Chapter A7

ROLE OF MICROGLIA AND MACROPHAGES IN EAE

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Abstract: Microglia and macrophages are related cell types that play an important role in the pathogenesis of MS and EAE. This chapters reviews the role of these cells in the normal brain and their contribution to inflammatory demyelinating disease, including their role in antigen presentation, co-stimulation, and production of cytokines and other inflammatory mediators

Key words: experimental autoimmune encephalomyelitis, macrophages, microglia, inflammation, antigen presentation, perivascular, parenchymal, meningeal

The microglia, a brain-resident, non-neuronal cell and the blood-derived or haematogenous macrophage represent two related cell types involved in key events in the development of pathology in Multiple Sclerosis and its autoimmune animal model, the Experimental Allergic Encephalomyelitis. Microglia and macrophages fulfil a variety of different functions, but they are also recognized for their ability to act as a particularly fine sensor of brain pathology. Both cell types are rapidly activated and recruited to sites of infection, neurodegeneration, stroke, autoimmune inflammatory models such as EAE and its presumptive human counterpart, multiple sclerosis. Microglia and macrophages are stimulated by a variety of cytokines, neurotransmitters, modulators and putative neurotoxins, extracellular matrix molecules and proteases present in the inflamed central nervous system. Moreover, both cell types are plastic in their morphology and cellular identity. The presence of dying cells and cell debris will cause a transformation of phagocytic microglia into a detached, rounded and migratory or amoeba-like (amoeboid) macrophage (Streit et al., 1988; Bohatschek et al., 2001). This also works in reverse: surrounded by a CNS environment, non-phagocytic macrophages freshly recruited from the blood stream will gradually develop

branched processes and transform into ramified microglia (Flugel et al., 2001, Bohatschek et al., 2001).

Activated microglia and macrophages synthesise a cornucopia of different cytokines, trophic factors, ECM components and neurotransmitter-like molecules that could exert a positive or damaging effect on the adjacent cells. They also interact with other cells of the immune system, particularly T-cells, which are recruited to the sites of CNS inflammation. Both in vitro and in vivo evidence also suggests that they may act as competent presenters of antigen, inducing and regulating the intensity of T-cell mediated inflammation and tissue injury. The aim of the current chapter will be to provide an overview on the different, related types of microglia and macrophages in the normal brain, describe their cellular and molecular response in EAE and multiple sclerosis and finally focus on their direct contribution to neuropathology in autoimmune demyelinating disease.

1. MICROGLIA AND MACROPHAGES IN THE NORMAL BRAIN

The normal central nervous system consists of several different non-neuronal cell populations that are related to monocytes and macrophages in the bone marrow and peripheral tissues, based on the presence of specific cellular differentiation markers such as the aMb2 integrin (CD11b/CD18), IgG receptors (CD16/CD32), IBA1 and so on. The brain microglia comprise the largest component, located inside the neural parenchyma. In the normal resting state, they are highly ramified cells, with extensive branches that can cover spaces of 30-50 μm in diameter. These resting microglia are territorial, in that their cell bodies or branches are rarely seen to adhere to one another, unlike white matter oligodendrocytes contacting one another like pearls on a string (Suzuki and Raisman, 1992) or protoplasmic astrocytes with extensive cell process to process contacts at astrocyte boundaries which allow the spread of intracellular ions and other small molecules from one astrocyte to the next (Nedergaard, 1994; Bushong, 2002).

The perivascular macrophages are located in between the blood vessel endothelia, occasional perithelial cells and the basal membrane that separates the blood vessel from the surrounding neural parenchyma, the Robin-Virchow space. Perivascular macrophages are typically slender elongated cells (elongated in the direction of the blood vessel axis) with broad but short processes that sometimes go around the blood vessel. Most perivascular macrophages are located around small to moderate blood vessels inside the central nervous system. Unlike the microglia, they do not show the elaborate

ramified structure typical of resting microglia, which could be due to spatial constraints, but also to a molecular and cellular micro-environment, different from that of neural parenchyma. These differences also extend to molecular markers, such as MHC2, cyclo-oxygenase or scavenger receptors, found on normal perivascular macrophages but not in resting microglia (Linnehan et al., 1999), and the paucity of the aMb2 integrin (Angelov et al., 1992).

