Central Nervous System Diseases and Inflammation

Thomas E. Lane • Monica Carson
Conni Bergmann • Tony Wyss-Coray
Editors

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Thomas E. Lane
Center for Immunology
Department of Molecular
Biology & Biochemistry
University of California
Irvine, CA
USA
tlane@uci.edu

Monica Carson
Department of Biomedical Sciences
University of California
Riverside, CA
USA
monica.carson@ucr.edu

Conni Bergmann
USC Keck School of Medicine Neurology
and Pathology
University of Southern California
Los Angeles, CA
USA
cbergman@usc.edu

Tony Wyss-Coray
School of Medicine
Department of Neurology and
Stanford University School of Medicine
Stanford, CA
USA
twc@stanford.edu

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Preface

Up until approximately 20 years ago, the idea that the central nervous system (CNS) and components of the immune system were dynamically interactive was considered impossible (or at least highly unlikely) as the CNS was judged an immunosuppressive environment based upon experimental evidence highlighting the survival of tissue grafts within the brain. Additional evidence supporting this viewpoint included (i) the presence of the blood–brain barrier (BBB) which provides a physical and physiological obstruction that is difficult for cells and macromolecules to cross, (ii) the relative absence of MHC class I and II expression on CNS cells like astrocytes and neurons, and (iii) lack of abundant antigen presenting cells (APC) which are required for the generation of an adaptive immune response. However, in spite of these obstacles, it is now well-accepted that the CNS is routinely subject to immune surveillance under both normal as well as diseased conditions. Indeed, activated cells of the immune system such as T and B lymphocytes and monocyte/macrophages readily infiltrate and accumulate within the CNS following microbial infection, injury, or upon development of autoimmune responses directed toward resident antigens of the CNS.

The importance of studying events surrounding the initiation and maintenance of neuroinflammation is now recognized by scientists and clinicians alike as critical not only in characterizing the complex mechanisms associated with host defense following infection but also in contributing to neurologic disease. Much of our understanding of neuroinflammation has been derived from numerous animal studies of neurologic disease including autoimmune models of demyelination such as experimental autoimmune encephalomyelitis (EAE) and transgenic mice, microbial e.g. virus and bacteria infections, spinal cord injuries in mice and rats, and mouse models of Alzheimer's to highlight just a few. Moreover, the underlying molecular and cellular mechanisms governing inflammation are just now being understood and the importance of chemokines and chemokine receptors in recruiting targeted populations of leukocytes into the CNS is now appreciated. In addition, resident cells of the CNS e.g. microglia are recognized as important mediators in regulating innate defense mechanisms as well as disease.

This volume highlights important advances in our understanding of different aspects of neuroinflammation with a concentration on specific areas focusing on

glial activation, molecular signals regulating inflammation and neurotoxicity, immune responses concentrating within the CNS, and the emergence of transgenic models of neurologic disease. It was the goal of the editors to provide timely and insightful comments on these particular aspects of neuroinflammation and disease while recognizing that it was impossible to adequately address other equally important and relevant aspects of neuroinflammation not covered but certainly deserving of attention. In addition, the editors feel this text will be useful for researchers, clinicians, as well as a valuable resource for students interested in the fascinating arena of neuroinflammation.

Thomas E. Lane
Irvine, CA
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Contents

Microglia: A CNS-Specific Tissue Macrophage

Shweta S. Puntambekar, Jonathan M. Doose, and Monica J. Carson

1 Introduction

The central nervous system (CNS) is a complex integrated organ comprised of:

1. Neurons (10% of total number of CNS cells and 50% of total cell mass).
2. Glia (90% of the total cell number but only 50% of the total mass of the CNS) (Simons and Trajkovic, 2006; Verkhratsky and Toescu, 2006; He and Sun, 2007).

Glia can be further divided into macroglia (oligodendrocytes and astrocytes) which are of neuroectodermal origin and microglia which are of mesenchymal origin. While neurons and macroglia are endogenous cells of the CNS, microglia and/or their progenitors appear to invade CNS tissue very early during embryonic development (Carson and Sutcliffe, 1999; Carson et al., 2004, 2006).

The primary function of all glia is to maintain the optimal operation of the CNS information circuit (the neuronal network). This involves: active regulation of the network's operations, ongoing maintenance to deal with normal wear and tear as well as active defense and repair following injury or pathogen attack (Carson and Sutcliffe, 1999; Carson et al., 2004, 2006). Neurons, macroglia and microglia all coordinately and dynamically participate in these processes. All of these cells also interact with CNS-infiltrating immune cells as part of their regulation of inflammatory responses in the CNS. Until recently, the importance of microglia in homeostatic CNS function was not fully recognized.

In this chapter, we will explore the experimental basis of the many suggested beneficial versus detrimental functions of microglia in both the healthy and injured/diseased CNS. We will also explore to what extent aberrant microglial function is due to primary microglial dysfunction versus primary dysfunctions in neurons and glia.

2 What are Microglia?

Microglia express most common macrophage markers and are often referred to as the tissue macrophage of the brain. In the healthy brain, microglia have a stellate morphology and are found in all areas of the brain and spinal cord (Fig. 1.1). As commonly

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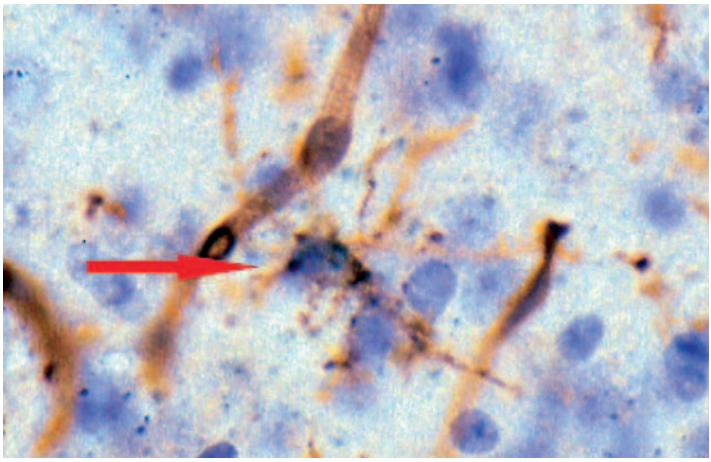

Fig. 1.1 A typical parenchymal microglia extending processes to all elements of its environment. Microglia and blood vessels are visualized in brown using tomato lectin. Nuclei are visualized in blue using hematoxylin (*See Color Plates*).

used in the literature, the term microglia has been applied to at least three different types of myeloid cells: parenchymal microglia, perivascular microglia and acutely blood-derived inflammatory macrophages that display a stellate morphology within the CNS. In this chapter, we will operationally define microglia as parenchymal cells that are largely from either a self-renewing population or only rarely replenished from adult bone marrow derived cells. Perivascular cells and myeloid cells which are acutely derived from the blood and which have the demonstrated potential to emigrate from the CNS shortly after entry (period of days to weeks), we will refer to as perivascular macrophages and CNS-infiltrating macrophages.

