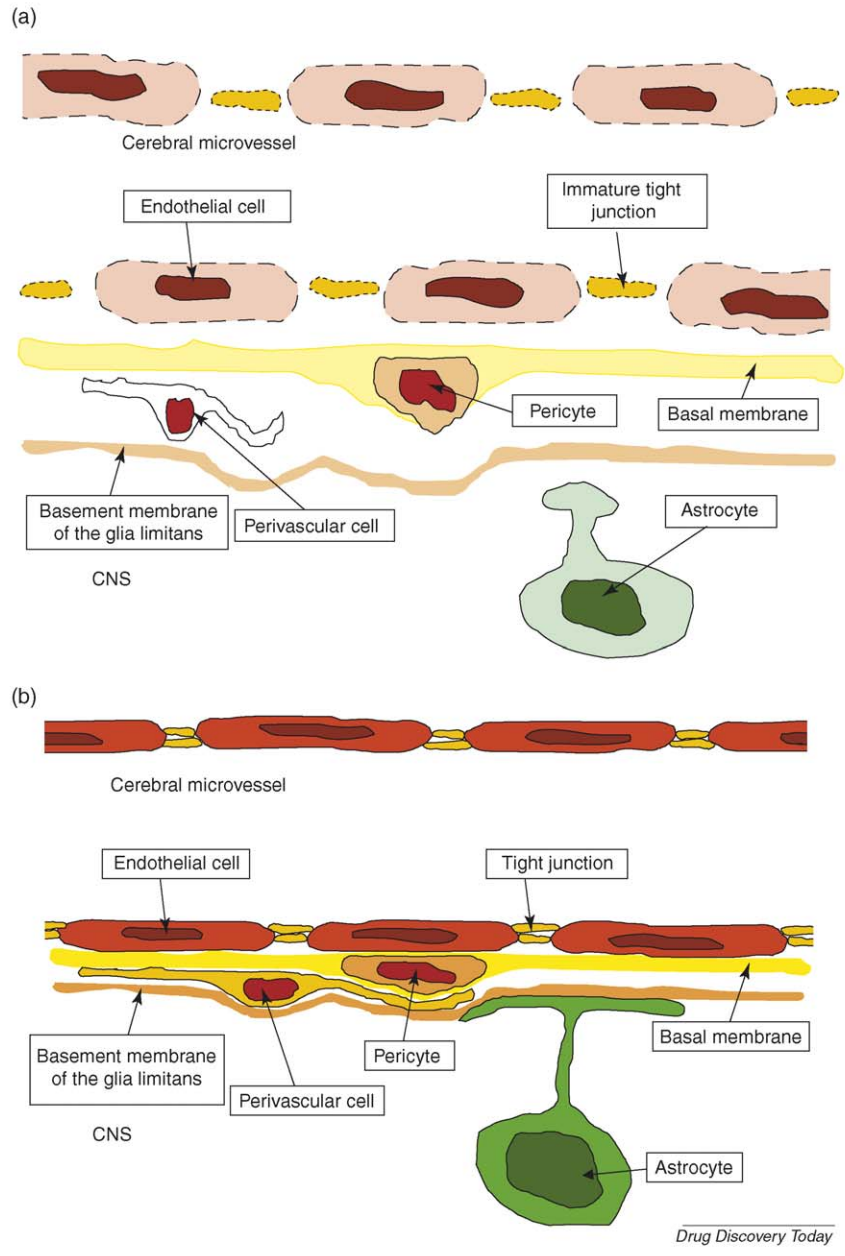

FIGURE 2

Histoanatomy of the blood–brain barrier in different developmental stages. (a) During early embryogenesis brain capillary endothelial cells have a large diameter, are irregular in shape and contain diaphragmed fenestrae. They are relatively permeable to small hydrophilic substances but probably not to macromolecules. During differentiation, brain capillary endothelial cells lose their fenestrae, become smaller and thinner-walled with a more regular shape **(b)**. Moreover, the interendothelial junctions expand and become more-complex. Because astrocytes form endfeet that are in close proximity to the endothelial cell membrane, they are the most probable candidates for inducing endothelial cells to develop typical BBB characteristics. Central nervous system (CNS) microvascular endothelial cells are also surrounded by a large number of pericytes, which are embedded in the basal membrane of BBB endothelial cells. However, they might contribute to the mechanical stability of the capillary wall.