The meningeal macrophages, a third group, are large and rounded cells located between meningeal epithelial cells and the basal membranes surrounding glia limitans, the astroglial lining encasing neural parenchyma. Immunohistochemically, meningeal macrophages are more closely related to perivascular macrophages, macrophages in chorio-epithelial and ventricular epithelial tissue and less to the highly ramified, resting microglia inside neural parenchyma. On the whole, the basal membranes of vessels meninges, ventricular and chorioepithelium, mark an anatomical border between two brain macrophage subpopulations: the ramified microglia inside the neural parenchyma that lacks intrinsic basal membranes, and perivascular, meningeal, ventricular or chorioepthelial cells sitting on the external side of the surrounding basal membranes.

Some publications also use the term "perivascular microglia", but the term is confusing, controversially defined, and frequently misleading. It is often used it as a synonym for rounded or process-poor perivascular macrophages (Linnehan et al., 1999; Stoll and Jander, 1999), even though these cells look very different from the ramified microglia. In others, it is used to denote microglia in the neural parenchyma with processes that contact blood vessels from the inside (Owens et al., 1998). Since each microglial cell covers a relatively large territory of well vascularized tissue, up to 50-70 μm in diameter, some contact is probably unavoidable, and labelling a microglial cell "perivascular", like that shown in figure 1C can simply reflect a particularly prominent process attached to a vessel co-stained by the same molecular marker.

Despite this clear anatomical partition, between microglia and macrophages, there is clearly at least some exchange between and plasticity in the individual compartments. Perivascular macrophages are gradually replenished by a pool of circulating monocytes, with a half-life of 1-2 months. A small population of macrophages migrate through basal membrane into neural parenchyma, to differentiate into ramified microglia, a process enhanced in different forms of neuropathology (Streit et al., 1989; Priller et al., 2001), including EAE (Fluegel et al., 2001). Resident microglia in the adult brain are themselves descendents of 2 waves of macrophage infiltration into neural parenchyma – a very early one, from the surrounding mesodermal tissue (Navascues et al., 1995; Cossmann et al., 1997; Kurz and Christ, 1998), then as a second wave, as "fountains of microglia" in CNS white matter during axonal pruning in the late fetus/newborn (Rio-Hortega cf Brockhaus et al., 1996), but this is followed by rapid differentiation,

arborisation and quiescence, turning into the resting, ramified phenotype. However, the same resident microglia can be rapidly activated by a host of different pathologies, lose branching and transform into amoeboid macrophages. Some debris-laden transformed microglia also appear to migrate from neural parenchyma into the perivascular, Robin-Virchow space, where they can stay for a very long time (Kosel et al., 1997), which could set the scene for an interaction with T-cells homing onto perivascular macrophages (Walter et al., 2001).

2. CELLULAR AND MOLECULAR RESPONSE IN EAE AND MS

The rapid recruitment of blood-borne monocytes, the activation of resident microglia and perivascular macrophages, together with the recruitment of T-cells, are among the most consistent changes observed in multiple sclerosis and its autoimmune animal models of experimental allergic encephalomyelitis (McCombe et al., 1994; Bruck et al., 1995; Ford et al., 1995; Li et al., 1996). Microglia display strong proliferative activity, particularly at the early active sites of demyelination (Matsumoto et al., 1992, Schonrock et al., 1998), and avid upregulation in their mitogen receptors, that is tuned down in later stages of the disease (Hulkower et al., 1993; Werner et al., 2002). Compared to neighbouring T-cells, microglia/macrophages show relatively little apoptosis (Nguyen et al., 1994; Smith et al., 1996; Bonetti et al., 1997) and much more proliferative activity (Ogmori et al., 1992). Interestingly, almost all of our knowledge of changes affecting brain microglia/macrophages in the human disease come from the post mortem analysis of the terminal stage, unlike the EAE models which allow to explore pathology at different phases of the disease - from the early preclinical stage, to onset of neurological symptoms, paralysis and remission, including the second and following bouts of the disease process in the relapsing forms of the EAE.

Recent increase in the use of diagnostic brain biopsies (Bruck et al., 1995; Lucchinetti et al., 2000), but particularly the introduction of the positron-emitting [^{11}C]-PK11195 in combination with positron emission tomography (PET) scanning has begun to change this situation. In brain tissue, the PK1195 binding site is highly selective for microglia and macrophages, it is rapidly activated in even in moderate forms of brain pathology (Stephenson et al., 1995; Banati et al., 1997) and can be used map the spatial pattern of microglial activation in multiple sclerosis (Banati et al., 1999; Cagnin et al., 2001). Compared with magnetic resonance imaging with or without Gadolinium, the PET-based technique shows a higher sensitivity with respect to identifying white mater regions at risk and provides a good

correlation with disease process and appearance of neurological deficit (Banati et al., 2000). These recent human data underscore the importance of brain macrophage activation as a diagnostic tool, to identify the localization and disease activity in multiple sclerosis.

