1 Immune Response in the Human Central Nervous System in Multiple Sclerosis and Stroke

Hans Lassmann

Division of Neuroimmunology, Center for Brain Research, Medical University of Vienna, Wien, Austria

Introduction

Traditional pathology provides a clear distinction between inflammatory and neurodegenerative disorders. Inflammatory diseases comprise a large spectrum of infectious and autoimmune diseases. In these conditions, a specific immune response against autoantigens or infectious agents is present, which induces inflammation and specific destruction of cells, which contain the inciting agent or autoantigen. In addition, cells and tissue components, which are present in the vicinity of the specific targets of the immune response, also get injured or destroyed by toxic products or mediators of the immune response, a process termed “bystander damage” (Wisniewski and Bloom, 1975). In contrast, in conditions of neurodegeneration or brain ischemia, the primary cause of cell and tissue injury is due to primary metabolic changes. Also, in these conditions, immune mediators, such as cytokines or activated cells of the immune system, as for instance granulocytes or activated macrophages and microglia, are involved in cell and tissue degeneration. This lead to the broad concept of “neuroinflammation” playing a major role in the pathogenesis of a wide spectrum of brain diseases and being a potential target for neuroprotective treatments (Craft et al., 2005, Ransohoff and Liu, 2007).

The Concept of Neuroinflammation

Any type of tissue injury in the central nervous system (CNS) is associated with local changes in the microenvironment, which are in part similar to those seen in inflammatory conditions. Cell injury in the CNS results in activation of microglia and astrocytes (Ransohoff and Brown, 2012). Furthermore, a similar activation of microglia can be induced even by functional changes in neuronal networks, such as for instance sustained overactivation of neuronal circuits in epileptic seizures (Xanthos and Sandkühler, 2013). Activation of glia is induced by different signals, including release of adenosine triphosphate (ATP) and its signaling through G-protein-coupled
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receptors, by direct neurotransmitter signaling or by the liberation of intracellular components from damaged cells, resulting in the activation of pattern recognition receptors (Iadecola and Anrather, 2011). An important consequence of astrocyte and microglia activation is the production of a wide spectrum of pro- and anti-inflammatory cytokines and growth factors. Thus, microglia and astroglia activation as a reflection of an inflammatory response to tissue injury may have beneficial as well as detrimental consequences for adjacent neurons and glia, depending on the type of the primary tissue injury and on the properties of the environment where it takes place (Griffiths et al., 2007).

In addition, tissue injury and the induction of proinflammatory cytokines and chemokines may lead to disturbance of vascular integrity at the blood–brain barrier, resulting in brain edema and the penetration of serum components into the CNS (Takeshita and Ransohoff, 2012; Erickson et al., 2012). Besides leakage of various additional proinflammatory factors such as complement components, the leakage of fibrin and its coagulation within the perivascular compartment plays an important role in this process. It has been shown in experimental studies that fibrin deposition in the brain augments the inflammatory process and/or the subsequent tissue injury. Thus, inflammatory processes in the brain are much milder in fibrinogen-deficient animals or in conditions of fibrin depletion in the plasma. Fibrin can activate microglia and macrophages through toll-like receptor signaling. In addition, fibrin interacts with microglia through specific binding to the integrin receptor CD11b/CD18, which amplifies the inflammatory process and its associated tissue damage (Davalos et al., 2012). Such a vascular inflammatory process may also recruit inflammatory cells from the circulation. Depending on the type of tissue injury and its local induction of different spectra of adhesion molecules and chemokines, different leukocyte populations will be recruited, such as granulocytes and monocytes, but also different subpopulations of T- and B-lymphocytes (Gorina et al., 2014; Ransohoff and Engelhardt, 2012). This vascular injury is an important component of the inflammatory reaction, giving rise to the cardinal features of an inflammatory response, which have been defined as tissue swelling due to edema (tumor), vasodilatation, and hyperemia (rubor and calor) and activation of sensory receptors (dolor). In the CNS, edema and tissue swelling are the most important consequence, as the brain can swell only to a limited degree due to restraints by the bony skull. Thus, edema results in increased intracranial pressure leading to disturbance of microcirculation and amplification of tissue damage by ischemia (Bor Seng Shu et al., 2013).

