＜招待講演 1—2＞多発性硬化症の臨床と病理のトピックス

Clinical and Pathological Topics of Multiple Sclerosis

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Abstract: Therapeutic options for patients with progressive multiple sclerosis are currently limited. This is in part due to lack of knowledge regarding the pathophysiology of the disease in this stage. This review summarizes recent findings, showing profound differences in the pathology between relapsing and progressive MS. Pathological hallmarks in progressive MS are slow expansion of pre-existing white matter lesions, massive cortical demyelination and extensive diffuse injury of the normal appearing white matter. As in relapsing MS also in progressive MS active tissue injury is invariably associated with inflammation, but inflammation seems to be trapped behind a closed blood brain barrier. Different immunological mechanisms are involved in tissue destruction in progressive MS, but inflammation induced mitochondrial injury appears to be a dominant pathway. Future therapeutic interventions will have to target inflammation, which is compartmentalized in the central nervous system. In addition, however, neuroprotective therapies may be necessary.

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Multiple sclerosis (MS) is defined as a chronic inflammatory demyelinating disease of the central nervous system, characterized by the formation of focal lesions within the white matter (Lassmann et al 2007). In the majority of the patients it starts with a relapsing disease course (relapsing remitting MS, RRMS), which is followed by a phase of secondary progressive disease (secondary progressive MS, SPMS). In a minority of patients the disease takes an uninterrupted progressive course from the beginning (primary progressive MS, PPMS). In the relapsing stage of the disease the pathological alterations in the brain are clearly associated with inflammation. New lesions are associated with profound leakage of the blood brain barrier, as seen in magnetic resonance imaging with contrast enhancement and anti-inflammatory, immunomodulatory or immunosuppressive therapies are beneficial. However, when patients reach the progressive stage of the disease contrast enhancing lesions become rare or are absent at all and anti-inflammatory treatments are largely ineffective, despite continuous clinical deterioration of the patients. For this reason it has been suggested that in the progressive phase the patients enter a neurodegenerative stage of the disease, which may become independent from inflammation (Trapp and Nave 2008). If true, this would have major consequences for therapy. Anti-inflammatory therapies would have to be replaced by new neuroprotective treatment strategies. For this reason it is currently of critical importance to elucidate the pathogenetic mechanisms, which operate in the progressive stage of the disease.

Pathology of progressive MS

The pathology in the brain and spinal cord of patients with RRMS is dominated by the presence of focal white matter lesions (Lassmann et al 2007). New lesions are associated with pronounced inflammation and blood brain barrier damage. In contrast, fresh active lesions are rare in patients, who died in the progressive stage of the disease. However, pre-existing focal demyelinated lesions show slow expansion at their margins, characterized by the presence of a rim of activated macrophages and microglia, very few of them containing early myelin degradation products as a sign of ongoing myelin destruction. In addition, widespread demyelination is also seen in the grey matter, in particular in the cerebral and cerebellar cortex (Kutzelnigg et al 2005). Cortical lesions are not evenly distributed, but they affect mainly infoldings of the brain surface, such as cortical sulci, the insular cortex and the cingulated cortex. In addition, massive cortical demyelination is also seen in the hippocampus, which may in part be associated with cognitive disturbances of the patients (Geurts et al 2007). Another feature of MS pathology, which is characteristic for patients, who died in the progressive stage of the disease, is a diffuse injury of the normal appearing white matter, which at late stages is also reflected by severe brain atrophy and dilatation of the cerebral ventricles. Thus, in the progressive stage of the disease pathology is not restricted to focal white matter lesions, but affects the

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global brain and spinal cord. Furthermore, there is little correlation between number, size and destructiveness of focal white matter lesions with cortical demyelination or diffuse white matter injury. This suggests that these different types of lesions at least in part develop independently from each other (Kutzelnigg et al 2005).