As yet there are no reagents able to distinguish acutely infiltrating macrophages from CNS-resident microglia in histological preparations. However, parenchymal microglia are unique in the adult in that they continue to express the very low levels of CD45 normally expressed during embryonic development of the hematopoetic system (Sedgwick et al., 1991; Ford et al., 1995; Carson et al., 1998). In the adult, all other nucleated differentiated macrophages and immune cells express high levels of CD45. While this is a useful biomarker to purify and separate CNS-resident microglia from CNS-infiltrating macrophages, it also indicates that these two populations have distinct functions. CD45 is an inhibitory receptor for CD22 (Mott et al., 2004; Han et al., 2005). While this CD45 ligand was long recognized as being expressed by B cells, Tan and colleagues have recently demonstrated than CNS neurons secrete a soluble form of CD22 from axonal terminals (Mott et al., 2004). Functionally, these authors also found that this was the mechanism by which neurons in culture were able to inhibit LPS-induced TNF-alpha production by microglia. Interestingly, the differential expression between microglia and macrophages suggests that CNS neurons may be more effective at inhibiting the functions of macrophages than those of microglia!

To date, most research on microglial function has focused on their roles during injury, pathogen infection or chronic neurodegeneration (Bechmann et al., 2001, 2005). In part, this focus is a consequence of the dramatic and rapid changes in microglial morphology and gene expression observed immediately following CNS injury or infection. Conversely, microglia in the healthy CNS have often been presumed to be quiescent and largely inactive. However, important non-defense oriented functions of microglia are suggested by the human disease referred to as Nasu–Hakola disease (Paloneva et al., 2000, 2001; Cella et al., 2003).

Nasu–Hakola disease is a genetic disorder leading to bone spurs, early onset cognitive dementia in the 1920s and death in the late 1930s. Positional cloning identified mutations in the TREM-2 pathway as the genetic cause of the disease (Paloneva et al., 2000, 2001; Cella et al., 2003). A primary neuronal defect was long speculated to be the cause of this neurological disorder due to the early onset of cognitive symptoms. However, we found that in the murine CNS TREM-2 RNA could only be detected in microglia (Schmid et al., 2002)! These data, in conjunction with similar findings from other groups illustrate that a primary disease of microglia has the potential to lead to a disease with primary psychological manifestations (not just primary inflammatory or autoimmune manifestations!) (Bouchon et al., 2001; Schmid et al., 2002; Daws et al., 2003; Melchior et al., 2006). As yet, it is unclear what functions are dysregulated in microglia with a dysfunctional TREM-2 pathway or how microglial dysfunction would lead to cognitive dementia after two decades of life.

3 What Do Microglia Do in the Normal CNS?

Recently, a variety of relatively non-invasive techniques, have been used to explore microglial function in the healthy CNS and to quantify changes in cellular activity upon acute injury(Davalos et al., 2005; Nimmerjahn et al., 2005). Using two-photon imaging of fluorescently labeled cells, Nimmerhan et al (2005) and Davalos et al. (2005) have monitored the extension and motility of microglia and their processes before and after introduction of a focal injury. In the healthy CNS, microglial cells had small, rod shaped cell bodies with many thin and highly ramified processes symmetrical extending from the cell body. Using time-lapse imaging, they observed that while microglial cell bodies remained relatively fixed, their processes were remarkably motile. The processes underwent continuous cycles of de novo formation and withdrawal, apparently surveying all elements of the CNS every 6 h!

As part of this analysis, the authors also noted that not all microglial processes were highly motile(Nimmerjahn et al., 2005). A subset of microglial processes provided a stable scaffold, perhaps anchoring the microglia in place. These data suggest that the branching of microglial processes may not be random and may serve to integrate homeostatic signals throughout the entire CNS. It is tempting to speculate that this may be a mechanism by which microglia help modulate the extensive network of neuronal synapses and functional plasticity of the healthy CNS.

Microglia are the resident immunocompetent cell of the CNS are provide the first line of defense in response to pathogens and neuronal injury. As such they can produce a wide variety of cytokines, proteases, reactive oxygen species as well regulate CNS-infiltrating T cells in an antigen-specific manner. Nimmerhan et al proposed that the constant microglial surveying of the brain is a necessary consequence of their defense functions (Nimmerjahn et al., 2005). Using a targeted disruption of the blood brain barrier (BBB), the authors demonstrated that microglial responses to injury were rapid (within minutes), spatially directed to the focal injury and not dependent on the presence of pathogenic molecules. Astrocytes are also an important neuronal support cell, playing a key role in CNS immune responses as well as in glutamate uptake in response to neuronal activity(Parpura et al., 2004; Volterra and Steinhauser, 2004). Somewhat surprisingly, these authors found that the basal motility of microglial processes was much higher than those of astrocytes (Nimmerjahn et al., 2005). In addition, while microglial processes rapidly extended toward an acute focal injury, astrocytic processes did not. These data provide a dramatic demonstration of the cell-type specific support provided by microglia and astrocytes.

4 What are Microglia Monitoring in the CNS?

Microglia are known to recognize pathogens using evolutionarily conserved pathogen recognition receptors, such as the Toll-like receptors (TLRs) (Lee and Lee, 2002; Schiller et al., 2006). However, the obvious question is how do microglia recognize changes in CNS function and neuronal health? And can they directly detect neuronal activity? Over 10 years ago, Neumann and colleagues demonstrated that blocking neuronal activity in slice cultures with the sodium channel blocker tetrodotoxin resulted in rapid microglial activation (Neumann et al., 1996, 1998). More recently, Nimmerjhan et al reported that application of the GABA receptor blocker bicucullin (resulting in an upregulation the neuronal activity) dramatically increased the region being sampled by microglia in the otherwise uninjured CNS (Nimmerjahn et al., 2005). How directly microglia detect neuronal activity is still a subject of debate. However, microglia do express inward rectifying potassium channels as well as receptors for many of the CNS neurotransmitters (Kettenmann et al., 1990, 1993; Schmidtmayer et al., 1994; Chung et al., 1999; Schilling et al., 2000).

Neuman and his colleagues found in their studies that microglia were responding in part to the neurotrophins being secreted by the neurons in these cultures (Neumann et al., 1996, 1998). Since then several neuronally expressed cues have been identified, including CD200, fractalkine, polyamines, CCL21 and ATP (reviewed in Carson et al., 2006, Melchior et al., 2006). Interestingly, these cues can be divided into those that are expressed by healthy neurons and that suppress pro-inflammatory microglial responses, and those that are expressed by damaged and/or dying neurons. These latter cues by in large augment the pro-inflammatory responses of microglia.