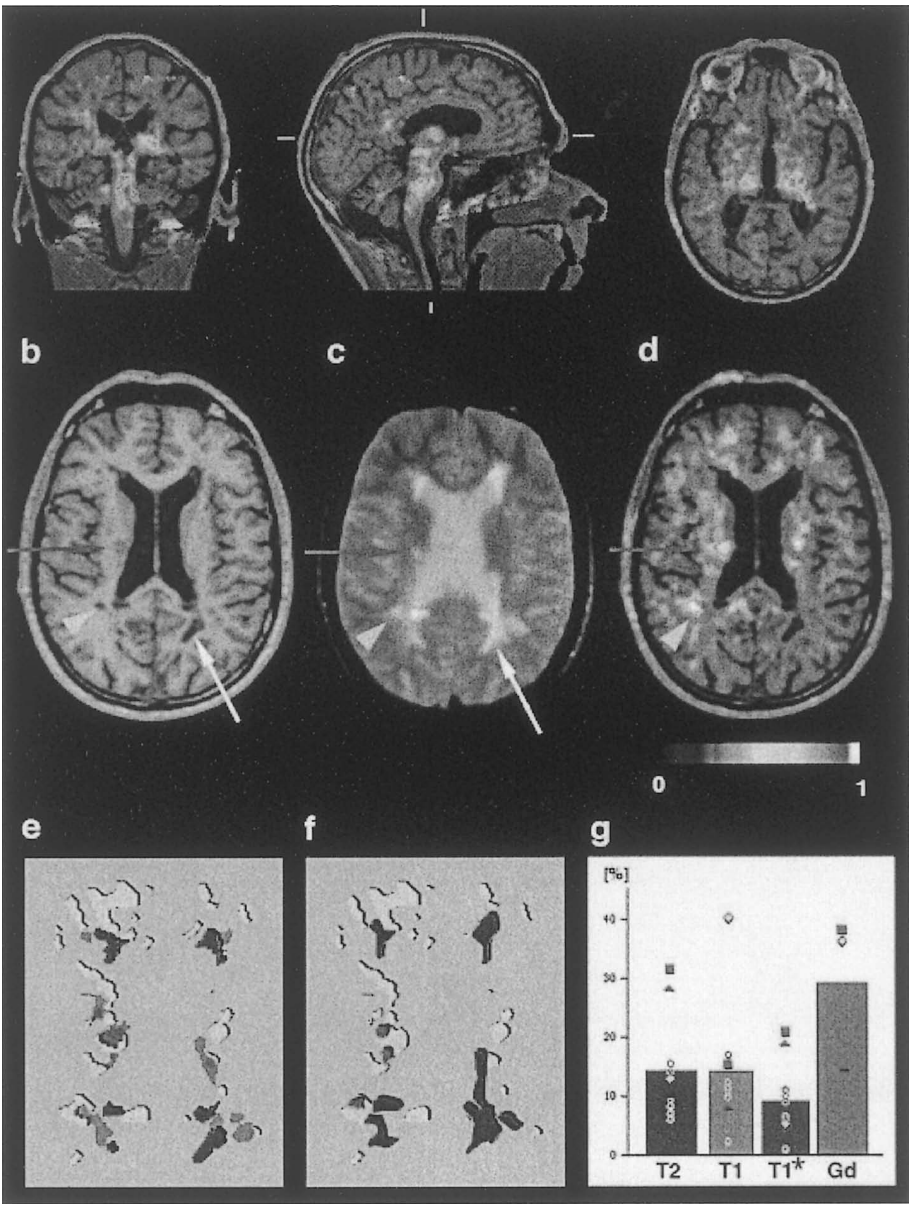

Figure 1. Detection of microglial activation in human patients with muliple sclerosis using positron emission tomography (PET) with [11C](R)-PK11195, combined with nuclear magnetic resonance imaging (MRI). All images follow the radiological convention, i.e. the left side of the image corresponds to subject's right side. (A) Three orthogonal views of

