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COMMENTARY

THE BLOOD–BRAIN BARRIER AND MULTIPLE SCLEROSIS

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Multiple sclerosis (MS†), a demyelinating disorder of the CNS, is the most important neurologic disease in young adults [1]. The myelin sheaths around the axons of the neuronal cells begin to disappear and their conductive properties are diminished markedly, resulting in motoric and sensory disturbances.

The blood–brain barrier is clearly involved in the disease process, and there are many indications that disruption of the blood–brain barrier is one of the early steps in the pathogenesis of MS. In this commentary the involvement of the blood–brain barrier in MS will be discussed. After a description of the methods to visualize the blood–brain barrier in MS patients, the animal models that have been developed to study MS are discussed, followed by the role of the blood–brain barrier in the pathogenesis of MS and, finally, the implications for treatment of the disease.

Under healthy conditions, the lumen of microvessels in the brain is covered by a thin sheet of endothelial cells, sealed together by tight junctions. Astrocytes encircle the brain capillaries with their processes and attach with these "endfeet" to the basement membrane shared with the endothelial cells. The processes are not sealed together, and small gaps allow passage of substances that have crossed the endothelial barrier. The astrocytes play an important role in the induction and maintenance of the specific barrier function of the cerebral endothelial cells [2]. The cerebral endothelial cells differ in many ways from peripheral endothelial cells. The cerebral endothelial cells possess narrow tight junctions, no fenestrae, and a paucity of vesicles [3]. The cells also contain a high density of mitochondria, which might suggest that the cells have a high metabolic activity due to the requirement for the regulation of selective permeability [4].

Disruption of the blood–brain barrier occurs in many pathologic conditions of the brain, such as tumours, hypertension, bacteraemia, seizures, hepatic coma, ischemia, and hyperosmolality. It is thought that disruption may occur because of a combination of increased intercellular leakage caused by the opening of tight junctions and enhanced pinocytosis [5].

Brown [6] studied a biopsy taken from a patient suffering from acute MS and concluded that in MS neither the number of mitochondria in the cerebral endothelial cells nor the morphology of the capillaries had been changed. However, a marked increase in the amount of pinocytotic vesicles in the cerebral endothelial cells was observed, which indicates an increased transport of blood-borne compounds across the blood–brain barrier. The tight junctions between the cerebral endothelial cells seemed not to be affected.

A clear, microscopically characterized pathological feature of MS is the selective destruction of the myelin sheaths without affecting the axons. This demyelination causes diminished nerve conduction and, consequently, different neurological deficits. The areas of demyelination, the plaques, are preferentially located in the periventricular white matter, the cervical part of the spinal cord, and the optic nerves [7].

When the pathology of the lesions is studied, two different types of lesions can be distinguished: acute and chronic. Adams *et al.* [8] characterized acute lesions using histochemical and immunocytochemical methods. These lesions showed the following characteristics which are partly connected with the blood–brain barrier disruption as observed by Brown [6]: edema, lymphocytic perivascular infiltration, plaque hypercellularity, plaque macrophage infiltration, and intra-macrophage myelin debris. Gay and Esiri [9] used immunocytological methods to study acute cases of MS and also found vessel wall damage in all the acute plaques observed, in

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† Abbreviations: MS, multiple sclerosis; CSF, cerebrospinal fluid; CT, computer-assisted tomography; HVDCT, high volume delayed computer-assisted tomography; Gd-DTPA, gadolinium-diethylenetriamine pentaacetic acid; MRI, magnetic resonance imaging; GABA, γ-aminobutyric acid; ACTH, adrenocorticotropic hormone; EAE, experimental allergic encephalomyelitis; CREAE, chronic relapsing experimental allergic encephalomyelitis; TNFα, tumour necrosis factor-α; and IL-2, interleukin-2.

combination with complement disposition, immune complex disposition, and infiltration of activated macrophages. In chronic plaques, myelin and oligodendrocytes disappear completely, and neither inflammation nor gliosis is present [17].