A dramatic of demonstration neuronally directed regulation is provided by the studies of Cardona et al. examining the function of fractalkine (Cardona et al., 2006). Fractalkine is a chemokine expressed as a transmembrane glycoprotein. It can be proteolytically cleaved from the membrane to generate a soluble fragment retaining the ability to bind its receptor (Cook et al., 2001). Within the CNS the expression of the fractalkine receptor (CXCCR1) is restricted to Iba1-positive cells (microglia and perivascular macrophages). Neurons (NeuN + cells), oligodendrocytes (NG2 + cell), and astrocytes (GFAP + cells) do not express CXCCR1 nor is the CXCCR1 promoter active in these cells (Cardona et al., 2006).

By comparing microglial responses in $Cx3cr1^{+/-}$ or $Cx3cr1^{-/-}$ mice, Cardona et al. were able to demonstrate the dual role of fractalkine to limit microglial responses and recruit microglia to the site of injury (Cardona et al., 2006). Systemic injection of LPS in mice lacking the fractalkine receptor resulted in a large increase in hippocampal neuronal cell death (Cardona et al., 2006). To prove that this was a consequence of dysregulated microglial function, microglia were isolated from the CNS of LPS-treated $Cx3cr1^{+/-}$ and $Cx3cr1^{-/-}$ mice. These cells were than transferred into the frontal cortex of wild-type littermates. Transferred wild-type microglia were highly motile and trafficked along white matter tracts throughout the wild-type recipient CNS. Strikingly, microglia from knockout mice failed to migrate and persisted at the injection site for at least 36 h following injection! Moreover, apoptotic neurons were observed at the site of injection only in animals injected with activated microglia lacking the fractalkine receptor (Cardona et al., 2006).

Cardona et al. (2006) identified IL-1B as playing a key role in the dysregulated responses in KO microglia. Coadministrating an IL1 receptor antagonist at the same time as the injection of KO microglia into wild-type mice significantly reduced the number of apoptotic neurons. Transfer of these microglia into the CNS of IL-1 receptor knock-out mice partially restored migration in $Cx3cr1^{-/-}$ microglia and completely prevented the previously observed neuronal apoptosis! Fractalkine is known to be a strong microglial chemoattractant. However, the restoration of migration suggests that fractalkine also serves to amplify microglial chemoattractant responses to other injury signals that are otherwise blocked by IL-1.

Cardona et al. (2006) further demonstrated that fractalkine regulates more than microglial responses to bacterial components such as LPS. They also examined microglial responses in the MPTP model of Parkinson's disease and in the transgenic $SOD1^{G93A}$ model of ALS. In both models, neuronal loss was much greater in $Cx3cr1^{-/-}$ mice than in $Cx3cr1^{+/-}$ mice.

5 Is it Important to Distinguish Between CNS-Resident Microglia and Acutely Infiltrating Macrophages?

Cardona et al. (2006) observed heightened cytokine responses to LPS in both peripheral macrophages (peritoneal macrophages) as well as in microglia from $Cx3cr1^{+/-}$ mice. Due to the experimental paradigm, they were able to specifically

examine the responses of microglia (the CD45low cells) separate from CNS-infiltrating macrophages (CD45high cells). In many studies, microglia and macrophages are grouped together in one population and their differential functions are not examined. However, over the last 10 years several studies have illustrated that these two populations are both molecularly and functionally distinct.

Initially, CNS-resident microglia have been distinguished from those that acutely infiltrate the CNS by their lower expression levels of many of the molecules required to interact with T cells (reviewed in Carson et al., 2006). Specifically, microglia tend to express lower levels of MHC and co-stimulatory molecules. As early as 1988, Hickey and Kimura (1988) demonstrated that antigen-specific interactions between CNS-microglia and myelin-specific T cell were not required to initiate or sustain destructive autoimmune responses during ***e***xperimentally induced ***a***utoimmune ***e***ncephalomyelitis (EAE), a rodent model of multiple sclerosis. This and several subsequent studies have definitively demonstrated that perivascular macrophages, CNS-infiltrating macrophages and/or dendritic cells are by themselves sufficient to trigger the onset and progression of EAE (Greter et al., 2005; McMahon et al., 2005). Conversely, antigen-specific interactions between CNS-resident microglia and myelin-specific T cells in the absence of peripheral antigen-presenting cells were by themselves insufficient trigger and sustain EAE. In part, this inability may be due to the failure of microglia to leave the CNS parenchyma at the same rates as perivascular macrophages or other CNS-infiltrating cells (Carson et al., 1999b).

6 Are Microglia Just an Incomplete or Redundant Macrophage Population?

From these types of studies, it may be tempting to refer to microglia as partial macrophages and to presume that they merely play redundant functions in CNS defense. Two studies, one using an EAE model and one using a facial axotomy model suggest otherwise.

In the first, Magnus and colleagues demonstrated that a B7 family member, B7 homologue-1 (also known as PD-L1) is abundantly expressed on the surface of CNS-resident microglia (Magnus et al., 2005). Microglial expression of PD-L1 is dramatically upregulated during the recovery phase of MOG- and PLP-forms of EAE and by direct treatment with IFNg. Several studies have revealed that PD-L1 acts in a negative feedback loop suppressing T-cell activation by decreasing IFNγ and IL-2 production and by down-regulating the expression ICOS, a T-cell activation marker (Magnus et al., 2005). PD-L1 knock-out mice developed more severe inflammation in the MOG-induced EAE (Latchman et al., 2004). Thus, while microglia may have comparatively weak APC function as compared to dendritic cells, their dialogue between peripheral immune cells and microglia through PD-L1 may not serve to amplify pro-inflammatory T cell responses. Rather, microglia may help to modulate local inflammation within the CNS by limiting the severity and

spread of pro-inflammatory T cell responses. Consistent with this conclusion is the observation that CNS-resident microglia produce much higher levels of molecules such as prostaglandins and NO that repress antigen-presentation and T cell activation than infiltrating peripheral immune cells (Carson et al., 1998, 1999a).

Using the facial axotomy model, Byram et al. (2004) have demonstrated an essential and non-redundant function of CNS-resident microglia. In this model, the facial motoneuron cell body resides within the CNS brainstem, while its axon transverses the skull to innervate the vibrissae in the face. Slicing the axon at the point it transverses the skull, causes the axon to withdraw and prevents it from subsequently regenerating and finding its natural target. Serpe et al. (1999) had previously shown that in this model, CD4 + T cells limits the rate of facial motoneuron cell death. In the study by Byram et al. (2004), the authors demonstrated that peripheral antigen-presenting cells (presumably macrophages and dendritic cells) were required to initiate a neuroprotective T cell response. While these cells clearly infiltrate the site of the facial motoneuron nucleus (FMN), they could not sustain the protective T cell response. CNS-resident cells (presumably microglia) were absolutely essential to either evoke or sustain the protective lymphocyte response!