Finally, inflammation may also be induced or augmented by specific mechanisms of adaptive immune responses. The prerequisite for such a scenario is that the organism has earlier mounted a specific response of T-lymphocytes or antibodies, which are directed against an antigen that is present within the CNS (Wekerle et al., 1986; Flügel et al., 2001). Inflammation, which is mediated by adaptive immune responses, is especially important in infectious and autoimmune diseases of the nervous system. The diverse patterns of neuroinflammation are summarized in Fig. 1.1.

Basic Principles of Immune Surveillance and Inflammation by Adaptive Immune Responses

The CNS has for long been viewed as an immune-privileged organ, which is shielded from the peripheral immune system by the blood–brain barrier and which does not express major histocompatibility complex (MHC) antigens required for antigen recognition by T-lymphocytes. This
Neuroinflammation versus inflammatory brain disease

- Neuroinflammation microglia activation
- Brain inflammation BBB disturbance
- Adaptive-immunity-mediated inflammatory disease

M2 microglia activation
- Neuroprotection

M1 microglia activation
- Propagation of tissue damage

Perivascular inflammation
- Edema
- Fibrin deposition
- Microglia activation
- Macrophage recruitment

Antigen recognition and activation of T-cells
- T-cell-mediated cytotoxicity
- Antibody-mediated damage
- Activation of microglia by T- and B-cell cytokines
- Macrophage recruitment

Figure 1.1 Inflammation of the CNS comprises a broad spectrum of tissue alterations including microglia activation, vascular inflammation with blood–brain barrier damage, and inflammation mediated by adaptive immunity. (See insert for color representation of this figure.)

concept, however, has been modified during recent years. T-lymphocytes can enter the normal brain through an intact blood–brain barrier in the course of immune surveillance (Wekerle et al., 1986). However, it is only the activated T-cell population, which is able to enter the normal CNS tissue. This implies that a small fraction of T-cells, when activated in the course of an infection, migrates into the brain or spinal cord in search for their specific antigen. When they do not find their cognate antigen, they quickly disappear from the brain tissue due to local destruction by programmed cell death (apoptosis; Bauer et al., 1998). Whether some of these T-cells can also migrate back into the bloodstream or into the lymphatic system is currently unresolved. However, when the specific antigen is present in the CNS and is presented in the perivascular or meningeal space by a macrophage population with features of dendritic cells, T-cells receive a further activation signal (Flügel et al., 2001, Mues et al., 2013). They then proliferate and expand clonally and produce additional proinflammatory cytokines and chemokines, which act on endothelia and promote the secondary recruitment of other leukocytes from the bloodstream, such as other T-cells, B-cells, and monocytes (Ransohoff and Engelhardt, 2012). This results in a first stage of perivascular and meningeal inflammation. Proinflammatory cytokines in this condition also activate local microglia and astrocytes, which amplify the inflammatory response through additional production of cytokines, chemokines, and proteases. This further amplifies the perivascular inflammatory response and allows the inflammatory cells to pass the subpial and perivascular astrocytic glia limitans and spread into the CNS parenchyma. Tissue injury can be induced directly by cytotoxic MHC Class I antigen-dependent cytotoxic T-cells (Saxena et al., 2008). These cells can recognize their specific antigen on all cells of the CNS, such as astrocytes, oligodendrocytes, and neurons, as all these cells express MHC Class I antigens in an inflammatory environment (Höftberger et al., 2004). The expression of MHC Class II antigens, which are necessary for antigen recognition by CD4 positive T-cells, is more restricted, being mainly...
present on macrophages, microglia, and occasionally on astrocytes. Thus, CD4\(^+\) T-cell-mediated inflammation mainly leads to the activation of macrophages and microglia, which are then responsible for the induction of tissue injury through the liberation of toxic immune mediators, such as reactive oxygen or nitrogen species, of cytotoxic cytokines, such as tumor necrosis factor alpha (TNF-\(\alpha\)), or of proteases and lipases (Jack et al., 2005). However, direct cytotoxicity can even be mediated by CD4\(^+\) T-cells. When highly activated, they may mediate cytotoxicity in a manner that does not depend on specific antigen recognition on the target cell (Nitsch et al., 2004).