VISUALIZATION OF THE BLOOD–BRAIN BARRIER

Several methods have been developed to visualize the blood–brain barrier and its function in MS patients: albumin measurements, computer-assisted tomography (CT) and magnetic resonance imaging (MRI). These techniques are very helpful in obtaining an insight into the disease process, and are widely used as diagnostic tools.

Determination of albumin in cerebrospinal fluid and serum

Albumin, measured in serum and cerebrospinal fluid (CSF), is considered to be a marker for the tightness of the junctions in the endothelium of the CNS. Albumin is a non-CNS protein, synthesized only in the liver, and not catabolized by the endothelial cells of the blood–brain barrier or transported across the blood–brain barrier. No abnormalities were found when serum albumin was measured in MS patients. Based on measurements of the CSF albumin concentrations, 23% of MS patients displayed an altered permeability of the blood–brain barrier [10]. In contrast, Lefvert and Link [11] reported that the ratio of CSF/serum albumin, which is also a measurement of blood–brain barrier damage, was not changed significantly in MS.

Computer-assisted tomography

In 35% of patients, MS lesions are detectable with CT. Areas of cellular hypodensity may be seen in the white matter, especially in the paraventricular region. These areas probably reflect the increased water content of the early plaques, associated with demyelination [12].

New and active lesions show contrast enhancement when twice the usual dose of contrast material is administered (high volume delayed CT, HVDCT). When the CT scan is made after a delay of 1 hr, enhancing lesions are seen in 60–80% of the patients with exacerbations [7, 13]. This enhancement may reflect alterations of the blood–brain barrier [12, 13].

MRI-gadolinium-DTPA

A promising technique for obtaining an insight into the actual disease process is gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA)-enhanced MRI. Gd-DTPA is a paramagnetic contrast agent and has the ability to cross a damaged blood–brain barrier [14]. In its conjugated form, Gd is distributed in the same manner as the diatrizoates (iodine-based contrast substances) [15]. The MS lesions are visualized by the enhancement of gadolinium, and this enhancement may reflect increased transendothelial vesicular transport [16].

ANIMAL MODELS OF MULTIPLE SCLEROSIS

Experimental allergic encephalomyelitis (EAE)

EAE has been widely used as an animal model of MS. Animals that suffer from EAE show different clinical symptoms, ranging from atonia of the tail to complete paralysis of the hind legs and overflow incontinence [17]. EAE can be induced in rats by injecting a homogenate of guinea pig spinal cord or myelin basic protein in complete Freud's adjuvant [17, 18].

The cerebral endothelial cells of the blood–brain barrier in the rats may be altered by sensitized lymphocytes and thereby lose their barrier properties. In EAE, the blood–brain barrier has been shown to be defective in two aspects: at the peak of the EAE attack, blood-borne substances that are normally excluded have been found to leak into the CNS. Furthermore, cellular infiltration, a prominent feature of EAE, indicates an abnormal cellular traffic across the blood–brain barrier [17]. When blood–brain barrier disruption was traced with [^{14}C]mannitol, the leakage across the blood–brain barrier was shown to start from the caudal region of the cord and spread cranially. The reason for this is not clear, but it fits well with the clinical data. From tracer studies with insulin and albumin and from electron microscopic studies, it was concluded that the tight junctions were disrupted in EAE, but the number of pinocytotic vesicles in the cerebral endothelial cells seemed to be normal. In contrast with the results obtained with the tracer studies, cellular infiltration showed no correlation with clinical signs. Even when the barrier function of the blood–brain barrier returned to normal, cellular infiltration could still be detected. No explanation has been given for this effect [17].

To study the role of the cerebral endothelial cells, Sternberger *et al.* [18] developed a monoclonal antibody directed against endothelial cells, which possess a barrier function. Using this antibody, it was possible to detect alterations in the barrier function in rats with EAE. In microvessels surrounded by inflammatory cells, there was no reaction with the antibody. The factors that cause this alteration in recognition are unknown. Several mechanisms are proposed by the authors: endothelial cells may be involved in immunity processes as antigen-presenting cells, which can activate T-cells, which then release mediators and cytokines that can alter the permeability of the blood–brain barrier. Another possibility of a role for cerebral endothelial cells in immunity is that endothelial cells, which may act as antigen-presenting cells, release mediators and cytokines by themselves.