7 Are CNS-Infiltrating Macrophages Always Bad for CNS Function?

In both the EAE and facial axotomy models just discussed, microglia express high levels of MCP-1/CCL2 (reviewed in Carson et al., 2006). Indeed, in many models of CNS injury and pathogen exposure, microglia are induced to express both MHC class II (a perquisite to present antigen to CD4 + T cells) and CCL2 (a potent macrophage chemoattractant). Since macrophages are highly effective producers of free radicals *AND* have demonstrated pro-inflammatory roles in EAE, can this be considered a beneficial response of microglia designed to maintain optimal CNS function? Two studies using murine models of amyloid/Alzheimer's disease (AD) pathology suggest that microglial production of CCL2 and thus microglial recruitment of macrophages to the CNS may be an essential mechanism to impede the rate of AD pathogenesis (Simard et al., 2006; Khoury et al., 2007).

Microglia and macrophages surround the amyloid plaques in both human AD tissue and in rodent models of AD. In the first study, Rivest and colleagues sought to identify the relative contribution of CNS-resident versus hematogenously derived macrophages in the cells surrounding the plaque (Simard et al., 2006). To this end, the authors generated bone marrow chimeric mice, in which the hematogenously derived macrophages express green-fluorescent protein (GFP) while CNS-resident microglia did not. Not unexpectedly, the authors found that early in the formation of amyloid plaques, peripheral macrophages were readily recruited into the CNS. Somewhat surprisingly, macrophage recruitment did not continue to increase with age and plaque deposition, rather the reverse. The authors subsequently illustrated that the peripheral macrophage population was more effective at phagocytosis than

the resident cells. From these data, the authors conclude that the late stage failure to recruit peripheral macrophages contributes to the progression of AD pathogenesis.

El Khoury et al. (2007) have recently confirmed and extended these studies. In this study, the authors studied amyloid responses in mice lacking the CCL2 receptor (ccr2 KO mice). They found that in ccr2 KO mice, fewer peripheral macrophages were recruited and that as a consequence amyloid pathogenesis developed much more rapidly, deposition within the vascular was much more severe and lethality occurred a much earlier ages than in mice expressing normal levels of ccr2. Interestingly, heterozygotes for the receptor expressed an intermediate phenotype, suggesting that the strength of the recruitment signal is carefully titrated in CNS immune responses!

8 Microglial Activation Gone Awry: Effects of Peripheral Infection and Aging!

In the AD studies just discussed, if may be that microglia are not effective in maintaining a sustained recruitment of macrophages. Recently, Cunningham and colleagues have presented a reciprocal problem in which peripheral inflammation may prime microglia to respond in an overly aggressive fashion to neuronal insults (Perry et al., 2002; Cunningham et al., 2005).

For this study, the authors chose to examine the effects of peripheral inflammation in a mouse model for transmissible spongiform encephalopathy prion disease (ME7) (Perry et al., 2002; Cunningham et al., 2005). In this ME7 model, mice are injected with symptomatic brain homogenates within the hippocampus and develop vacuolation, loss of hippocampal CA1 neurons and extracellular deposition of an insoluble protein (PrP^{Sc}) (Perry et al., 2002; Cunningham et al., 2005). However in contrast to many neurodegenerative models, microglial activation is atypical and mostly characterized by an overexpression of the anti-inflammatory cytokine, TGF-b (Cunningham et al., 2005).

ME7 mice were subsequently challenged with LPS, by either direct injection into the CNS or into the peritoneal cavity. Microglia in control prion brains (ME7 injected with saline) had a very similar appearance to those receiving prions and LPS intracerebrally (Cunningham et al., 2005) but expressed very different patterns of cytokine expression. Those challenged with LPS ICV expressed much higher levels of IL-1b and inducible nitric-oxide synthase (iNOS) (Cunningham et al., 2005). In addition, much higher numbers of neutrophils were found in the CNS of mice receiving LPS icv. Strikingly, the authors found that LPS injection intraperitoneally (ip) exacerbated the levels of IL-1b, COX-2, and TNF. Furthermore, neuronal cell death was doubled in ME7 mice receiving peripheral LPS injection. The precise mechanism underlying the observed exacerbation is as yet not fully defined. It is likely to be due to multiple mechanisms including vagal nerve stimulation from the spleen to the hypothalamus, induced BBB alterations and systemic increases in chemokine and cytokine levels.

9 So What Goes Right and What Goes Wrong with Microglial Activation?

Microglia are found in all mammalian brains and spinal cords. Conversely, there are no spontaneous animal models in which microglia are severely deficient or absent. These two facts suggest an evolutionarily conserved function. However, like all myeloid cells, microglia are highly plastic and are able to summate cues from all aspects of their environment (Fig. 1.2). Thus, at any point in time, the phenotype of an individual microglia is determined as a function of its environmental cues. This observation suggests two possible outcomes:

1. Microglial phenotypes are likely to be unstable and highly heterogeneous throughout the CNS and throughout the lifespan of the individual as a direct consequence of the many different local CNS microenvironments.
2. Dysfunctional microglial responses may be a direct consequence of dysfunctional neurons and macroglia.

Lastly, in contrast to most peripheral macrophage populations, CNS-resident microglia are relatively long-lived. Thus their dysfunction may have more long-lasting consequences than for other peripheral macrophage populations. Recently Sierra et al. have demonstrated that with age, microglial pro-inflammatory responses do become more robust (Sierra et al., 2007). As yet it remains unexplored if this is a primary dysfunction of the aged microglia or a consequence of the aging neuronal and macroglial population providing inappropriate regulatory cures! In the end, it is apparent that for most of us, for most of our lives, microglial activation is either a benign or a beneficial event. However, for therapeutic intervention, it is important to discern whether the dysfunction apparent in many chronic neurodegenerative diseases is due to inherent deficits in microglia or whether inappropriate microglial activation is a consequence of a dysfunctional CNS microenvironment!

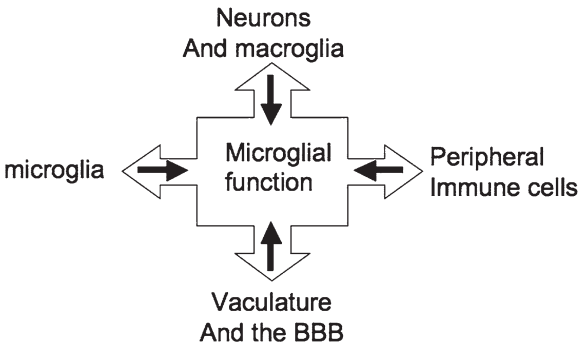

Fig. 1.2 Microglial phenotype and function are not stable and are determined by their interactions with their environment.