Downregulation of the inflammatory response is of critical importance for protecting the CNS against uncontrolled immune-mediated damage. This is in part achieved by highly efficient destruction of T-cells within the brain and spinal cord through apoptosis (Bauer et al., 1998, Flügel et al., 2001). This process eliminates both antigen-specific T-lymphocytes and secondarily recruited T-cells. It is highly efficient in conditions of acute T-cell-mediated brain inflammation and allows persistence of the inflammatory process only as long as there is a continuous influx of T-cells from the circulation into the lesions. The molecules involved in the induction of T-cell apoptosis are currently not well defined. In addition, brain inflammation is further controlled by the recruitment of regulatory T-cells (Tregs) into the inflamed CNS tissue (O’Connor and Anderton, 2008; Fransson et al., 2012) as well as by downregulation of immune response due to activation of the pituitary/adrenal axis (MacPhee et al., 1989). Clearance of the inciting trigger, such as the infectious agent, also terminates inflammation by the lack of further antigen presentation and T-cell activation. Taken together, these mechanisms grant that the brain is controlled by a strictly regulated immune response, which keeps collateral damage as small as possible.

Immune-mediated damage of the brain by specific antibodies in general is low or absent, as they penetrate the normal blood–brain barrier only to a very limited degree. In addition, antibodies require interaction with complement or activated effector cells such as granulocytes or macrophages to induce tissue damage, factors that are not present in the normal CNS (Vass et al., 1992). However, circulating autoantibodies, directed against foreign or self-antigens, which are present on the extracellular surface of cells, may become pathogenic, when they reach the brain in an inflammatory environment, for instance induced by a T-cell-mediated inflammatory response (Linington et al., 1988). T-cell-mediated inflammation not only opens the blood–brain barrier, but also activates local macrophages and microglia and induces granulocyte and macrophage recruitment from the circulation by inducing local chemokine secretion. It further stimulates the production of complement and inhibits the production of complement-inhibitory proteins. These additional factors allow efficient antibody-mediated cell destruction (Pohl et al., 2013).

Data obtained in diseases, which are mediated by autoantibodies against neurotransmitter receptors or cell surface channels, suggest that even antibodies alone may induce disease and damage in the CNS. In this case, high titers of autoantibodies are present in the circulation, and the antibodies exert their pathogenic role by direct binding to the channels or receptors, thus acting more analogous to a pharmacological agonist or antagonist than as an immunological tool (Hughes et al., 2010). In this situation, massive functional disturbances are seen despite only sparse or absent inflammation and structural tissue damage (Bien et al., 2012).

As mentioned previously, recruitment of T- and B-lymphocytes into the CNS may also occur secondarily to tissue injury, for instance in ischemia or in neurodegenerative diseases
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(Gelderblom et al., 2009). However, the mere presence of lymphocytes within a brain lesion does not necessarily imply that they are pathogenic. When there is no activation signal within the CNS tissue, such cells are inert bystanders. Such T-cell infiltrates in brain lesions are potentially pathogenic when they are locally activated, for instance by recognizing specific autoantigens. This is indicated, when the respective T-cells locally proliferate, show clonal expansion, or express activation markers (Liesz et al., 2013a).

### Inflammation in the Central Nervous System of Patients with Multiple Sclerosis

Multiple sclerosis (MS) has originally been defined as an inflammatory demyelinating disease, suggesting that the formation of brain and spinal cord lesions in this disease is driven by the inflammatory process (Lassmann et al., 2007). Focal plaques of primary demyelination, reflected by complete loss of myelin, but partial preservation of axons and neurons, are the hallmark of MS pathology. Demyelinated plaques are present in the white matter as well as in the grey matter, such as the cerebral and cerebellar cortex (Peterson et al., 2001) and the deep grey matter nuclei (Vercellino et al., 2009), including the basal ganglia, the thalamus, and hypothalamus (Huitinga et al., 2004). In addition to the focal pathology, reflected by demyelinated plaques, there are also diffuse changes in the brain, consistent of small perivascular demyelinated lesions, diffuse axonal injury and neurodegeneration, generalized microglia activation, and diffuse astrocytic scar formation in the entire white and grey matter of the brain and spinal cord (Kutzelnigg et al., 2005). This finally leads to profound tissue loss and atrophy in the entire CNS. While focal demyelinated lesions in the white matter dominate the pathology of patients with early stages of relapsing remitting MS, cortical demyelination and diffuse damage of the white and grey matter are most prominent in patients during the later progressive stage of the disease (Kutzelnigg et al., 2005).