The use of EAE as a model for MS has been criticized: the histological lesions are different between MS and EAE, because in EAE many more inflammatory cells are present in the brain parenchyma, while the large centrifugal demyelinating plaques that are characteristic for MS are not present. EAE has been suggested to be primarily a vasculopathy, with secondary demyelination [19].

Chronic relapsing experimental allergic encephalomyelitis

Chronic relapsing experimental allergic encephalomyelitis (CREAE), a modification of acute EAE, is characterized pathologically by inflammation, gliosis, and scattered plaques of demyelination throughout

the spinal cord and, to some extent, within the brain. Clinically, the disease is characterized by an acute phase of hind limb weakness and paralysis 10–13 days after inoculation with guinea pig spinal cord homogenate in Freud's adjuvant. In the post-acute phase, most animals are free from clinical signs, but 45 days after inoculation clinical relapses occur, followed by a series of relapses and remissions [20].

Tsukada *et al.* [19] described a new model for CREAE, induced in guinea pigs by inoculation with the membrane fraction of rat cerebral endothelial cells, which was free from myelin basic protein. These endothelial cells were grown *in vitro* and showed a high expression of γ-glutamyltranspeptidase, which is specific for cerebral endothelial cells. Some animals developed a relapsing–remitting course of the disease. The primary pathological feature of these animals in the acute phase was infiltrates of mononuclear cells, attached to the endothelial cells. After 40 days, severe loss of myelin was observed in the white matter. These areas of demyelination were centred around blood vessels, as in MS. It is not known which membrane fraction is responsible for the demyelinating, encephalitogenic reactions.

Hawkins *et al.* [21, 22] studied lesions in guinea pigs with EAE and CREAE with Gd-DTPA MRI. Gd-enhancement was always accompanied in EAE by active, perivascular inflammation and in CREAE by active, perivascular inflammation and demyelination. There was a direct relationship found between the clinical relapse and Gd-enhancement in CREAE-affected animals. These observations suggest that blood–brain barrier breakdown is important in the development of clinical signs in the inflammatory, demyelination state in CREAE.

From studies on CREAE-affected guinea pigs with blood–brain barrier markers such as lanthanum, nitrate and gadolinium-nitrate, it has been shown that the blood–brain barrier breakdown in this disease is probably mediated by a metabolic change in the cerebral endothelial cells, associated with increased vesicular transport. The tight junctions seemed not to be affected in CREAE [23].

A mouse model of CREAE has been developed and used to study the action of the immunosuppressive agent Brequinar sodium, a 4-quinoline carboxylic acid derivative. This agent proved to be effective only in periods of relapse, when the blood–brain barrier was disrupted. It was suggested that, to be effective as a therapeutic, the compound has to cross the blood–brain barrier and act on the disease-inducing cells, the T-cells, activated within the CNS [24].

The characteristics of EAE, CREAE, and MS are summarized in Table 1.

PATHOGENESIS OF THE ACUTE MS PLAQUE

Many studies have been performed to elucidate the first steps in the pathogenesis of the plaques in MS. These studies have been performed in the animal models EAE and CREAE, and in MS patients, using immunocytochemical methods and MRI. The availability of Gd-MRI, in particular, has provided much evidence about the first steps of pathogenesis.

Role of the blood–brain barrier in pathogenesis

Pathological studies. Brown [6] demonstrated, in his morphometric analysis of the capillaries of an MS patient, that the cerebral endothelial cells contain a higher number of pinocytotic vesicles.

In a study of the normal white matter in MS patients, Allen *et al.* [25] found a strong astrocytic proliferation, some sclerosis in the blood vessels, and some perivascular inflammation. The astrocytic proliferation could be due to blood–brain barrier disruption, as cerebral endothelial cells and astrocytes are in close contact and strongly influence each other. Inflammation mediators, released by infiltrating leucocytes, may activate the astrocytes to proliferate. Another possibility is that the astrocytic proliferation is the preliminary step and that this proliferation causes blood–brain barrier disruption.