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Mechanisms of Microglial Activation by Amyloid Precursor Protein and its Proteolytic Fragments

S.A. Austin and C.K. Combs

1 Reactive Microglia are a Characteristic Histopathology of Alzheimer's Disease Brains

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease (Selkoe, 2005). Histologically, it is characterized by the deposition of extracellular senile plaques composed primarily of beta amyloid (Aβ) peptides and intracellular inclusions, termed neurofibrillary tangles, made up of primarily hyperphosphorylated tau protein (Braak and Braak, 1997a, b; Grundke-Iqbal et al., 1986; Selkoe, 2001). In addition, AD brains demonstrate significant neuron loss and abundant gliosis (McGeer et al., 1986). The mechanisms by which these pathology occur, however, is debatable. It has been hypothesized that inflammatory events contribute to both the histological and behavioral progression of disease (Akiyama et al., 2000). The histological data demonstrating gliotic changes in AD brains as compared to age-matched controls certainly supports the notion that microglia, in particular, may mediate the changes that are observed. Reactive microglia with swollen bodies and shortened, thickened processes are histologically identified in close association with the fibrillar or congophilic plaques in the AD brain (Itagaki et al., 1989; Miyazono et al., 1991). Although the percentage of microglia associated with fibrillar plaques is greater, they are also localized, in a more ramified phenotype, with the diffuse plaques (Itagaki et al., 1989; Mattiace et al., 1990; Sasaki et al., 1997). These data suggest that microglia develop a specific reactive phenotype in association with plaques as Aβ undergoes a transition from a nonfibrillar to fibrillar, congophilic conformation (Sheng et al., 1997). In fact, some studies suggest that microglia are involved in the earliest stages of plaque deposition perhaps even dictating where plaques are depositing in the brain (Griffin et al., 1995; Sheng et al., 1995, 1998). Moreover, AD brains have increased protein levels of several proinflammatory mediators commonly associated with reactive microgliosis, including cytokines: interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, activated complement components, and cyclooxygenase (COX)-2 when compared to controls (Akiyama et al., 2000; Dickson et al., 1993; Eikelenboom et al., 1989; Luterman et al., 2000; Mrak and Griffin, 2000; O'Banion et al., 1997; Strauss et al., 1992; Xiang et al., 2006;). Strikingly similar observations have been made while

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examining transgenic mouse models of disease over the last decade. The majority of the mouse models that have been created over-express human mutant forms of the amyloid precursor protein (APP) and/or mutant forms of the proteins responsible for gamma secretase cleavage of APP, presenilin (PS) 1 and PS2. These animal models have consistently demonstrated that reactive microgliosis occurs in association with fibrillar plaque formation as detected histologically with multiple immuno-markers (Morgan et al., 2005). Collectively, a voluminous body of data strengthens the proposition that APP and its proteolytic fragments are involved in not just plaque deposition but also the reactive microgliosis observed in AD brains.

2 Amyloid Precursor Protein and its Relationship to Alzheimer's Disease

APP is a ubiquitously expressed type I transmembrane protein that structurally resembles a cell surface receptor (Kang et al., 1987). The x-ray and crystal structure of the extracellular domain suggests that the protein can homodimerize in a cis (on the same cell surface) or trans (opposing cell surfaces) fashion (Rossjohn et al., 1999; Wang and Ha, 2004). Indeed, multimerization of APP occurs in neuronal cell lines basally (Scheuermann et al., 2001) and following ligand dependent stimulation (Lu et al., 2003). Furthermore, cross-linking APP with antibodies against the extracellular domain stimulates changes in intracellular signaling in vitro reminiscent of ligand dependent-receptor activation (Hashimoto et al., 2003; Okamoto et al., 1995). APP binds to extracellular matrix components including collagen (Beher et al., 1996) and laminin (Kibbey et al., 1993) as well as proteoglycans (Williamson et al., 1995) suggesting a role in mediating cell adhesion. A role as an adhesion receptor is further supported by the fact that APP levels increase on the neurite surface of differentiating neurons (Hung et al., 1992) and localize to points of focal adhesion in cell culture (Sabo et al., 2001). The short cytoplasmic tail contains a Y(682)ENPTY(687) (695 numbering) motif commonly employed by cell surface receptors as a docking site for SH2 and PTB domain containing proteins. Not surprisingly, several adaptor proteins including FE65, X11, JIP-1b and Shc have reported associations with this domain in a variety of different paradigms further supporting its role as a cell surface receptor (Borg et al., 1996; Bressler et al., 1996; Matsuda et al., 2001; Tarr et al., 2002).

As already mentioned, clinical interest in APP derives from the fact that its proteolytic processing leads to generation of the Aβ peptides that accumulate as extracellular plaques in AD brains (Masters et al., 1985). Moreover, a variety of APP missense mutations have been identified which result in a rare, autosomal dominant form of AD (Cai et al., 1993; Chartier-Harlin et al., 1991; Hendriks et al., 1992; Mullan et al., 1992; Murrell et al., 1991). The best characterized consequence of these mutations is an alteration in proteolytic processing of APP leading to elevated secretion of the longer Aβ peptide, amino acids 1–42 (Citron et al., 1992, 1994; Scheuner et al., 1996; Suzuki et al., 1994;). This peptide forms the fibrillar core of the amyloid plaques in AD brains (Jarrett et al., 1993). Additionally, the fibrillar

peptide is potently toxic to neurons in a variety of paradigms (Lorenzo and Yankner, 1996; Pike et al., 1993). These collective data led to the formulation of the amyloid cascade hypothesis which proposed that fibrillization of the amyloid peptide is a key event in the pathophysiology of disease and critically important in the death of neurons leading to dementia (Hardy and Higgins, 1992).

3 Fibrillar Aβ is an Activating Stimulus for Microglia

Because Aβ peptide forms the fibrillar core of the senile plaques in both sporadic and autosomal dominant disease, it has been hypothesized that fibrillar plaque deposition represents a mechanistically critical process in disease progression (Hardy and Higgins, 1992; Jarrett et al., 1993). As already mentioned the fibrillar peptides exhibit a direct toxic action on neurons and the biology of this process, although certainly of relevance to AD, is outside of the scope of this discussion. On the other hand, the close association of reactive microglia with amyloid plaques as they transition from diffuse to fibrillar dense core (mature) plaques has suggested that fibrils are direct stimuli for activating microglia. Indeed, a large body of data exists demonstrating that fibrillar peptides stimulate microglia to acquire a reactive, neurotoxic phenotype.