Active demyelination and neurodegeneration in the MS brain are invariably associated with inflammation, consistent of infiltrates of the tissue by T- and B-lymphocytes and by macrophages (Fig. 1.2; Frischer et al., 2009). Most prominent, however, is the profound

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**Figure 1.2** Inflammation in multiple sclerosis. (a) Inflammation in active multiple sclerosis lesions is associated with demyelination, reflected by the loss of blue myelin staining in the lesion. (b, c) Infiltration of the tissue with CD8+ T-lymphocytes (b) and CD20+ B-lymphocytes (c) (black cells) in the active lesion edge. The zone of initial demyelination at the lesions edge shows profound microglia activation with intense expression of NADPH oxidase (brown cells; p22phox). (d) CD8+ T-lymphocytes are also present in the lesion center (black cells). Most inflammatory cells are macrophages with low expression of NADPH oxidase (brown cells). (See insert for color representation of this figure.)
activation of the local microglia population (Jack et al., 2005). Several arguments speak in favor for a pathogenic role of this inflammatory response. T- and B-cells in the lesions show an activated phenotype and clonal expansion (Babbe et al., 2000; Obermeier et al., 2011), most likely due to proliferation following the encounter with their cognate antigen in the lesions. Furthermore, active lesions in the white matter of patients with MS show contrast enhancement as a consequence of inflammation-induced blood–brain barrier damage (Miller et al., 1988; Gaitan et al., 2011). Most importantly, anti-inflammatory or immunomodulatory treatments have a beneficial effect, in particular in patients in the early relapsing stage of their disease (Wiendl and Hohlfeld, 2009).

There is still some debate, whether inflammation also drives neurodegeneration in the progressive stage of the disease, because in such patients, lesions with contrast enhancement are rare and current anti-inflammatory treatments are no longer effective. Thus, a widely proposed concept for the pathogenesis of the progressive stage of MS is that inflammation in early relapsing disease initiates a cascade of events, which leads to demyelination and neurodegeneration in the progressive stage and becomes independent of the original inflammatory response (Trapp and Nave, 2008). However, detailed pathological studies showed that active tissue injury in the progressive stage is associated with T- and B-cell infiltrates in the CNS and that in patients in whom lymphocyte infiltration in the brain has declined to levels seen in age-matched controls, no active demyelination is found and neurodegeneration also is reduced to levels seen in the respective controls (Frischer et al., 2009). Yet, the nature of the inflammatory response appears to be different between early and late stages of MS. While in new active lesions in acute and relapsing/remitting MS, inflammation is associated with massive blood–brain barrier damage, inflammation in the progressive stages occurs at least in part behind a closed or repaired blood–brain barrier (Hochmeister et al., 2006). Furthermore, aggregates of inflammatory infiltrates, which consist of T-cells, B-cells, and plasma cells and may even form lymph follicle-like structures, are present in the meninges and perivascular spaces (Serafini et al., 2004). The extent of meningeal inflammation and the formation of inflammatory aggregates correlate well with the extent of active cortical demyelination and neurodegeneration (Magliozzi et al., 2007).

The Nature of the Inflammatory Response in Actively Demyelinating Lesions in MS

Profound inflammation, consisting of T-cells and B-cells, is a characteristic feature of actively demyelinating MS lesions. Active demyelination and neurodegeneration are associated with the presence of activated macrophages and microglia, which are present in close contact with degenerating myelin sheaths and axons (Prineas and Graham, 1981; Ferguson et al., 1997; Trapp et al., 1998). However, inflammation in active lesions appears to occur as a two-step phenomenon. In the initial stage, termed initial (Marik et al., 2007) or pre-phagocytic lesions (Barnett and Prineas, 2004), lymphocyte infiltration is moderate or sparse and the lymphocytic population mainly consists of MHC Class-I-restricted CD8+ T-cells. In contrast, when myelin sheaths have been destroyed and taken up by microglia and macrophages, inflammatory infiltration is much higher and a wide spectrum of different leukocyte populations is found, including CD4+ and CD8+ positive T-cells, B-cells, hematogeneous macrophages, and a variable number of plasma cells (Marik et al., 2007; Henderson et al., 2009). Thus, a small number of T-cells (mainly CD8+
cells) appear to enter the brain in the course of immune surveillance, encounter their specific antigen, and start the lesions through microglia activation. However, when myelin and oligodendrocytes get destroyed, intracellular and myelin components are liberated into the extracellular space and provide an additional proinflammatory stimulus. This, then, leads to secondary amplification of the inflammatory process in the lesions.

It has, however, been questioned, whether the mild T-cell infiltration in initial lesions is sufficient to drive the demyelinating process. The alternative interpretation of these findings is that initial demyelination occurs independently from adaptive T- and B-cell responses and that inflammatory cells are secondarily recruited into sites of pre-existing tissue injury, where they then may amplify demyelination and neurodegeneration (Barnett and Prineas, 2004; Henderson et al., 2009).