Relation between inflammation and demyelination or neurodegeneration in MS

As mentioned above contrast enhancement in magnetic resonance imaging is rare and anti-inflammatory therapies are largely ineffective in the progressive stage of MS. It is, thus, important to analyse, whether progressive tissue injury in this stage of the disease develops independently from inflammation. We have addressed this question recently in a detailed quantitative study, enumerating T-lymphocytes, B-lymphocytes, plasma cells and macrophages in the lesions of patients with acute or relapsing disease in comparison to those in the progressive stage in relation to active demyelination and progressive axonal degeneration (Frischer et al 2009). As expected, inflammation was most pronounced in classical actively demyelinating lesions, followed by slowly expanding lesions. However, there was still significant inflammation also in inactive lesions and in the normal appearing white matter. Overall, we found a highly significant correlation between inflammation and active demyelination or acute axonal injury. This was not only seen in patients with acute and relapsing MS, but also in those with progressive MS. Interestingly, in a subset of aged patients who died at very late stages of the disease, inflammation in the brain declined to levels seen in age matched controls. In these patients no active or slowly expanding lesions were present and acute axonal injury too decreased to levels seen in age matched controls. This suggests that the disease process in MS may die out after long standing disease course. Ongoing neurodegeneration in such patients appears to be related to aging and age associated confounding diseases, such as Alzheimer's disease or vascular disease (Dal Bianco et al 2008, Frischer et al 2009).

The relation between inflammation and active tissue injury in cortical lesions deserves a special comment. It has been noted in several studies before, that within the cortex of MS patients inflammation is sparse or absent, despite ongoing demyelination and axonal and neuronal injury. However, profound inflammation is seen in the meninges, which cover the cortical and in particular subpial demyelinating lesions (Serafini et al 2004). This indicates that the lesions are driven by soluble products, produced within inflammatory cells in the meninges, which diffuse into the cortex and damage myelin and axons either directly or indirectly through microglia activation. The shape and topographical distribution of cortical lesions in MS patients is fully consistent with this hypothesis.

All these data provide strong evidence that ongoing demyelination and axonal injury in MS patients is invariably associated with inflammation and this is particularly also the case in patients with progressive disease. Therefore, the question arises, why in such patients no contrast enhancement is seen in magnetic resonance imaging and why anti-inflammatory treatments are ineffective.

Inflammation in progressive MS becomes trapped behind a closed (or repaired) blood brain barrier

Since it became clear, that brain inflammation is prominent in patients, who died in the progressive stage, it appears necessary to analyse in more detail the relation between inflammation and blood brain barrier injury. To address this issue we studied blood brain barrier leakage by analysing the extravasation of fibrin and by a specific marker for leaky brain endothelial cells in relation to inflammation in MS lesions (Hochmeister et al 2006). As expected, we found profound disturbance of the blood brain barrier in active lesions in patients with acute or relapsing MS. In progressive MS the situation was more complicated. Mild blood brain barrier disturbance, which appears to be below the detection limit for contrast enhancement in magnetic resonance imaging, was seen around many brain vessels even in completely inactive plaques. More importantly, however, numerous brain vessels with profound perivascular inflammatory infiltrates were seen, which showed no evidence for serum protein leakage or increased permeability of the endothelial cells. This suggests that inflammation in the progressive stage of the disease is compartmentalized in the brain and hides behind a closed blood brain barrier. In line with this observation it was recently shown that in patients with progressive MS dense aggregates of inflammatory cells, which form structures that resemble lymph follicles, are present in the meninges and in the perivascular space of large cerebral vessels (Serafini et al 2004). Retention and homing of inflammatory cells within the compartment of the central nervous system may be driven by the local production of certain cytokines and chemokines, which are normally expressed within lymph nodes. Thus, in inflamed brain tissue of MS such molecules can be produced by astrocytes as well as by leucocytes within the lesions.

These data suggest that inflammation in the progressive stage of MS hides behind a closed blood brain barrier and becomes independent from peripheral immune control. Under

such circumstances it is not surprising that current anti-inflammatory treatments, which have very limited capacity to pass the intact blood brain barrier, are ineffective. It also explains the lack of contrast enhancement in magnetic resonance imaging.