smaller and thinner-walled with a more regular shape. Moreover, the interendothelial junctions expand and become more-complex [20]. Interestingly, typical BBB markers, such as the glucose transporter Glut-1, the non-receptor tyrosine kinase lyn and P-glycoprotein, are expressed early during brain angiogenesis (when brain vessels are not yet fully matured) [23,24].

The mechanism of BBB differentiation is not completely understood. It has been demonstrated by transplantation experiments that the formation of the BBB is induced by the developing neuroectoderm during embryogenesis [25]. It has been investigated *in vivo* whether or not the different cell types surrounding the BBB endothelium can promote BBB differentiation. In particular, astrocytes have been suggested to be necessary for the induction of the BBB characteristics, because they form endfeet that are in close proximity to the endothelial cell membrane [26]. Moreover, CNS microvascular endothelial cells are also

surrounded by a large number of pericytes. However, they are probably not involved in BBB endothelial cell differentiation, but rather might contribute to the mechanical stability of the capillary wall [27].

It has been shown by many research groups (both *in vivo* and *in vitro*) that, in contrast to epithelium, the integrity of the BBB is strictly dependent on ambient factors provided by the CNS microenvironment [28]. This has been supported by a recent study, employing the *in vitro* BBB model that was proposed by Cecchelli *et al.* [29], where bovine brain capillary endothelial cells (BBCECs) in co-culture with rat astrocytes display many BBB characteristics [30]. In this model, co-cultured BECs not only form a tight permeability barrier and a high electrical resistance but also show typical functional characteristics of BBB endothelium transport systems, such as the transcytosis of low-density lipoprotein (LDL) or transferrin, required *in vivo* to supply the brain with nutrients [29]. Removal of astrocytes from the co-culture results in an increased permeability across BBCECs and opening of the BBB endothelial tight junctions to horseradish peroxidase, as detected by electron microscopy [30].

Breakdown of the barrier function of the BBB in inflammatory MS lesions was considered to be the result of changes in the CNS microenvironment [15]. This process can be mimicked with the BBB co-culture model by removal of the astrocytes from the BECs – because it has been shown that removal of astrocytes leads to an 'opening' of permeability barrier capacities in BECs [30].

Tight-junction molecules at the blood–brain barrier

A complex network of tight junctions (between highly specialized endothelial cells in the capillary vessel wall of the CNS) is the morphological basis of the BBB [20]. These tight junctions restrict the paracellular diffusion of small hydrophilic molecules between BECs, and distinguish brain endothelium from endothelium of other organs [20,21].

In recent years, several tight-junction-associated proteins have been characterized. Occludin was the first integral membrane tight-junction-associated protein identified [31]. However, knockout mice subsequently demonstrated that occludin is not essential for proper tight-junction formation [31]. By contrast, claudins are essential for this process [32]. To date, the claudin family consists of at least 24 integral membrane proteins that have been described in different species and have relatively similar molecular structures. In BBB tight junctions claudin-5 and, recently, claudin-3 proteins have been detected, claudin-12 was identified at the mRNA level [33,34]. There is some doubt about reports that claudin-1 is expressed in BBB tight junctions. Most probably, these results are due to a crossreactivity between the previously used anti-claudin-1 antibody and claudin-3 [30,34]. The functional relevance of claudin-5 for proper BBB function has been shown in claudin-5-deficient mice [33]. However the development of cerebral blood vessels in these mice was not altered, and the mice died soon after birth owing to failure of the BBB, thus failing to provide a barrier for small molecules (<800 Da).