Both T- and B-cells show clonal expansion in the MS brain, and for B-cells, this is reflected by the presence of oligoclonal intrathecal antibody synthesis (Skulina et al., 2004; Obermeier et al., 2008). Regarding T-cells, the most pronounced clonal expansion is seen for MHC Class-I-restricted CD8\(^+\) cells, which also dominate in initial lesions stages. Genome-wide association studies in patients with MS versus controls identified a large number of different genes to be associated with MS susceptibility (Sawcer et al., 2011). Most of them have putative functions in the immune system. Finally, anti-inflammatory or immunomodulatory treatments are beneficial at least in early disease stages (Wiendl and Hohlfeld, 2009).

Studies on local cytokine and chemokine expression in MS lesions are limited but consistent with an inflammatory response, which is driven by T- and possibly B-lymphocytes. Active lesions show the expression of various adhesion molecules (Washington et al., 1994; Allavena et al., 2010; Cavrol et al., 2008; Ifergan et al., 2011; Larochelle et al., 2012) and chemokines (Trebst et al., 2001; Kivisakk et al., 2004), which are instrumental for leukocyte migration through the blood–brain barrier (Steiner et al., 2010). Antigen-specific activation of T-cells in MS lesions is also indicated by the expression of activation antigens (Pohl et al., 2013; Annibali et al., 2010), the presence of costimulatory molecules (Windhagen et al., 1995; Gerritse et al., 1996) or autoantigen and MHC complexes (Krogsgaard et al., 2000), and by the local expression of various pro- and anti-inflammatory cytokines (Mycko et al., 2003; Tzartos et al., 2008, 2011). This has so far been mainly described in classical active lesions. Detailed studies on the phenotype of lymphocytes in different stages of the disease and in relation to the activity of inflammation and neurodegeneration are still missing.

**Macrophages and Microglia in MS Lesions**

Much of our knowledge on the role of microglia and macrophages comes from experimental studies performed in rodents. However, there are species-related functional differences between rodent and human microglia, which involve cytokine signaling, response to innate immunity stimuli, and effector functions (Smith and Dragnow, 2014). Thus, to understand microglia function in brain disease, analysis of their function in human disorders is important.

There is good agreement that active demyelination and axonal injury in MS occurs in close apposition with activated microglia and macrophages (Prineas et al., 2001; Lassmann, 2011). In the normal-appearing white matter around actively demyelinating lesions, microglia nodules are
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seen, which are in close contact with myelinated nerve fibers. In initial lesions, massive microglia activation is associated with oligodendrocyte apoptosis and initial changes of myelin disintegration. Myelin is then taken up by phagocytic cells. Toward the lesion center, there is a continuous transition between cells with microglia and macrophage phenotype, suggesting that most of the phagocytic cells in the lesions come from the microglia cell pool. Furthermore, dystrophic axons within and around active MS lesions are seen in close contact with microglia or macrophages (Ferguson et al., 1997). Activated microglia and macrophages in initial and active MS lesions express a variety of markers, including MHC class I and Class II molecules (Höftberger et al., 2004), Fc-receptors (Ulvestad et al., 1994), and markers associated with phagocytosis, such as CD68 (Brück et al., 1995). Most importantly, they highly express components of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX1 and NOX2) complexes, suggesting high oxidative burst activation. In contrast, expression of inducible nitric oxide synthase (iNOS) is sparse or absent in initial lesions, but it is upregulated on a subset of macrophages in established lesions. When myelin has been taken up in macrophage-like cells, they lose their expression of NADPH oxidase but retain the expression of phagocytosis-related molecules, such as CD68 or CD163 (Fig. 1.2; Fischer et al., 2012, 2013). As, however, shown in spinal cord injury, this conversion from an M1 to an M2 phenotype of macrophages in response to myelin phagocytosis can be counteracted by the presence of TNF and by iron loading of macrophages in the lesions (Kroner et al., 2014).

Unrelated to the presence of demyelinated lesions, there is also a general activation of microglia in the normal-appearing white matter in patients with MS and to a lower extent also in the white matter of age-matched controls (Lassmann, 2011). Recent studies suggest that microglia in the normal-appearing white matter of patients with MS are in an alerted state, which may be transformed into a cytotoxic state by the additional presence of proinflammatory cytokines (Vogel et al., 2013). Global microglia activation may in part reflect anterograde and retrograde neuronal and axonal degeneration due to lesions in other brain areas. This may explain why new MS lesions are more frequently seen in areas, which receive axonal input from distant regions, affected by other MS-related pathology (Kolasinski et al., 2012). In addition, global microglia activation in the MS brain also reflects diffuse neurodegeneration in the normal-appearing white and grey matter.