In a study of acute plaques, using (immuno)-histochemical methods, Adams *et al.* [8] found perivenular lymphocytic infiltration in all the plaques, and no lymphocytes at the edge of acute lesions. There were many activated T-cells present, as well as many activated macrophages. These activated cells have also been found perivascularly at the edges of old lesions and in "normal" white matter of MS patients. From these observations Adams *et al.* [8] concluded that perivascular lymphocytic cuffing is the first step in the pathogenesis of the plaques. This "vasculitis" results in blood–brain barrier damage with leakage of fibrinogen, IgG and complement, fibrin deposition and possibly astrocytic proliferation. Myelin may be damaged by the inflammatory reaction, as a so-called "innocent bystander." This can be caused by the release of myelin-damaging enzymes by the activated leucocytes or by the deposition of immune complexes on myelin or a

Table 1. Characteristics of EAE, CREAE and MS

	EAE	CREAE	MS
Origin of the disease	Autoimmune	Autoimmune	Not known
Plaques, areas of demyelination	No	Yes	Yes
Blood–brain barrier, tightness of the junctions	Decreased	Normal	Normal
Blood–brain barrier, pinocytotic vesicles	Normal	Increased	Increased
Cellular infiltration into the brain	Very large	Large	Large

combination of these processes. However, the exact mechanism of demyelination is not known.

These findings were supported by a study of Gay and Esiri [9], who, in addition to confirming the results of Adams *et al.* [8], reported the deposition of immunoglobulins and complement factors on particles in the vessel wall and in macrophages. This may indicate that there is an unknown, specific antigen present in these complexes, which could activate adhesion of leucocytes and phagocytosis.

From these pathological studies, it can be concluded that the increase in blood–brain barrier permeability, caused by a higher number of pinocytotic vesicles, is an early step in the pathogenesis of the MS plaque. Furthermore, the infiltration of lymphocytes into the brain, across the blood–brain barrier, appears to be very important for the development of the disease.

Studies using Gd-MRI. With Gd-enhanced MRI, a very useful method for studying the role of the blood–brain barrier in the pathogenesis of acute MS plaques has been developed. Grossman *et al.* [14] showed that with the use of Gd-MRI it is possible to distinguish between active and chronic plaques, and that Gd-MRI is a sensitive technique for the detection of blood–brain barrier abnormalities.

To detect the functional and histological changes that correspond with the lesions seen using Gd-MRI, Hawkins *et al.* [21] reported a serial study of the blood–brain barrier changes in EAE and CREAE. When compared with traditional low transport markers such as horseradish peroxidase, Gd-DTPA enhancement was seen in regions with blood–brain barrier breakdown. Gd-DTPA enhancement in CREAE was always accompanied by perivascular inflammation and demyelination; in EAE, there was only inflammation. Therefore, it might be expected that Gd-enhancement in MS reflects inflammation and not necessarily demyelination. Furthermore, in an animal model of demyelination, which was induced by cuprizone, there was no inflammation and no blood–brain barrier breakdown present. This indicates that the blood–brain barrier breakdown in CREAE and MS may not be caused by demyelination, but that blood–brain barrier disruption is indeed of primary importance in immune-mediated demyelination [26].

Katz *et al.* [27] were the first to study the correlation between Gd-enhanced lesions and pathology in a patient suffering from acute MS. The enhanced areas showed dense perivascular lymphocytic cuffs and mononuclear infiltration at the margins. This indicates that in enhanced regions where blood–brain barrier disruption occurs, a strong inflammatory reaction exists.

Gd-MRI in MS patients. Using Gd-MRI, Miller *et al.* [28] showed in a serial study of MS patients that in recognizable new lesions or new parts of existing lesions blood–brain barrier impairment was always present. When the blood–brain barrier was disrupted in these new, acute plaques, edema could be expected, and this was confirmed by MRI. In chronic plaques, however, although there was no enhancement seen and therefore it may be assumed that the blood–brain was restored, edema was observed by MRI. This edema proved to contain less protein than the edema in acute plaques. Probably, there was still blood–brain barrier leakage that was not detectable with Gd-DTPA. From trypan-blue injections in the brain after the death of MS patients, it is known that there is also some blood–brain barrier disruption in chronic plaques [29]. It is also possible that, after the acute phase, the blood–brain barrier is restored and that during this restoration process, water can still leak through the vessel wall, while proteins and gadolinium cannot.