[11C](R)-PK11195 images co-registered and overlaid on the MRI of Patient 9, showing spinothalamic tract-associated [11C](R)-PK11195 signals extending through the brainstem and pons into the thalamus. (B-D) T1-weighted (B) and T2-weighted (C) MRI and [11C](R)-PK11195 PET (overlaid onto T1-weighted MRI) (D) of Patient 9 show lesions in all different spin-echo MRI sequences that partially overlap with areas of significantly increased [11C](R)-PK11195 binding (red arrow). The white arrow points to a `black hole' in an area that appears strongly hypointense in the T1-weighted MRI and has little binding of [11C](R)-PK11195. Note, however, that a similar black hole (yellow arrowhead) adjacent to the right occipital horn of the lateral ventricle shows significant [11C](R)-PK11195 binding. (E-F) Demonstration of the definition of the MRI lesion load masks in Patient 9 (purple, T1-weighted MRI lesions excluding black holes; blue, black hole only; green, gadolinium-enhancing areas; dark grey (in F), T2-weighted MRI lesions; red, areas of overlap between significantly increased [11C](R)-PK11195 binding and MRI-defined areas of pathology); yellow, areas of increased [11C](R)-PK11195 binding and no overlap with any MRI-defined pathology. (G) Average percentage volume of the MRI-defined lesions overlapping with increased [11C](R)-PK11195 binding. The red square represents Patient 8 and the red triangle Patient 6, who were both in relapse at the time of the scans. The yellow diamond represents Patient 9, who had secondary progressive multiple sclerosis. T1*, black holes. Reproduced from Banati et al., Brain. 123:2321-37, 2000.

On the biochemical level, macrophage and microglial activity in MS and EAE is associated with a strong upregulation of molecules involved in antigen presentation, myelin and tissue breakdown, production of reactive oxygen substances. They also synthesize components of the complement cascade, cytokines, growth factors and neurotrophins, chemotactic molecules, excitotoxins and apoptosis-inducing substances, and their receptors. These molecules, reviewed in the following paragraphs, as well as in more detail in the preceding and following chapters of this book, are involved in inducing and regulating the level of macrophage activation, interaction with encephalitogenic lymphocytes, mediating damage to myelin, axons and oligodendrocytes, as well as inducing the repair of the injured white matter.

ANTIGEN PRESENTATION

Major Histocompatibility Complex. T cells are known to recognize their specific antigen when associated to the class I or class II molecules of the major histocompatibility complex, abbreviated as MHC1 and MHC2 (for a review see Zinkernagel and Doherty, 1997). This recognition is aided by the binding of T-cell accessory molecules CD4 and CD8, expressed by the T-helper (mainly CD4+) and the T-suppressor/cytotoxic (mainly CD8+) lymphocytes, to their respective MHC2 or MHC1 ligands (Fleury et al., 1991; Miceli and Parnes, 1991). Despite the strong MHC class-selectiveness in the presentation of specific antigen – MHC1 for the endogenous (cytoplasmic) and MHC2 for the exogenous (phagocytosed) antigen, recent studies point to the existence of alternative and highly effective pathways for the presentation of exogenous antigen via MHC1 (Reimann et al., 1994;

Larsson et al., 2001). In addition, non-classical MHC1-like molecules such as CD1 can also present antigen, particularly glycolipids, to the CD8+ as well as to the CD4-CD8- (double null) T-lymphocytes (Sugita et al., 1998).

The rapid upregulation in the major histocompatibility complex antigens were amongst the first set of molecular changes described in multiple sclerosis as well as in different forms of experimental allergic encephalomyelitis using immunohistochemical techniques. Here, the upregulation of MHC2 was clearly restricted to reactive microglia, macrophages and blood-borne leukocytes, neighbouring GFAP+ astrocytes were MHC2 negative (Konno et al., 1989; Boyle and McGeer, 1990). Unlike MHC2, CD1 expression was not found on parenchymal macrophages or microglia, but rather on perivascular leukocytes and particularly, on the GMCSF+, hypertrophic astrocytes surrounding MS plaques (Battistini et al., 1996). Interestingly, the absence of overlap between MHC2 and CD1b suggests a high level of cell type selectivity in the presentation of MHC2 and CD1b-dependent antigens (Cipriani et al., 2003).

Up to now, most reports on antigen presentation and EAE have concentrated on MHC2, leading to the common assumption that only CD4+ lymphocytes are encephalitogenic. However, studies using beta2-microglobulin-deficient mice do show that MHC1 is involved in mediating EAE elicited by adoptively transferred, encephalitogenic CD8+ T-cells (Sun et al., 2001). This effect is direct and not mediated by some indirectly stimulated host CD4+ lymphocytes, a point demonstrated using the RAG1-/, immunodeficient mice (Sun et al., 2001). In the majority of cases, antigen presentation by CD1+ cells appears to inhibit EAE (Lider et al., 2001; Miyamoto et al., 2001), although this depends on the cytokine requirements of the specific form of EAE (Jahng et al., 2001). In the case of MHC2, most initial reports tended to emphasize that microglial expression of MHC2 may present antigen to encephalitogenic T-cells, needed to initiate or promote the inflammatory and demyelinating process (Hayes et al., 1987; McGeer et al., 1988). More recent studies have focused on their counter-regulatory and immunosuppressive properties. EAE-inducing effects are now attributed to perivascular macrophages.