Since peptide stimulation often requires interaction with a cell surface protein, many groups have worked to identify putative Aβ "receptors" on cells within the nervous system. Aβ has been shown capable of interacting with a truly diverse set of cell surface proteins including parent APP (Lorenzo et al., 2000; Van Nostrand et al., 2002; Wagner et al., 2000) the receptor for advanced glycation end products (RAGE) (Yan et al., 1996), scavenger receptor A (El Khoury et al., 1996), CD36 (Coraci et al., 2002; Moore et al., 2002), CD47 (Koenigsknecht and Landreth, 2004), β1 integrins (Koenigsknecht and Landreth, 2004), glypican (Schulz et al., 1998), *N*-Methyl-D-Aspartate (NMDA) receptors (Bi et al., 2002), α-7 nicotinic acetylcholine receptors (Wang et al., 2000), serpin-enzyme complex receptor (Boland et al., 1995), *N*-formyl peptide receptor-like (FPRL) 1 (Yazawa et al., 2001), and the insulin receptor (Xie et al., 2002a). Importantly, a number of these studies were performed using microglia and microglial cell lines offering some insight into the mechanism by which the peptide interact with the cell surface of microglia. These studies demonstrate that the Aβ peptide has the potential, particularly in its fibrillar form, to interact with a large array of structurally and functionally distinct proteins suggesting that fibril-cell interactions may be somewhat nonspecific.

An additional direction of research has focused not on the cell surface Aβ interaction but rather the subsequent intracellular signaling response driving acquisition of the reactive phenotype. Although the elucidated pathways have been determined from unique cell systems including primary microglial cultures, microglial cell lines, and monocytic cell lines, there are common aspects of the response. For example, fibrils stimulate a transient increase in activity of a number of tyrosine kinases including Fyn, Lyn, Syk, focal adhesion kinase (FAK), and pyruvate kinase (PYK) in stimulated THP-1 monocytes and primary rodent microglia (Bamberger et al., 2003; Combs et al., 1999, 2001; McDonald et al., 1997, 1998). Subsequent

to the increase in tyrosine kinase activities, the cells release calcium from intracellular stores (Combs et al., 1999) and a number of serine threonine kinases are activated. For example, members of the mitogen activated protein (MAP) kinase family, extracellular signal regulated kinases (ERKs), c-Jun N-terminal kinase (JNKs), and p38 (Combs et al., 2001; Giri et al., 2003; McDonald et al., 1998) protein kinase C (PKC) (Combs et al., 1999), and RSK1/2 (McDonald et al., 1998) have all been reported to be activated by fibrillar stimulation of monocytes or microglia. In addition, subsequent changes suggestive of altered transcription occur after fibril stimulation including nuclear factor kappa beta (NFκB) activation, increased c-fos levels, and increased phosphorylation of CREB (Combs et al., 2001; McDonald et al., 1998).

It is not surprising, then, that fibrillar Aβ stimulation leads to increased secretion of several proinflammatory molecules from monocytes and microglia. Fibrils stimulate increased protein secretion and/or mRNA levels of several cytokines from human monocyte cell lines, microglial cell lines, primary rodent microglia and primary human, fetal and adult microglia including TNFα, IL-8, IL-6, MCSF, MIP-1α, IL-1β, matrix metalloproteases 1, 3, 9, 10, and 12 (Combs et al., 2000, 2001, Floden and Combs, 2006; Franciosi et al., 2005; Gasic-Milenkovic et al., 2003; Giri et al., 2003; Lue et al., 2001; Twig et al., 2005; Walker et al., 2001, 2006; Yates et al., 2000). In addition to cytokine secretion, fibrillar Aβ also stimulates secretion of superoxide anion from both human and mouse microglia via increased activity of plasmalemmal NADPH oxidase (Bianca et al, 1999; Wilkinson et al., 2006). Fibrillar stimulation also increases secretion of glutamate (Floden et al., 2005; Noda et al., 1999) and D-serine (Wu et al., 2004) demonstrating that oxidative and excitotoxic species may facilitate microglial mediated neuron death.

However, the identity of the neurotoxic agent(s) generated by Aβ fibril stimulated microglia appears to vary between paradigms ranging from excitotoxins, to cytokines, to oxidative damage dependent death (Banati et al., 1993, 1999; Combs et al., 2001; Floden et al., 2005; Giulian et al., 1995; Ii et al., 1996; Kingham and Pocock, 2001; Li et al., 2004; Monsonego et al., 2003; Tan et al., 2000; Xie et al., 2002b). It is likely that a variety of secreted factors contribute to the eventual loss of neurons that occurs either in the culture paradigms or in vivo following Aβ fibril stimulation of microglia. It will be important to identify which factors, if any, are truly generated by microglia in AD brains to determine the accuracy of in vitro modeling of microglial-dependent inflammatory changes during disease.

4 Oligomeric Aβ is an Activating Stimulus for Microglia

One of the criticisms of the amyloid cascade hypothesis, as originally proposed, has long been a clear lack of correlation between dementia rating and the numbers of fibrillar plaques in the brain (Lue et al., 1999; McLean et al., 1999; Morris et al., 1996). Indeed, it has been suggested that synaptic loss and gliosis precede plaque deposition (Martin et al., 1994). A similar observation has been con-

firmed in studies employing mouse hAPP transgenic lines. For example, Westerman et al. (2002) observed cognitively normal aged APP_{SWE} mice in spite of high concentrations of insoluble Aβ aggregates leading them to suggest a soluble Aβ form is responsible for neuronal deficit. An additional study using mice overexpressing APP_{IND} and $APP_{SWE,IND}$ demonstrated decreased presynaptic marker immunoreactivity and impaired synaptic transmission prior to plaque deposition (Hsia et al., 1999). Finally, using APP_{IND} and $APP_{SWE, IND}$ as well as APP_{WT} overexpressing cells Mucke et al. (2000) demonstrated that presynaptic marker immunoreactivity correlates inversely with levels of Aβ and plaque load correlates independently of Aβ levels. Collectively these data suggest that the fibrillar, insoluble form of the peptide may not be the most relevant species for mediating neuronal death/dysfunction.