In a serial study using unenhanced MRI and Gd-MRI, Bastianello *et al.* [30] characterized the lesions of four patients with MS. It was found that enhancement occurred in new lesions and that it was a transient phenomenon. Gd-enhancement persisted in only 30% of the lesions after 1 month, indicating restoration of the blood–brain barrier within a few weeks.

To determine how early blood–brain barrier breakdown occurs, Kermode *et al.* [31] performed serial studies with MRI and Gd-MRI and coupled the data obtained with clinical symptoms. It was found that blood–brain barrier disruption precedes the appearance of lesions in unenhanced MRI. In one case, blood–brain barrier disruption clearly preceded the clinical symptom.

Harris *et al.* [32] performed a serial study in six patients with a mild form of MS. Although these patients were more or less clinically stable during the research period, Gd-enhancement could be seen in all six. It also seems that in relapsing–remitting patients, disease activity persists. In accord with other authors, it is concluded that blood–brain barrier disruption is a very early step in plaque formation.

Methylprednisolone, a synthetic glucocorticoid, is able to diminish Gd-enhancement dramatically. This suggests that methylprednisolone causes a more rapid restoration of the blood–brain barrier. However, in a serial study, all the lesions seen on the first scan remained visible on the second unenhanced MRI scan. There are two explanations for this phenomenon. First, blood–brain barrier disruption may not be very important in the pathogenesis of MS. It is only important in the onset of a new lesion, but not in the development of the disease. Although the integrity of the blood–brain barrier improved under the influence of methylprednisolone, lesions were still present and the long-term prognosis was not improved by the therapy. A second possibility is that the disruption of the blood–brain barrier is a crucial factor in the pathogenesis, but that the lesions can develop despite methylprednisolone therapy because of the delay between the start of blood–brain barrier disruption and the start of the therapy. The last option suggests that blood–brain barrier disruption is the beginning of an irreversible cascade of events leading to demyelination [33]. In a serial study using HVDCT and unenhanced MRI, Koopmans *et al.* [13] suggested that blood–brain barrier disruption is not necessarily followed by plaque formation. However, Gd-MRI, which is more sensitive for detecting blood–brain barrier disruption, was not used.

Recently, Barkhof *et al.* [34] reported Gd-

enhancement in the meninges of a patient with clinically defined MS. The meninges were free from myelin and, therefore, the inflammation was possibly triggered by blood–brain barrier disruption but certainly not by myelin. Grossman [35] stated in a reaction to this report that this conclusion was ambiguous. There were no consistent CSF findings, the patient was completely asymptomatic with regard to the meningeal involvement, and finally he suggested that the meningeal involvement might be caused by a lumbar puncture.

All the studies, performed with the use of Gd-MRI reflect that blood–brain barrier disruption is an early step in the development of the disease. However, it is still not clear whether this is the start of an irreversible cascade of events that lead to demyelination, or whether it is possible to restore the blood–brain barrier and for the myelin sheaths to remain unaffected.

Cause of blood–brain barrier disruption

Different mechanisms have been proposed as being responsible for blood–brain barrier disruption in the early stage of MS. Immune complexes, colocated in the vessel wall with specific antigens, could trigger lymphocytes to adhere and migrate into the brain.

The upregulation of different adhesion molecules in MS has been described [36, 37]. These adhesion molecules are probably involved in the increase in adhesion and migration of lymphocytes into the brain. However, it is not clear how this upregulation occurs. It could be a primary cause of blood–brain barrier disruption, but the upregulation of the adhesion molecules also could occur under the influence of cytokines released by activated T-cells. The induction of adhesion molecules on the cerebral endothelial cells would then only be a secondary reaction.