In the cytoplasm tight junctions are interconnected with the cellular cytoskeleton via three different zonula occluden molecules (ZO-1, ZO-2, ZO-3) [32]. In recent studies of brain specimens, it has been shown that the localization of claudin-3 (but not of claudin-5, occludin or ZO-1) is altered under pathologic conditions that are accompanied with BBB breakdown, such as in the animal model of MS, experimental autoimmune encephalomyelitis (EAE) and in human glioblastoma multiforme [34].

Pharmacology of interferon-β

Two different types of interferons have been described, type I interferons (including IFN-α, IFN-β, IFN-κ and IFN-τ, IFN-ω) and the type II interferon IFN-γ [35]. Interestingly, it was suggested that the proinflammatory type II interferon IFN-γ might be helpful in the treatment of MS (based on its immunomodulatory and antiprolifertative properties, and also on a positive treatment effect on EAE after intraventricular administration of IFN-γ in rats) [36]. However, in an early treatment study IFN-γ promoted clinical exacerbations in MS patients [37]. In contrast to IFN-γ, IFN-β exhibits anti-inflammatory effects. IFN-β provides four main mechanisms of action, namely immunomodulation, antiviral activity, antiproliferative effect and cell differentiation [35]. Several studies demonstrated beneficial effects of IFN-β in MS patients [38–40].

At present, IFN-β-1a and IFN-β-1b, two different kinds of recombinant human IFN-β, are used in the treatment of MS patients (Box 2). IFN-β-1a is expressed by Chinese hamster ovary (CHO) cells and its molecular structure and glycosilation pattern are both identical to human IFN-β [38,39]. IFN-β-1a can be applied in two different preparations in MS treatment: Avonex™ (Biogen, Cambridge, MA, USA) and Rebif™ (Ares-Serono, Geneva, Switzerland) [38,39]. By contrast, IFN-β-1b (Betaseron™, Berlex, Montville, NJ, USA and Betaferon™, Schering, Berlin, Germany) is not glycosylated because it is expressed by *Escherichia coli* [40].

We have to mention here that the specific activity (as measured in MIU) is not comparable between the different IFN-β preparations because several different bioassays are available to determine the specific activity of the IFN-βs [41]. To investigate the relative bioavailability of different IFN-β preparations in MS patients, as well as in healthy individuals, the antiviral activity of IFN-β was measured using biologic markers such as neopterin, human Mx protein and 2′,5′-oligoadenylate synthetase. These biomarkers are produced in the human body after application of exogenous IFN-β, and they are

BOX 2

Interferon-β treatment of multiple sclerosis

Two different kinds of recombinant human interferon-β (IFN-β), IFN-β-1a and IFN-β-1b, are used in the treatment of the relapsing–remitting course of multiple sclerosis (RRMS; Box 1), whereas in the secondary progressive form of MS (SP-MS) only IFN-β-1b has been approved. Normally, treatment will be initiated after patients have experienced at least two relapses within the previous two years. However, there is increasing evidence that an earlier treatment onset, even after the first relapse, will be helpful.

The three different preparations of IFN-β differ in terms of dosage, dose frequency and administration route (subcutaneously or intramuscularly). They should be applied as long-term treatments. IFN-β treatment reduces the relapse rate by ~30%, ameliorates relapse severity and leads to a delay of the progression of disability in RRMS patients, as compared with untreated patients. Moreover, IFN-β treatment leads to a reduction in the number, and cumulated area, of MS lesions (also called disease burden) in cranial MRI. They are able to postpone the neurological deficits of the individual patients for up to several years.

suggested to correlate with the biologic effect of IFN-β. Moreover, the antiviral biomarkers are relatively easy to determine, as compared with the immunomodulatory effect of IFN-β – quantitative measurement of which is almost impossible. Two studies supported by Schering (Berlin, Germany) found more or less the same pharmacodynamic effects or an enhanced bioactivity (as determined by neopterin, human Mx protein, 2′,5′-oligoadenylate synthetase and sVCAM-1 induced by IFN-β-1b, as compared with IFN-β-1a [42]. By contrast, a study performed by Serono measured the biologic activity of IFN-β-1a and IFN-β-1b using an *in vitro* bioassay system, and reported antiviral activity that was 14-times stronger for IFN-β-1a than IFN-β-1b [41]. The authors concluded that these *in vitro* findings could also have clinical implications. However, one has to be very careful when drawing conclusions about the clinical efficacy of IFN-β in MS (by measuring the antiviral activity) because it is not clear whether there is any correlation between the immunomodulatory efficacy of IFN-β in MS treatment and its antiviral activity *in vitro*.