Studies by Hickey and Kimura using bone marrow chimaeras clearly show that MHC2 expression by perivascular macrophages is sufficient for antigen presentation and onset of severe EAE, following the transfer of encephalitogenic CD4+ T-cells (Hickey and Kimura, 1988). On the other hand, high level of microglial MHC2 corresponds with resistance to EAE in different strains of rats (Sedgwick et al., 1993; Klyushnenkova et al., 1997). Exposure to microglial MHC2 also appears to induce T cell apoptosis, unlike the perivascular macrophages that promote T-cell survival (Ford et al., 1996; Klyushenkova et al., 1997). Presentation of antigen to non-encephalitogenic T-cells appears to play an important part in preventing excessive autoimmunity. Thus, adoptive transfer of encephalitogenic T-cells in mice

with severe combined autoimmunity (scid) leads to a much more severe and recurrent form of EAE compared with immunocompetent mice (Jones et al., 1999). The particularly strong and profuse upregulation of MHC2 on parenchymal microglia during early remission (Konno et al., 1989; McCombe et al., 1992) thus appears to make an important contribution in strengthening the immunosuppressive action of microglial MHC2.

Accessory Molecules. In addition to processed antigen embedded in MHC, effective antigen presentation requires the presence of co-stimulatory or accessory molecules on the surface of the antigen presenting cell, engaging their receptor counterparts on the T-lymphocyte. These accessory molecules belong to several different families of cell surface glycoproteins including B7, CD40, ICAM1-3 and the aXb2 integrin. aXb2 is a cell type-specific marker of professional, antigen-presenting cells (APC) also known as dendritic cells (Brocker et al., 1997; Suter et al., 2000).

MS is associated with a strong upregulation of CD40 (Gerritse et al., 1996; Laman et al., 1998; Weinberg et al., 1999) and B7.1 on the perivascular macrophages and microglia (Williams et al., 1994; De Simone et al., 1995). Inhibition or neutralization of CD40 and B7.1 prevented induction of EAE (Kuchroo et al., 1995; Gerritse et al., 1995; Weinberg et al., 1999; Becher et al., 2001). In the case of B7.1 this effect depended on the presence of IL4 (Kuchroo et al., 1995). Resting microglia already express moderate levels of B7.2 (Dangond et al., 1997), and its inhibition may enhance the severity of EAE (Kuchroo et al., 1995). Interestingly, combined inactivation of B7.1 and B7.2 strongly reduced the pathology and severity of neurological symptoms in adoptively transferred EAE (Chang et al., 1999). Both systems, CD40 and B7, appear to complement each other. Combined inactivation of the B7 receptor CD28, and inhibition of CD40 leads to a particularly strong resistance to the induction of EAE (Grivin et al., 2002).

Activated and phagocytic brain-resident microglia do express co-stimulatory molecules such as ICAM1-3, aXb2 or B7.2 in a variety of pathological conditions (Bo et al., 1996; Werner et al., 1998, Bohatschek et al., 1999; Kloss et al., 1999). However, most studies concur that the majority of the accessory molecule-positive cells in MS and EAE that present antigen to T-lymphocytes are hematogenous in origin and concentrated in the perivascular infiltrates (Williams et al., 1994; De Simone et al., 1995; Gerritse et al., 1996; Laman et al., 1998; Weinberg et al., 1999). Adoptive transfer of encephalitogenic T-cells strongly enhances the influx of bone marrow-precursors of dendritic cells to the site of CNS inflammation. Moreover, these newly recruited dendritic cells, with the appropriate MHC molecules, are fully sufficient to induce inflammation and myelin destruction in mice following adoptive transfer of rat bone marrow and rat encephalitogenic T-cells (Subramanian et al., 2001). This point is also

underscored by recent study using bone marrow chimaeras between the CD40 wild type (CD40+/+) and CD40 null (CD40-/-) animals, with the latter normally resistant to EAE (Becher et al., 2001). Replacement of the CD40+/+ bone marrow with that from a CD40-/- animal, turning perivascular macrophages and newly recruited dendritic cells to CD40-/-, while retaining CD40+/+ microglia, prevents the appearance of EAE in almost all animals. On the other hand, the transfer of CD40+/+ bone marrow to the irradiated, CD40-/- host, causes an almost complete recovery of EAE susceptibility in the host, with just a minor delay (+20%) and reduction in the maximal severity of clinical symptoms (-25%), compared to normal, CD40+/+ animals receiving a CD40+/+ bone marrow transplant (Becher et al., 2001).