From all these data, it is assumed that activated microglia and macrophages play a major role in the induction of demyelination and tissue degeneration in the brain of patients with MS. However, astrocytes take part in this process as well. As reviewed recently, they are involved in propagating and controlling inflammation. Furthermore, functional impairment of astrocytes in active lesions augments demyelination, oligodendrocyte death, and axonal injury (Brosnan and Raine, 2013). Reactive astrocytes in MS lesions lose their cell polarity, which results in the loss of connexins, the excitatory amino acid transporter EAAT2, and the water channel aquaporin 4 (Masaki et al., 2013). This impairs energy supply to oligodendrocytes and axons and increased excitotoxicity and may also propagate brain edema.

Mechanisms of Demyelination and Neurodegeneration in MS

There are many different acute and chronic inflammatory diseases of the CNS, but the widespread primary demyelination leading to large plaques of myelin destruction with axonal preservation
and reactive gliosis is a specific feature of MS pathology. This is best illustrated by the presence of cortical demyelinated lesions, which besides in MS are only seen in conditions of virus infection of oligodendrocytes (Fischer et al., 2013; Moll et al., 2008). Thus, in MS, there must be a specific trigger responsible for the induction of demyelination.

Experimental studies show that immune-mediated tissue injury in inflammatory demyelinating diseases can be mediated by several different mechanisms, involving T-lymphocytes, B-cells, and antibodies, as well as activated macrophages or microglia. This is also reflected by heterogeneous patterns of demyelination seen in active plaques of different patients with MS (Lucchinetti et al., 2000). Applying organotypic tissue culture, it was found that serum and in part also cerebrospinal fluid contains demyelinating activity (Bornstein and Appel, 1965). Experimental data indicate that this demyelinating factor may be an autoantibody response directed against antigens expressed on the surface of myelin or oligodendrocytes (Linnington et al., 1988). However, this may not be the case in MS. Depletion of immunoglobulins from MS sera did not abolish demyelinating activity in most tested MS sera (Grundke Iqbal and Bornstein, 1980). Recently, it was shown that supernatants from activated B-cells may contain a demyelinating activity, which could not be ascribed to immunoglobulins (Lisak et al., 2012). Thus, these data indicate that a soluble factor produced by inflammatory cells may be involved in the induction of demyelination in the MS brain, but the nature of this factor remains unknown.

All these different patterns of demyelination in MS lesions, however, have in common the close association between activated macrophages and microglia with active cell and tissue injury. However, microglia and macrophage activation alone does not explain the selectivity of demyelination seen in MS lesions in the cortex and white matter. Active tissue injury in the MS brain is associated with profound oxidative injury, reflected by the presence of oxidized lipids, proteins, and DNA (Bizzozero et al., 2005; van Horssen et al., 2008). Most profound evidence for oxidative injury has been found in oligodendrocytes and myelin, as well as in axons and degenerating neurons. In fact, massive accumulation of oxidized phospholipids was seen in apoptotic oligodendrocytes and in neurons and in fragmented axons and dendrites, and this was associated with DNA oxidation and fragmentation (Haider et al., 2011; Fischer et al., 2013). Oxidative injury appears to be driven by oxidative burst in activated microglia and amplified by mitochondrial injury (Mahad et al., 2008) and liberation of iron from damaged myelin and oligodendrocytes (Hametner et al., 2013; Lassmann et al., 2012).

### Inflammation in Stroke Lesions

The primary cause of tissue injury in stroke lesions is hypoxia and ischemia. However, a number of data suggest that an inflammatory reaction within the lesions may amplify functional disturbance and structural damage (Gelderblom et al., 2009). This assumption is mainly based on evidence from experimental models, in which various different anti-inflammatory treatment strategies have been shown to reduce clinical deficit and lesion size. However, in contrast to the situation in MS, anti-inflammatory or immunomodulatory treatments have failed the test in clinical stroke trials so far (Amanthea et al., 2013). Several potential explanations have been forwarded for this unsatisfactory situation. Firstly, experimental research has shown that inflammation in the brain not only is detrimental, but also is beneficial depending on the exact timing.
of the treatment and the nature of the initiating event (e.g., permanent or transient vessel occlusion, ischemic vs hemorrhagic stroke vs intracerebral hemorrhage). Exact timing of treatment can easily be achieved in experimental models but is very difficult to accomplish in the setting of a complex human trial. Secondly, patients with stroke or with traumatic brain injury go through a phase of generalized immunodepression, which reaches a peak during the first days after CNS injury and is also responsible for an increased susceptibility for systemic infections during this phase. Additional immunosuppression during this phase may increase systemic side effects and dangers (Dirnagl et al., 2007). Finally, information regarding the exact nature of the inflammatory response in human stroke lesions currently is limited and in part controversial.