Interestingly, recent data suggests that nonfibrillar Aβ conformations may be more reliable indices of disease progression. An oligomeric form of Aβ has been shown to accumulate in vivo and, more importantly, is elevated in AD brains in correlation with degree of behavioral deficit (Lue et al., 1999; McLean et al., 1999). The oligomeric form of the peptide can vary from dimers to high molecular weight SDS-stable oligomers and also arises in vitro from Aβ secreted into the culture media (Gong et al., 2003; Podlisny et al., 1995; Xia et al., 1997). Importantly, much like their fibrillar derivatives, the oligomers are neurotoxic, stimulate gliosis, produce cognitive dysfunction, and decrease long-term potentiation (LTP) both in vitro and in vivo (Chromy et al., 2003; Cleary et al., 2005; Hu et al., 1998; Klyubin et al., 2005; Lambert et al., 1998; Roher et al., 1996; Walsh et al., 2002; Wang et al., 2002). The low molecular weight dimeric/trimeric multimer of Aβ is reportedly able to mediate reversible inhibition of LTP generation (Klyubin et al, 2005; Walsh et al., 2005), impairment of cognitive dysfunction (Cleary et al., 2005) and microglial-dependent neuron death (Roher et al., 1996). A similar study using monomeric-tetrameric preparations demonstrated robust toxic effects on neuronal cell lines using both Aβ1-40 and Aβ1-42 (Dahlgren et al., 2002). Similarly, higher molecular weight multimers have demonstrated direct neurotoxic effects in paradigms ranging from rodent hippocampal slice cultures (Chong et al., 2006) to cell lines (Chromy et al., 2003; Demuro et al., 2005) to human fetal neuron cultures (Deshpande et al., 2006). Not surprisingly, these high molecular weight multimers have been observed to directly bind to neurons in both diseased brains and rodent hippocampal neuron cultures (Kokubo et al., 2005; Lacor et al., 2004). More recently, a dodecamer, Aβ*56, has been specifically characterized to increase in vivo in the brains of Tg2576 mice correlatively with the appearance of cognitive deficit and induce a reversible spatial memory deficit when microinjected into rat brain (Cleary et al., 2005; Lesne et al., 2006). This plethora of new data has revised the amyloid cascade hypothesis to now state that AD is initiated by neurotoxic stimulation provided by soluble Aβ peptide in its oligomeric rather than fibrillar form (Selkoe, 2002). Unfortunately, the mechanism by which oligomers stimulate neuron loss and glial activation is still unclear.

There is some data demonstrating that increasing oligomer concentrations correlate with microgliosis in vivo in transgenic rodent brains (Gordon et al., 2002;

Koistinaho et al., 2002). In agreement with this observation several in vitro studies have begun characterizing the ability of nonfibrillar Aβ peptides to stimulate microglia. Although the culture paradigms as well as multimeric state have varied between laboratories, a common theme with these studies is that oligomeric stimulation promotes acquisition of a proinflammatory phenotype. As already mentioned, while many studies have characterized putative receptors for fibrillar Aβ, it remains unclear how the oligomeric peptides interact with microglia. Using purified cultures of mouse microglia we have observed increased protein phosphotyrosine levels upon stimulation with the low molecular weight dimer/trimer Aβ1-42 oligomers (unpublished observations). This is similar but not identical to the signaling response initiated by stimulating microglia with fibrillar peptides (Combs et al., 1999). For example, we have not observed any increase in MAP kinase activities upon stimulation with these dimer/trimer oligomers (unpublished observations). There is, however, still a paucity of data describing the extent of the stimulated signaling response in microglia following treatment with not only the low molecular weight dimer/trimer oligomers but also the larger multimers.

On the other hand, the reactive phenotype produced by oligomer stimulation is better characterized. Using rat astrocyte cultures (95–98%astrocytes/2–5% microglia) two different studies demonstrated that the low molecular weight oligomers (White et al., 2005) and soluble Aβ (Hu et al., 1998) stimulate proinflammatory changes including increased protein and mRNA levels of inducible nitric oxide synthase (iNOS) and IL1-β, increased iNOS activity, and increased TNFα secretion. In a similar study Manelli et al. (2006) demonstrated that this mixed glia paradigm produced neurotoxins when cocultured with coverslips of primary rat cortical neurons. Although these data do not prove that the changes in proinflammatory protein expression and neurotoxicity are via oligomer-microglia interaction a study by Roher et al. (1996) demonstrated that rat mixed hippocampal neuron-glia cultures exhibited toxicity when treated with low molecular weight oligomers (dimer/trimer) only when microglia were present.

Other studies have used purified cultures of microglia to characterize the effects of oligomer stimulation on activation. A recent report showed that Aβ1-42 monomer-24mer preparations stimulate rat microglia cultures to secrete IL-1α and interferon-γ (IFN-γ) (Lindberg et al., 2005). A similar study by Takata et al. (2003) showed that rat microglia cultures increase secretion of TNFα, IL-6, and nitric oxide upon stimulation with low molecular weight Aβ1-40 oligomers. Even the Aβ25-35 fragment in its nonfibrillar form has recently demonstrated the ability to activate rat microglia to increase TNFα secretion (Hashioka et al., 2005). Using a dimer/trimer preparation of Aβ1-42, we have observed a similar activating response using cultures of purified mouse microglia. Oligomer stimulation results in increased expression of CD68, increased secretion of IL-6, TNFα, keratinocyte chemoattractant chemokine (KC), and decreased secretion of monocyte chemoattractant protein-1 (MCP-1) (Floden and Combs, 2006; unpublished observations). Moreover, these low molecular weight species are toxic to neurons only in the presence of microglia similar to prior work (Roher et al., 1996; unpublished observations). Therefore, although oligomeric peptides have direct effects on neuron activity and viability these collected studies above suggest that oligomeric

peptides, much like their fibrillar counterpart, may mediate a portion of their detrimental effects through microglia activation. It remains to be seen whether different multimeric states have unique stimulatory abilities for microglia.

5 The N-terminal Secreted Fragment of APP, sAPP, is an Activating Stimulus for Microglia

It is now appreciated that additional cleavage products of APP besides the Aβ peptides also mediate distinct, physiologic effects on cells. APP can be processed along two distinct, competing pathways to release a large secreted N-terminal portion of the protein (sAPP). ADAM 10 and TACE are involved in alpha secretase cleavage of APP resulting in generation of a soluble, 612 amino acid, N-terminal fragment of APP (sAPPα) which is released into the extracellular space (Esch et al., 1990; Haass et al., 1991; Sinha and Lieberburg, 1999; Weidemann et al., 1989). The aspartic proteases, BACE1 and BACE2, represent the beta secretase activities responsible for generation of sAPPβ required for the proteolytic processing to generate the Aβ peptides (Bennett et al., 2000; Sinha et al., 1999; Vassar et al., 1999). Much like, the fibrillar form of Aβ, sAPP has a host of effects on neurons. For example, sAPPα has direct protective effects on cultured neurons in response to excitotoxic challenge that is a hundred fold more protective than sAPPβ (Barger and Mattson, 1997; Furukawa et al., 1996). This effect involves increased guanylate cyclase activity and increased NFkB activation as well as decreased NMDA receptor-mediated calcium influx (Barger and Mattson, 1995, 1996; Furukawa et al., 1996; Furukawa and Mattson, 1998). In addition, sAPPα has a demonstrated ability to stimulate increased neurite outgrowth in neuronal cells via a tyrosine kinase stimulated signaling response (Jin et al., 1994; Mook-Jung and Saitoh, 1997).