CYTOKINES

Perivascular cuff macrophages, parenchymal macrophages and microglia show a strong upregulation for a long list of inflammation-associated, soluble cytokines, including interleukin-1/IL1 (Bauer et al., 1993), interleukin-10/IL10 (Jander et al., 1998; Hulshoff et al., 2002), transforming growth factor beta-1 (Kiefer et al., 1998; De Groot et al., 1999), macrophage-colony stimulating factor/MCSF (Hulkower et al., 1993; Werner et al., 2002), granulocyte-macrophage colony-stimulating factor (GMCSF), interleukin-12 (IL12) and tumour necrosis factor-alpha/TNFa (Hulkower et al., 1993; Renno et al., 1995; Bitsch et al., 1998; Laman et al., 1998; Fischer and Reichmann, 2001). There is also an upregulation of related cell surface molecules such as FAS and FAS-ligand (FasL), which are members of the TNF superfamily (Ouallet et al., 1999). Interestingly, expression is frequently focused to specific and different subpopulations of macrophages and microglia (Bitsch et al., 2000; Juedes et al., 2000). For example, the aMb2-positive microglia/macrophages expressing the dendritic cell marker aXb2 integrin secrete high amounts of IL12, while those negative for aXb2 produce GMCSF and TNFa (Fischer and Reichmann, 2001).

A subpopulation of these macrophage-produced cytokines and related molecules, such as IL10, TGFb1, or FasL has been shown to inhibit or prevent EAE (Rott et al., 19994; Stevens et al., 1994; Zhu et al., 2002; see also Wyss-Coray et al., 1997). However, a majority have a strong disease-promoting activity (Waldburger et al., 1996; Taupin et al., 1997; Marusic et l., 2002). Neutralization of TNF-alpha (Selmaj et al., 1991; Korner et al., 1997), IL1 (Jacobs et al., 1991), IL12 (Leonard et al., 1995) with antibodies or soluble receptors suppresses EAE. Genetic deletion of IL1R1, GMCSF, TNFa or IL12p40 has a similar effect, conferring resistance to EAE (Schiffenbauer et al., 2000; McQualter et al., 2001; Matejuk et al., 2002; Murphy et al., 2002; Gran et al., 2002; see however Liu et al., 1998).

Moreover, studies using bone marrow chimaeras between TNFa+/+ and TNFa-/- mice show that it is the TNFa which is produced by blood-borne leukocytes that plays a decisive role in the onset and severity of EAE (Murphy et al., 2002).

Importantly, moderate overexpression of TNFa or IL12, using the astrocyte GFAP promoter (Stalder et al., 1998; Pagenstecher et al., 2000) has been shown to lead to CNS inflammation and neurological disease. In the case of TNF, it causes overt demyelination and axonal damage very similar to that observed in EAE and MS. The fact that this demyelination is also observed in TNFa-overexpressing scid mice lacking T and B-cells (Stalder et al., 1998) strongly suggests that these cytokines not only promote the initial immune response, but also appear to play a crucial in the final steps following antigen recognition, that actually cause the brain pathology and neurological dysfunction.

CHEMOKINES

The activated macrophages and microglia produce a variety of chemotactic molecules including members of the chemokine family, but also many other chemoattractant factors such as secretoneurin (Storch et al., 1996), leukocyte chemotactic factor/LCF (Schluesener et al., 1996), endothelial-monocyte-activating polypeptide II (Schluesener et al., 1997) and chemotactic peptide-10/CP10 (Deininger et al., 1999). Amongst the chemokines, there is a strong increase in the macrophage chemotactic protein/MCP1 (Hulkower et al., 1993; Simpson et al., 1998, Jee et al., 2001), monocyte inflammatory protein 1 alpha/MIP1a (Balashov et al., 1999), neurotactin or fractalkine/CX3CL1 (Pan et al., 1997), TCA3 (Murphy et al., 2002), CCL19 and the macrophage-derived chemokine/CCL22 (Columba-Cabezas et al., 2002, 2003), which act as chemotactic ligands for macrophages, T-lymphocytes or both. There is also an upregulation for a list of macrophage chemokine receptors, such as CCR1, the receptor for MIP1a (Rottman et al., 2000; Trebst et al., 2001), CCR2, CCR3 and CCR5 (Simpson et al., 2000), CCR8 (Trebst et al., 2003), CXCR4 and CX3CR1 (Jiang et al., 1998), and the receptors for IL8 and N-formyl-Met-Leu-Phe/FMLP, particularly on foamy, phagocytic macrophages (Muller-Ladner et al., 1996).