Neutralizing anti-IFN-β antibodies that occur after the onset of IFN-β treatment, and the impact they have on the treatment of MS patients, has been another controversial topic. Neutralizing antibodies (NAbs) can cause life-threatening conditions, as it is known for Nabs against erythropoietin and thrombopoietin [43,44]. However, NAbs induced by IFN-β have less-severe effects. The prevalence of NAbs against IFN-β in the serum of MS patients has varied from 7% to 42% in different studies, depending on the type of IFN-β preparation used, the dosage, the dose frequency and the route of administration [43,44]. NAbs occur more frequently and in higher concentrations after treatment with IFN-β-1b; however, NAbs against IFN-β disappeared in 52% of MS patients treated with IFN-β-1b, as compared with only 19% during long-term IFN-β-1a treatment [45]. Although there is growing evidence that NAbs have a negative effect on the clinical course and the MRI-defined disease burden, the impact of NAbs on the clinical evolution of MS is still a highly controversial topic.

Effects of interferon-β treatment in multiple sclerosis patients

In recent years, different forms of recombinant human IFN-β have been approved for clinical treatment of RRMS (Boxes 1, 2) [38–40]. It has been shown in different studies that IFN-β leads to clinical benefits and to a reduction of disease-attributed pathologies in cranial MRI scans.

It is not known how these benefits are achieved. It has been hypothesized that IFN-β acts as an immunomodulator downregulating proinflammatory (and upregulating anti-inflammatory) cytokines, particularly in the peripheral blood [46]. Changes in the composition of AM in the peripheral blood of patients receiving IFN-β have been discussed as an additional direct or indirect mechanism [47]. Moreover, there is *in vitro* evidence that IFN-β might lead directly or indirectly to a stabilization of the BBB in RRMS patients (Table 1) [48,49]. Supporting this theory, serial MRI scans from IFN-β-treated MS patients show an early reduction in the appearance of Gd-enhancing lesions within a few weeks, as compared with the placebo group [9]. This MRI-defined stabilization of the BBB was found to be much earlier than it would be expected by sole immunomodulatory effects, which are only suggested to work after several months.

The results of different studies investigating the influence of IFN-β treatment on the serum concentration of soluble AM are controversial [47,50–52]. Our group studied the influence of IFN-β-1b treatment on the cell-surface bound forms of ICAM-1 and

TABLE 1

Evidence on how interferon-β acts on the blood–brain barrier (BBB)
Multiple sclerosis patients
Reduction of Gadolinium-enhancing lesions (⇒ reduction of BBB dysfunction) early after onset of interferon-β (IFN-β) treatment
Long-term stabilization of the expression of cell-surface bound adhesion molecules (AM) on peripheral blood leukocytes (as compared with untreated patients showing a continuous decrease of AM expression) ⇒ indirect evidence for an IFN-β-derived stabilization of the BBB and, therefore, a reduced transmigration of leukocytes with enhanced expression of AM
Animal experiments
Reduction of perivascular infiltrates in acute rat experimental allergic encephalomyelitis (EAE) after IFN-β treatment
In vitro
Increase of the transendothelial resistance of bovine BBB endothelial cell monolayers in a BBB co-culture model
Decrease of the paracellular permeability of bovine BBB endothelial cell monolayers in a BBB co-culture model
Prevention of permeability increase after two different stimuli mimicking BBB changes in multiple sclerosis (MS) – changes in the microenvironment and application of histamine – in a bovine BBB co-culture model
No effect on the decrease of transendothelial resistance induced by lipopolysaccharide in a bovine BBB co-culture model
Pre-incubation of interleukin (IL)-1β- and IFN-γ-activated rat brain endothelial cells with IFN-β • reduced expression of proinflammatory adhesion molecules [intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1)] • reduced transendothelial migration of monocytes
Pretreatment with IFN-β ⇒ reduction of the transendothelial migration of human T helper 1 lymphocytes across human brain endothelial cells (no effect on adhesion molecule expression on human brain endothelium)
Change of the morphology of brain capillary endothelial cells after IFN-β in a bovine co-culture model ⇒ round, cobblestone-like
Immunostaining for tight junctions associated proteins claudin-3, claudin-5, occludin, zonula occluden (ZO)-1 and ZO-2 at the cellular borders in bovine brain capillary endothelial cells ⇒ continuous and more homogeneous
Reversion of H_2O_2 induced translocation of ZO-1 and ZO-2 from the cell to cell contacts into the cytoplasm of human brain endothelial cells