**Microglia Activation and Macrophage Response**

Microglia activation mediates the first neuroinflammatory response in cerebral ischemia and intracerebral hemorrhage. The microglia response in experimental stroke lesions has been summarized in detail in a recent review (Taylor and Sansing, 2013); this will be covered in Chapter 5 and will, thus, only be shortly summarized in this chapter. First activation occurs as a result of ischemic cell destruction, mediated besides other mechanisms by the release of ATP and HMGB1 (high-mobility group box 1) into the extracellular space. Importantly, the patterns of microglia activation are different in the ischemic core and the peri-infarct zone (penumbra). In the infarct core, primary microglia activation results in an anti-inflammatory or neuroprotective phenotype during the first hours, but a substantial number of these cells die within the next days due to ischemia (Fig. 1.3). The remaining cells together with macrophages, recruited from the circulation are instrumental in phagocytosis of tissue debris. With increasing time after the acute ischemic event, however, the phenotype of microglia in part changes into a proinflammatory (M1) phenotype. This is associated with the production of different cytokines, such as interleukin (IL)-1ß, TNF-α, IL-6, transforming growth factor β, and IL-10 (for detailed review see Lambertson et al., 2012). In contrast, in the peri-infarct zone, proinflammatory inflammatory microglia activation dominates (Taylor and Sansing, 2013). The expression of enzymes involved in the production of reactive oxygen and nitric oxide species, such as NADPH oxidase or iNOS together with free radical production through damaged mitochondria results in profound oxidative injury in the lesions, which in the penumbra leads to patterns of demyelination and neurodegeneration, which are in part similar to those seen in MS lesions (Aboul-Enein et al., 2003) and which may also be amplified by similar age-dependent mechanisms. When there is an additional hemorrhagic component or extensive vasogenic brain edema, proinflammatory microglia activation is further enhanced (Taylor and Sansing, 2013).

**Granulocyte Infiltration**

Granulocyte infiltration into stroke lesions is an early event, and the products secreted by activated granulocytes are toxic for the CNS tissue in vitro (Mena et al., 2004; Gronberg et al., 2013). However, the importance of granulocyte infiltration in stroke lesions has been challenged in a recent systematic study on experimental models and human tissue. The study showed that
in conditions of pure ischemia, granulocyte infiltration is sparse and that granulocytes mainly remain in the meninges and perivascular spaces. In human stroke as well as in experimental stroke models, the perivascular glia limiting membrane was suggested to be a tight barrier, impeding granulocyte movement from the perivascular space into the brain parenchyma (Enzmann et al., 2013). However, when there is an additional hemorrhagic component, more pronounced granulocyte recruitment and their dispersion into the damaged brain parenchyma are seen (Kalimo et al., 2013). Thus, therapies blocking infiltration and activation of granulocytes in human patients with stroke may only be applicable to a subset of the total patient population (Fig. 1.3). However, augmentation of tissue injury by neutrophils not necessarily means that these cells have had to enter the CNS through the blood–brain barrier. When they adhere to cerebral endothelial cells, the latter may be damaged by the secretion of reactive oxygen species and neutrophil-derived proteases. This may lead to disturbance of microcirculation and brain edema, which by itself may augment tissue injury (Segel et al., 2011).
The Role of Lymphocytes

Lymphocytes accumulate with time in stroke lesions. In experimental stroke lesions, few of them are present already at very early stages and gradually increase in numbers, reaching a plateau after about 5 days (Gronberg et al., 2013). T-cells within the lesions consist of both CD4+ and CD8+ cells. Furthermore, a substantial number of these cells show clonal expansion. This is seen not only in the brain and lesions, but also in draining lymph nodes and spleen (Liesz et al., 2013a, 2013b, 2013c). Interestingly, clonal expansion of T-cells is a late event, occurring mainly between 7 and 14 days after induction of the lesions. Whether proliferation and clonal expansion is driven by recognition of a cognate antigen or by a nonspecific stimulus is currently unknown. However, systemic autoimmune reactions can be triggered as a consequence of stroke (Vogelgesang and Dressel, 2011; Becker et al., 2011), possibly through leakage of brain antigens into draining lymph nodes (Planas et al., 2012).