From a study in rats with EAE, it became clear that tumour necrosis factor-α (TNFα) can accelerate the disease [38]. Sharief and Thompson [39] studied the relation between blood–brain barrier disruption and TNFα levels in serum and CSF of MS patients. Blood–brain barrier disruption was measured using the albumin quotient (albumin in CSF/albumin in serum), and a correlation was found between TNFα levels and albumin quotient in patients with active MS. It was suggested that TNFα is involved in blood–brain barrier disruption, as TNFα is known to have a toxic effect on cerebral endothelial cells and is able to increase leucocyte adherence to cerebral endothelial cells. It remains possible, however, that high TNFα levels are a result of inflammation and blood–brain barrier disruption. A comparable result was obtained in a study of the levels of interleukin-2 (IL-2) and the soluble IL-2 receptor (sIL-2R) in the CSF in correlation with the serum and CSF albumin [40]. Also in that study the degree of blood–brain barrier damage correlated with the intrathecal levels of IL-2 and sIL-2R, but it was not possible to determine a causal relationship. More studies must be performed to investigate the role of TNFα and other cytokines in correlation with Gd-MRI, which is a more appropriate method to measure blood–brain barrier disruption.

TREATMENT

Impairment of the blood–brain barrier has various consequences in respect to therapy, as can be seen in the case of baclofen, which is used to treat spasticity in MS. Different drugs are used to diminish blood–brain barrier permeability, such as glucocorticoids, which can reduce edema, and immunoregulators, which can prevent lymphocytes from entering the brain. In EAE and CREAE, different therapies have been tested to modulate the immune response.

Baclofen

Baclofen [4-amino-3-(*p*-chlorophenyl)butyric acid] has been widely used to combat the spasticity that often occurs in MS. It is a γ-aminobutyric acid (GABA) analogue that selectively acts on the GABA-B receptor. It primarily restricts calcium influx into the presynaptic terminal, thereby reducing the presynaptic transmitter release [41].

The transport of baclofen across the blood–brain barrier occurs normally by the stereo-selective large neutral amino acid carrier [42]. However, in about a quarter of the patients with severe spasticity, oral baclofen in the normal maximal dose (<80 mg/day) does not have any effect. Therefore, higher oral doses are proposed for MS patients with severe spasticity [43]. Another possibility is the use of intrathecal baclofen to surmount the rather low ability to cross the blood–brain barrier. In a group of 28 patients with severe, untreatable spasticity, all had considerable benefit from the treatment [41].

From these results it can be concluded that baclofen still has difficulties crossing the blood–brain barrier in MS, in spite of the fact that the blood–brain barrier is affected in an early stage of the disease. Probably, the carrier system is still functional and is the only way for baclofen to enter the CNS.

Glucocorticoid therapy

Glucocorticoids generally have an inhibiting effect on infections, especially in the CNS, by preventing the penetration of leucocytes. Furthermore, the IgG production of B-lymphocytes is restrained.

In MS patients, glucocorticoid production after endogenous adrenocorticotropic hormone (ACTH) induction is diminished and shows strong inter- and intra-individual fluctuations. It has been reported by different groups in double-blind studies that ACTH can shorten the time of relapse. The mechanism of this shortening is not known, but possibly the glucocorticoids, released after ACTH administration, have a beneficial effect on the edema in the brain. No difference was observed in the remitting period of the disease between treated and non-treated patients. When administered over a short period, ACTH does not produce severe side-effects [44].

The most effective form of glucocorticoid therapy seems to be high doses of methylprednisolone given intravenously [44]. CT scans (HVDCT) showed that in patients treated with high intravenous doses of methylprednisolone, a diminished contrast enhancement in plaques could be observed, even within 8 hr. It was suggested that this is due to the stabilizing

effect of corticosteroids on the blood–brain barrier. This reduction of enhancement is not specific for MS, because corticosteroids have a similar effect on a variety of cerebral lesions, including primary and metastatic tumours and encephalitis [45].