ICAM-3 on mononuclear cells [53]. We found stable expression levels of cell-surface bound AM on blood mononuclear cells over an 18 month period of IFN-β-1b treatment. Untreated MS patients, however, showed a continuous decrease in the expression of cell-surface bound AM expression. These findings indicate that stabilization of the expression of cell-surface bound AM on blood mononuclear cells can indicate the beneficial effects of IFN-β therapy in MS patients, and might be used as a surrogate marker for treatment efficacy in MS.

In vitro and in vivo studies on the impact of interferon-β on the blood–brain barrier

In vivo, many different kinds of factors, such as cells (e.g. astocytes and other glial cells, pericytes, leukocytes from the peripheral blood, neurons, etc.) and soluble factors of the extracellular matrix and from the peripheral blood of CNS capillaries, can influence BECs. Owing to this complex situation, it is difficult to determine the direct influence of IFN-β on BECs *in vivo*. Therefore, *in vitro* investigations are a more viable option. Using the *in vitro* co-culture BBB model, according to Cecchelli *et al.* [29], an IFN-β-mediated dose-dependent stabilization of the BBB was found (Table 1) [54]. Moreover, two different pathophysiological stimuli (changes in the microenvironment, histamine), relevant in the pathogenesis of BBB disruption in MS patients, were simulated *in vitro* [14,15]. With both conditions (changes in the microenvironment and histamine) BBB dysfunction was prevented by pretreating BECs with IFN-β.

These data are in line with results from an older study [49] that used a BBB model consisting of BBCECs and rat astrocytes, which were grown on both sides of a collagen-coated filter membrane. In this study, IFN-β showed an increase in the transendothelial electrical resistance (TEER), a measure for the barrier function of endothelial cells. However, the authors suggested an indirect mechanism of IFN-β action, such as an increased sensitivity for immunomodulatory effects of endogenous glucocorticoids, because overnight pretreatment of 1000 U/ml IFN-β did not protect against the decrease in TEER induced by lipopolysaccharide (LPS). This only partially contradicts the hypothesis that IFN-β has a direct stabilizing effect on the BBB, because the addition of LPS, which activates the innate immune system via toll-like receptors *in vivo,* might have been too strong an inflammatory stimulus to investigate the subtle effects of IFN-β-induced prevention of BBB breakdown [55]. The cytoplasmic signaling pathway of the toll-like receptor involves the Toll/IL-1 receptor (TIR) domain, which activates a unique signaling module and utilizes central signaling pathways such as the activation of nuclear factor-κB (NF-κB) [56]. The main signaling module employed by the TIR family consists of MyD88, IL-1 receptor associated kinase (IRAK) family members and Tollip. According to our knowledge, it has not been demonstrated that TIR family members are downstream targets of the type I interferon receptor (IFNR) signaling cascade [57].