For a potential design of immunosuppressive or immune-regulatory treatment strategies, it has to be remembered that many different lymphocyte population infiltrate the brain in stroke lesions, some of them having detrimental and others beneficial actions. Thus, this includes CD4+ or CD8+ effector T-cells, Tregs, as well as B-cells and natural killer cells (Gelderblom et al., 2009). In general, CD4+ T-cells appear first followed by CD8+ cells and B-cells (Gronberg et al., 2013). Transfer of autoreactive CD4+ Th1- or Th17-cells at the time of cerebral ischemia augments clinical disease (Zierath et al., 2013), and blockade of T-cell entry into the brain or peripheral T-cell depletion during early stages of reperfusion reduces infarct size in experimental animals (Kraft et al., 2013; Xiong et al., 2013), although the beneficial effect is different between different models (Xiong et al., 2013). However, stroke lesions are also infiltrated by Tregs (Stubbe et al., 2013), and experimental studies have shown a beneficial effect of different regulatory T- and B-cell populations in clinical outcome or lesion size (Engelbertsen et al., 2013; Li et al., 2013; Offner and Hurn, 2012; Liesz et al., 2013b). These studies argue that recruitment of Tregs or B-cells into the lesions reduces neuroinflammation and subsequent tissue damage. This view is contradicted by another study, which showed reduced infarct size after peripheral Treg depletion. Tregs interacted with cerebral endothelial cells in the process of transmigration through the vessel wall, and this interaction caused microvascular dysfunction and thrombosis (Kleinschnitz et al., 2013).

Dynamics of Inflammation in Human Stroke Lesions

Information on the inflammatory response in human stroke lesions is currently limited (Mena et al., 2004; Kalimo et al., 2013). Overall, the response appears to be similar to that seen in experimental models, but the stage-dependent changes in inflammation are less distinct. Four different stages have been defined (Mena et al., 2004). The first, which occurs 1–2 days after disease onset, is characterized by acute neuronal injury and shows a low but variable infiltration by polymorphonuclear leukocytes and some microglia activation (Fig. 1.3a–1.3j). This is followed by a stage of acute inflammation (3–37 days after disease onset), reflected by infiltration of the tissue by granulocytes, lymphocytes, and macrophages, and a phase of chronic inflammation (10–53 days) with dominance of lymphocyte and macrophage infiltrates (Fig. 1.3k–1.3n). Finally,
in the resorption phase (26 days to years after disease onset), only macrophages are seen within the lesion in variable numbers. There are several possible reasons for the profound overlap in the dynamics of inflammation in human stroke lesions. Owing to the severe vascular pathology in aged patients with stroke, lesions may not occur as a single event but may progressively or recurrently enlarge with time. In addition, human stroke lesions are rarely purely ischemic but may have in different patients a hemorrhagic component of variable intensity. Finally, human stroke lesions may be complicated by additional comorbidities, such as for instance systemic infections or sepsis.

Conclusions

Neuroinflammation is an important pathophysiological process involved not only in classical inflammatory diseases of the nervous system such as MS, but also in other neurodegenerative conditions such as stroke. In MS, anti-inflammatory treatments have shown clear beneficial effects, when applied during the early stage of the disease. However, in patients with stroke and even in patients with MS who have entered the progressive phase of the disease, such therapeutic strategies have failed so far. One of the reasons for this unsatisfactory situation is that neuroinflammation has both detrimental and beneficial effects, depending on the nature of the primary insult, the nature of the immune response, and the microenvironment within the lesions. Currently, most of our knowledge on brain inflammation in disease comes from experimental studies, while information on the nature and time course of the inflammatory response as well as on the composition, activation state, phenotype, and function of inflammatory cells in brain lesions of patients is very limited. Only when these processes are well defined in human brain diseases and when proper paraclinical markers are developed, which allow to monitor these processes in vivo, anti-inflammatory treatment of patients will have a realistic chance of success.

References


NEUROINFLAMMATION: NEW INSIGHTS INTO BENEFICIAL AND DETRIMENTAL FUNCTIONS


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