Many of these macrophage-derived chemokines are produced by hematogenous macrophages, invading the inflamed CNS (Miyagishi et al., 1997; Sorensen et al., 1999; Matejuk et al., 2002) and are controlled by inflammation–associated cytokines such as TNF (Matejuk et al., 2002; Murphy et al., 2002). Studies using genetically deficient animals and/or neutralizing antibodies also show that the chemoattractive molecules play an important role in the pathology of EAE. Transgenic deletion of CCR1, the receptor for MIP1a, CCR2, the receptor for MCP1, or CCR8, the receptor

for TCA3, strongly reduce the susceptibility, onset and severity of EAE (Rottman et al., 2000; Huang et al., 2001; Murphy et al., 2002). A similar inhibitory effect is also observed with the antibody inactivation of MCP1 (Karpus et al., 1995). That these molecules are produced in the brain has suggested that brain-resident cells, particularly microglia, may also play a role in the induction of EAE (Murphy et al., 2002). However, this point is contentious and needs to be confirmed, using bone marrow chimaeras. The fact that many of these chemokines and chemokine receptors are located on blood-borne macrophages, particularly in perivascular cuffs, could argue against a major contribution by microglia.

REACTIVE OXYGEN SPECIES AND SIGNALLING ENZYMES

Acute inflammatory diseases such as EAE and multiple sclerosis are associated with strongly augmented production of reactive oxygen species (ROS), particularly by activated brain macrophages (Ruuls et al., 1995). Brain macrophages show increased deposition of iron (LeVine, 1997), myeloperoxidase (Nagra et al., 1997) and inducible NO synthase/iNOS in multiple sclerosis (Bagasra et al., 1995; De Groot et al., 1997) and in EAE (Van Dam et al., 1995; Tran et al., 1997). Similar upregulation is also observed in viral models of CNS demyelination, e.g. with Theiler's murine encephalomyelitis virus (Oleszak et al., 1997). In combination with peroxide radicals the synthesis of NO will lead to the formation of peroxynitrite, which is toxic for oligodendrocytes (Mitrosic et al., 1996). In vitro, oxidative stress causes macrophages to become toxic (Bartnik et al., 2000), and scavengers of ROS or their precursors such as uric acid or catalase are known to inhibit EAE (Ruuls et al., 1995; Kean et al., 2000).

Nonetheless, brain macrophages also produce a string of molecules that reduce oxidative stress. Perivascular macrophages, and to lesser extent microglia, show high levels manganese superoxide dismutase in EAE (Qi et al., 1997). Expression of metallothioneins 1&2/MT1&2 (Espejo et al., 2001) reduces the high susceptibility to EAE, shown in the MT1&2-/- mice (Penkowa et al., 2001). Multiple sclerosis and EAE also cause increased macrophage synthesis of stress protein heme oxygenase-1 (HO1) that produces CO (Emerson and LeVine, 2000; Schluesener and Seid, 2000), the inducer of cGMP-synthesizing enzyme guanyl cyclase (Brune and Ullrich, 1987). This increased HO1 degrades the pro-oxidant heme groups, but also reduces the availability of the NADPH cytochrome P450 reductase that is needed for the production of superoxide (Emerson and LeVine, 2000). Inducers of HO1 such as hemin, reduce the severity, and HO1 inhibitors enhance the severity of EAE (Liu et al., 2001).

Macrophage iNOS and NO synthesis and their effects in EAE have been a focus or particular attention. In most cases, early pharmacological inhibition of nitric oxide synthase has been shown to reduce EAE (Cross et

al., 1994; Brenner et al., see however Ruuls et al., 1996). Late application interferes with the recovery process and enhances relapsing activity (Okuda et al., 1998; O'Brien et al., 2001). Interestingly, NO production by brain microglia and macrophages strongly inhibits T-cell proliferation (Juedes and Ruddle, 2001).