It is also conceivable that IFN-β treatment blocks transendothelial migration of pathogenetic leukocytes from the peripheral blood into the CNS. Pre-incubation of IL-1β- and IFN-γ-activated rat BECs with IFN-β led to a reduced expression of proinflammatory AM and to a diminished transendothelial migration of monocytes *in vitro* (as well as to a reduction of perivascular infiltrates in acute rat EAE *in vivo*) [48]. It was concluded that IFN-β exerts direct anti-inflammatory effects on BECs that can contribute to reduced lesion formation – as observed in MS patients. A recent *in vitro* study also demonstrated that pretreatment with IFN-β led to a reduction of the transendothelial migration of human T lymphocytes across human BECs [58]. By contrast, it was shown that IFN-β has an impact on Th1 cells and not on the expression of AM on BECs.

Possible molecular mechanisms of interferon-β at the blood–brain barrier

Type I interferons comprise a phylogenetic, relatively old, family of cytokines and most cells of the body possess the innate ability to fight viruses, after activation by their cell-surface type I IFNR. Considering the involvement of IFN-β in the innate antiviral defense, it is tempting to speculate that the biologic function of IFN-β-mediated stabilization of the BBB is to further close the barrier to prevent pathogens from crossing it and infecting the CNS [57].

Type I IFNR is composed of two chains, designated α and β, and transduces signals via the classical Jak–Stat pathway [57]. In addition, other signaling pathways are activated by type I IFNR, including the phosphatidylinositol (PI)3-kinase pathway, which affects many downstream target proteins such as protein kinase C (PKC), mitogen-activated protein kinase (MAPK), AKT, p90 ribosomal S6 kinase (p90RSK), S6 kinase (S6K) and extracellular regulated kinase (ERK). We suggest that one or more of these pathways might be responsible for the stabilizing effect that was determined by applying an *in vitro* co-culture BBB model [54]. Supporting this hypothesis, it was found that IFN-β was able to block the histamine-induced increase in permeability across human endothelial cells [59]. It is known that transient short-term hyperpermeability induced by histamine involves calcium–calmodulin-dependent activation of the myosin light chain (MLC) kinase. Several different mechanisms (e.g. receptor blockade by H1 antagonists) and pathways (including elevation of cyclic AMP as well as PKC, Rho and Rho kinase inhibition) have been suggested to prevent histamine-induced permeability in endothelial cells. However, the exact intracellular mechanism responsible for the stabilization of barrier function in BECs remains to be elucidated.

To find out whether IFN-β exerts a direct effect on the molecular composition of tight junctions, the distribution of the tight-junction-associated proteins claudin-3, claudin-5, occludin, ZO-1 and ZO-2 was investigated by immunofluorescence staining [54]. In conditions with low paracellular permeability (e.g. after addition of IFN-β) the shape of the individual BECs was more round and cobblestone-like, and the staining for tight-junction-associated proteins at the cellular borders was continuous and more homogeneous. Kuruganti *et al.* [60] investigated the effects of inflammatory mediators mimicking mechanisms of BEC dysfunction in MS. They found that H_2O_2 induced a reversible translocation of ZO-1 and ZO-2 from the cell-to-cell contacts into the cytoplasm of human BECs. This relocalization could be reversed by IFN-β. In brain specimens of MS patients, ZO-1 was located at the cell borders in normal white matter; whereas, in inflammatory MS lesions staining for ZO-1 was diffuse. The authors concluded that the findings seen in their *in vitro* experiments mimic the situation in active MS lesions. These findings provide evidence that IFN-β-derived BBB stabilization is achieved by changes in the molecular structure of endothelial tight junctions.

Conclusions and future prospects

The β-IFNs are the first drugs used in MS therapy that have effectively improved the long-term course of the disease. However, a lot of questions remain to be answered. Although it seems clear that IFN-β leads to a direct stabilization of the BBB, the exact molecular mechanisms of this action are not yet understood. The understanding of these molecular mechanisms will not only improve our understanding of the pathogenesis of the disease but will also be helpful for the development of new, promising, specific drugs that effectively stabilize the BBB – presumably with lower rates of systemic side effects. Such specific drugs could be useful not only for MS but also for a broad spectrum of other neurological diseases with BBB dysfunction such as stroke, meningitis or brain tumors.

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