Mechanisms of tissue injury in the progressive stage of MS

Based on the data discussed above it is likely that also in the progressive stage of the disease tissue injury is driven by the inflammatory process. Many different immune mechanisms have been suggested so far to be important for the formation of MS lesions, including (cytotoxic) T-cells, specific auto-antibodies and mechanisms of innate immunity, mainly mediated by activated macrophages and microglia. A potential role of auto-antibodies is suggested by deposition of immunoglobulin and activated complement in active white matter lesions. So far, however, attempts to identify specific pathogenic auto-antibodies in the blood or cerebrospinal fluid of patients with progressive MS were only in part successful. One example are antibodies against neurofascin, which in experimental models may induce axonal injury, in case they reach the nervous tissue in an inflammatory environment (Mathey et al 2007).

Many studies so far have shown that active demyelination and acute axonal injury occur in close association with activated macrophages or microglia. It thus seems that these cells are the main effectors for immune mediated injury in MS. Another important issue regarding mechanisms of tissue injury in MS lesions is the selective vulnerability of different components of the nervous tissue. Besides predominant myelin and oligodendrocyte destruction thin calibre axons are particularly vulnerable. Recent findings indicate that mitochondrial injury is a major driving force for tissue destruction in MS lesions (Dutta et al 2006). Fulminate acute MS lesions may reveal a pattern of tissue injury, which closely reflects hypoxic brain damage and in such lesions profound alterations of selective components of the mitochondrial respiratory chain are seen (Mahad et al 2008). Essentially similar mitochondrial changes are also present in the active zone of slowly expanding lesions in progressive MS and there is a compensatory increase in mitochondrial number and function within inactive parts of the lesions (Mahad et al 2009). Thin calibre axons are particularly vulnerable in partial mitochondrial injury, since mitochondrial density is low in relation to the high energy demand of these fibres. Similarly, oligodendrocytes are particularly vulnerable to mitochondrial injury as seen by their selective destruction in cuprizone intoxication. Activated macrophages and microglia can produce factors, which are toxic for mitochondria. These are mainly oxygen and nitric oxide radicals (Smith and Lassmann 2002). Whether radical production by other cells, such as neurons themselves or astrocytes, is also involved is currently unresolved. Energy deficiency in axons has deleterious consequences (Stys and Trapp 2009). The energy dependent sodium pump is essential to remove intra-axonal sodium and restore excitability. Furthermore, intra-axonally accumulated sodium is replaced by calcium ions due to reverse operation of the sodium/calcium exchanger. Intra-axonal calcium accumulation activates calcium dependent proteases, such as calpain, which may then dissolve the axonal cytoskeleton and lead to disturbance of axonal transport and axonal degeneration. Axonal changes seen in MS lesions are consistent with such a mechanism of neurodegeneration.

Consequences for therapy

Our data suggest that anti-inflammatory or immunomodulatory treatment should be effective also in the progressive stage of the disease, provided it reaches inflammation, which is trapped behind an intact blood brain barrier. An additional problem, however, is that detailed mechanisms of the immune reaction within such chronic progressive brain lesions are still incompletely understood. Thus, it is not clear so far, whether immune cell activation in trapped inflammation in the central nervous system is similar or different compared to that responsible for the formation of new lesions in the acute or relapsing state.

Another option is neuroprotective therapy. From the data, available so far, anti-oxidants appear to be good candidates to be tested in the future. Alternatively, blockade of different sodium or calcium channels, as already tested in first clinical trials, is a reasonable option. To be effective, however, also these drugs have to reach the central nervous system behind a closed blood brain barrier, they have to be safe and they have to lack side effects, which prevent long-term treatment over years. Finally they have to stand the test in well designed clinical trials. Thus, despite our improved knowledge of the pathogenesis of progressive MS, translation into effective therapies is not expected in the near future.

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