The effect of high-dose intravenous methylprednisolone has also been studied with the use of Gd-MRI. Barkhof *et al.* [33] showed that blood–brain barrier integrity improved after high-dose methylprednisolone, and that this improvement correlated well with the clinical status of the patients. These results agree with those obtained in a Gd-MRI study by Burnham *et al.* [16] of seven patients displaying acute symptoms of MS, who were treated with methylprednisolone. To study the mechanisms behind the effect of high-dose methylprednisolone, Barkhof *et al.* [46] compared the CSF composition of 16 patients with results obtained with Gd-MRI. It was found that methylprednisolone interferes with the active disease process, as determined by Gd-MRI, and with the demyelination process, as determined by the level of myelin basic protein in CSF. It is possible that methylprednisolone has a direct effect on the process responsible for the demyelination. Another possibility is that methylprednisolone interferes only non-specifically with the mediators of the disease or that it acts directly on the blood–brain barrier, both processes tending to prevent leucocytes, which might be responsible for demyelination, from entering the brain.

From a serial study performed with Gd-MRI, Miller *et al.* [47] concluded that the rapid reduction of blood–brain barrier abnormalities after treatment with high doses of methylprednisolone is the cause of the evident clinical improvement during this therapy.

Immunoregulators

Many treatments involving the immune system have been proposed, since the immune system plays an important role in MS. Some immunosuppressors, such as azathriapine and cyclophosphamide, have been used, as well as total lymphoid radiation. Although it seemed that in small groups of patients there was an improvement, immunosuppressors are not suitable for long-term treatment because of their severe side-effects. It was shown that probably interferon-α and interferon-β could have a beneficial effect on exacerbations, while interferon-γ actually aggravates the disease [48]. Interferon-γ may exacerbate the disease due to the fact that it is able to increase the expression of different antigens on endothelial cells and astrocytes. This can lead to an increase of the migration of lymphocytes in the CNS across the blood–brain barrier.

New therapeutic possibilities

In EAE, different therapies have been tested to decrease disease activity. In respect to the blood–brain barrier, one of the most promising possibilities is the blockade of different adhesion molecules. In this way, leukocytes are prevented from entering the CNS. Monoclonal antibodies against VLA-4, a member of the β1 integrin family, diminished the binding of leukocytes to EAE brain vessels *in vitro* and the accumulation of leukocytes in the CNS *in vivo* [49]. Macrophages were blocked from entering the CNS after treatment with monoclonal antibodies against Mac-1, a β2 integrin, and this treatment suppressed clinical signs of EAE [50].

The possibility of producing reshaped humanized monoclonal antibodies, in which mouse anti-human peptide sequences responsible for antigen binding are inserted onto a human immunoglobulin, promises to be very useful in this respect. The monoclonals retain immunological specificity, but anti-idiotypic responses that may limit efficacy in long-term treatment are strongly diminished [51].

Another possibility of interfering early in the disease process is to influence the cytokine mechanisms, which are involved in blood–brain barrier breakdown and the recruitment of inflammatory cells towards the CNS [52].

CONCLUSION

The mechanism of the disruption of the blood–brain barrier during the pathogenesis of MS is still unclear, but certainly the immune system becomes activated. Genetic and environmental factors probably play a role in the activation of the immune system against unidentified antigens. This activation causes an increase of cellular infiltration across the blood–brain barrier. The mechanism that causes the increase in lymphocyte infiltration and in pinocytotic vesicles is not completely understood. Activated T-lymphocytes releasing cytokines could be important in this process. It is known that cytokines increase the number of adhesion molecules present on cerebral endothelial cells and that these adhesion molecules could promote the adhesion and migration of lymphocytes into the brain. Another related possibility is that cytokines directly influence the endothelial cells, causing increased transport across the blood–brain barrier.

Disruption of the blood–brain barrier could also be caused by the deposition of particles (antigens) and immune complexes on the vessel wall. These immune complexes, together with complement factors, can activate macrophages and lymphocytes. Activated leucocytes can now enter the brain.

From all the studies performed in recent years, especially with the use of Gd-MRI, it can be concluded that the disruption of the blood–brain barrier is necessary for the development of the disease. After barrier opening, edema and inflammation are produced in the CNS, and this can lead to demyelination, although demyelination does not occur after every inflammation. The mechanism of demyelination has not been elucidated.

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