The importance of this point was illustrated by studies in the interferon gamma receptor deficient (IFNgR-/-) mice, that are normally unable to recover following the induction of EAE, and die from severe demyelinating illness (Willenborg et al., 1999). In vitro analysis showed that supernatants from IFNgR+/+ macrophages inhibit the proliferation of encephalitogenic T-cells while IFNgR-/- macrophages lack this ability. Moreover, the inhibitory effects of IFNgR+/+ macrophages could be suppressed by an inhibitor of iNOS, underscoring the importance of NO in regulating the T-cell response. Interestingly, bone marrow chimaeras between IFNgR+/+ and IFNgR-/- show that the presence of IFNg receptors on just one, blood-borne or brain resident component, was sufficient to prevent the normally lethal outcome following the induction of EAE in IFNgR-/- mice (Willenborg et al., 1999).

FUNCTIONAL ROLE

Both brain–derived microglia and blood borne macrophages are crucially involved in many consecutive stages of autoimmune demyelination in experimental allergic encephalomyelitis and multiple sclerosis. They play an important role as antigen-presenting cells in the initial demonstration of antigen (Hickey and Kimura, 1988; Jones et al., 1999), and secondary recruitment of T-cells, granulocytes and macrophages (Huitinga et al., 1995). They also produce a long list of potentially damaging substances, including reactive oxygen species, NO and peroxynitrite, TNFa, interleukin-1b and excitotoxins (see above). As a case in point, interleukin 1b has been shown to activate mixed glial cell cultures to produce glutamate agonist neurotoxins that cause oligodendroglial cell death (Takahashi et al., 2003). MS-associated inflammation leads to a strong increase in glutamate receptors on axons in the centre of CNS lesions and on neighboring, reactive astrocytes (Geurts et al., 2003), which could predispose them to damage by the glial-derived excitotoxins. Axonal damage is particularly intense at early disease stages of MS (Kuhlmann et al., 2002), which could correspond to particularly intense microglial activation (Bitsch et al., 2002). Microglia and macrophages are also the chief debris-removing cells that eliminate damaged myelin, resulting in the widespread loss of axonal covering the CNS white matter. Last, but not least, microglia and macrophages appear to play a decisive role in the induction of remission, as well as resistance to the induction of the disease (Konno et al., 1989; McCombe et al., 1992; Willenborg et al., 1999).

A key question raised with the respect to microglia and macrophages is the relative contribution of blood-derived versus brain resident cell populations to the overall pathology during the process of autoimmune destruction of myelin. This question is particularly appropriate, since activated, and particularly phagocytic microglia share most of molecular markers with the blood-borne macrophages that enter the damaged brain (Streit et al., 1988; Bruck et al., 1995; Raivich et al., 1999). Most studies concur that blood-derived macrophages are crucial in the induction of EAE. Removal of bone marrow monocyte precursors and circulating macrophages with clodronate reduces the parenchymal influx of new macrophages, interferes with lymphocyte recruitment and microglial proliferation (Bauer et al., 1995; Plofriet et al., 2002). The same macrophage depleting treatment also blocks adoptively transferred EAE (Huitinga et al., 1995; Tran et al., 1998). Similar, disease-abolishing results were also obtained in bone marrow chimaeras, when bone marrow-derived macrophages (but not microglia) lacked the appropriate MHC antigens, accessory molecules (CD40) or cytokines such as TNFa that promote demyelinating disease (Hickey and Kimura, 1988; Stalder et al., 1998; Subramanian et al., 2001; Becher et al., 2001; Murphy et al., 2002), underscoring the significance of blood derived macrophages.

Nonetheless, there are several lines of evidence that begin to shed light on the importance of brain resident microglia at different stages of the demyelinating disease. The initiation of the autoimmune response is an important case in point. For example, the acute transfer of rat encephalitogenic T-lymphocytes and appropriate antigen presenting cells (APC) to scid mice causes a delay phase of approximately 8 days before the onset of EAE (Subramanian et al., 2001), pointing to the importance of local APC in the early initial stages. This point is underscored by the very rapid microglial response to the adoptive transfer of EAE, in this case the induction of microglial amyloid precursor protein, within 24 hours after the infusion of encephalitogenic T-cells in animals with the appropriate MHC antigens (Banati et al., 1995). Studies using bone marrow chimaeras also show that microglia are much more effective removers of myelin than blood-derived macrophages (Rinner et al., 1995). Finally, brain-resident microglia also play a decisive role in limiting the extent of demyelination, preventing lethal outcome and inducing disease remission (Konno et al., 1989; McCombe et al., 1992; Willenborg et al., 1999). Here, new insights into the function and molecular signals of macrophages and microglia, their interaction with each other, as well as with T-lymphocytes, axons and myelin-producing oligodendroglia, could pave the way to introducing new and more effective therapies to the human demyelinating disease.

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