Almost paradoxically, sAPPα also has a demonstrated ability to robustly stimulate microglial activation. Although the signaling pathway is not completely determined, is has been demonstrated that sAPPα stimulation of microglia involves increased MAP kinase activities. Specifically, treatment of rat microglia with sAPPα leads to increased levels of active ERKs, p38 kinase, and JNKs (Bodles and Barger, 2005). In addition, sAPPα or sAPPβ stimulation of primary microglia as well as the N9 microglia cell line increases NFκB activity (Barger and Harmon, 1997). As might be expected, these changes lead to increased expression or activity of a host of proinflammatory products including iNOS and IL-1β, and reactive oxygen species (Barger and Harmon, 1997; Barger et al., 2000; Bodles and Barger, 2005; Li et al., 2000). Importantly, the production of proinflammatory proteins is dependent upon activity of JNK and p38 kinases and not ERKs since specific inhibitors of JNK and p38 MAP kinases but not ERKs attenuate the sAPPα-induced increase in iNOS protein levels and activity (Bodles and Barger, 2005). Besides cytokine secretion, sAPPα also stimulates microglia to secrete glutamate via the cystine-glutamate antiporter (Barger and Basile, 2001; Ikezu et al., 2003).

Based upon the identity of the secretory products described above, it is not surprising that the secretions from sAPPα or sAPPβ stimulated microglia are toxic to

rodent neuron cultures (Barger and Basile, 2001; Barger and Harmon, 1997; Ikezu et al., 2003). The toxicity can be prevented by a superoxide dismutase (SOD) mimetic, MnTBP, specific inhibitors of neuronal nitric oxide synthase (nNOS), specific inhibitors of iNOS, and the NMDA receptor antagonist, MK-801 (Barger and Basile, 2001; Ikezu et al., 2003). Taken together, these data suggest that sAPP-stimulated microglia induce neuron death via combined oxidative and excitotoxic mechanisms. Therefore, although alpha secretase cleaved APP, sAPPα, is a demonstrated neurotrophic factor, it can also drive microglia to acquire a reactive, neurotoxic phenotype. It remains to be seen which of these opposing actions will dominate the in vivo function of sAPP.

6 Full Length APP can Act as a Proinflammatory Receptor on Microglia

We have thus far reviewed the accumulating data describing the ability of APP proteolytic fragments to stimulate microglial activation. Far less information is available regarding the function of full length APP in microglia. This is somewhat surprising since microglia serve as the second major producer of Aβ peptides behind neurons (Banati et al., 1993). It is relevant to discuss microglial APP in the context of this discussion since work by ourselves as well as others has suggested that is behaves as a proinflammatory receptor on monocytes and microglia. It has been known for some time that APP mRNA can be found within microglia of human brains (Schmechel et al., 1988). However, it has also been reported that plaque associated microglia in the AD brain have no detectable APP mRNA (Scott et al., 1993). The more definitive assessment of protein, however, has confirmed that microglia not only express APP but also upregulate protein levels in response to particular stimuli. In vitro cultures of purified rat microglia have verified they can express all isoforms of APP (Haass et al., 1991; LeBlanc et al., 1991). However, basal APP levels are low compared to neurons and very little of the protein is localized to the plasmalemma (Haass et al., 1991; LeBlanc et al., 1991). Not surprisingly, then, in vitro rat microglia studies have demonstrated that very little to no Aβ peptide or sAPP is generated by microglia (Haass et al., 1991; LeBlanc et al., 1991). These data suggest that the holoprotein may function differently in microglia compared to neurons.

However, other in vitro studies have demonstrated that APP protein levels are readily upregulated in microglia upon specific stimulation. For instance, human monocytes differentiated to macrophage in vitro increase their APP protein levels (Bauer et al., 1991). Microglia cultures stimulated with activating ligands like lipopolysaccharide (LPS) or prostaglandin E2 (PGE2) also increase APP, particularly on the cell surface (Pooler et al., 2004; unpublished observations). Using a mouse microglia line, BV-2, Monning et al. (1995) have demonstrated that when microglia express cell surface APP they are fully capable of secreting sAPP fragments. More importantly, this occurs in response to microglial adhesion to extracellular substrates like fibronectin and poly-L-lysine (Monning et al., 1995).

Perhaps the most compelling microglial APP data is derived from a series of in vivo studies demonstrating that APP immunoreactivity increases acutely and transiently within microglia following a variety of insults. For instance, transection of facial or sciatic nerves in rats results in increased microglial APP immunoreactivity within 6h post lesion not only in the affected nucleus but also in areas of afferent projection (Banati et al., 1993). Lesion of the entorhinal cortex in rats produced a similar, transient profile of increased microglial APP immunoreactivity in the dentate gyrus (Banati et al., 1994). However, microglial APP expression is also responsive to a broader range of insults beyond axotomy. Both an experimental autoimmune encephalomyelitis (EAE) model as well as a transient ischemia model in mice result in an elevation of microglial APP immunoreactivity that lasts for several days-weeks (Banati et al., 1995a, b). Collectively, these data, together with the observation that the structure of APP resembles a cell surface receptor (Kang et al., 1987), suggest that APP has a role in regulating acquisition of a reactive phenotype in microglia.

We have begun work in support of this hypothesis by characterizing the signaling response stimulated by plasmalemmal APP in primary mouse microglia and the human monocytic cell-line THP-1 (Sondag and Combs, 2004, 2006). Utilizing two different stimulation paradigms we have found that APP is associated with a classic tyrosine kinase-based proinflammatory signaling response leading to acquisition of a reactive phenotype in these cells. By plating these cells onto a type I collagen substrate we have modeled β1 integrin-mediated adhesion-dependent activation. In addition we have used an antibody, 22C11, against the N-terminus of APP to cross-link cell surface APP to simulate ligand binding. Both paradigms stimulate increased protein phosphotyrosine levels in microglia and THP-1 cells indicative of increased tyrosine kinase activity. APP pull-down co-immunoprecipitations have shown that the Src family tyrosine kinase, Lyn, and the tyrosine kinase, Syk, are recruited to a complex with APP upon substrate adhesion or antibody cross-linking (Sondag and Combs, 2004). In addition, substrate adhesion but not antibody cross-linking recruits APP to a multireceptor signaling complex with β1 integrin along with Syk and Lyn (Sondag and Combs, 2004). Subsequent to increased tyrosine kinase activity, we observed activation of the MAP kinase family following both adhesion and antibody cross-linking (Sondag and Combs, 2004, 2006).

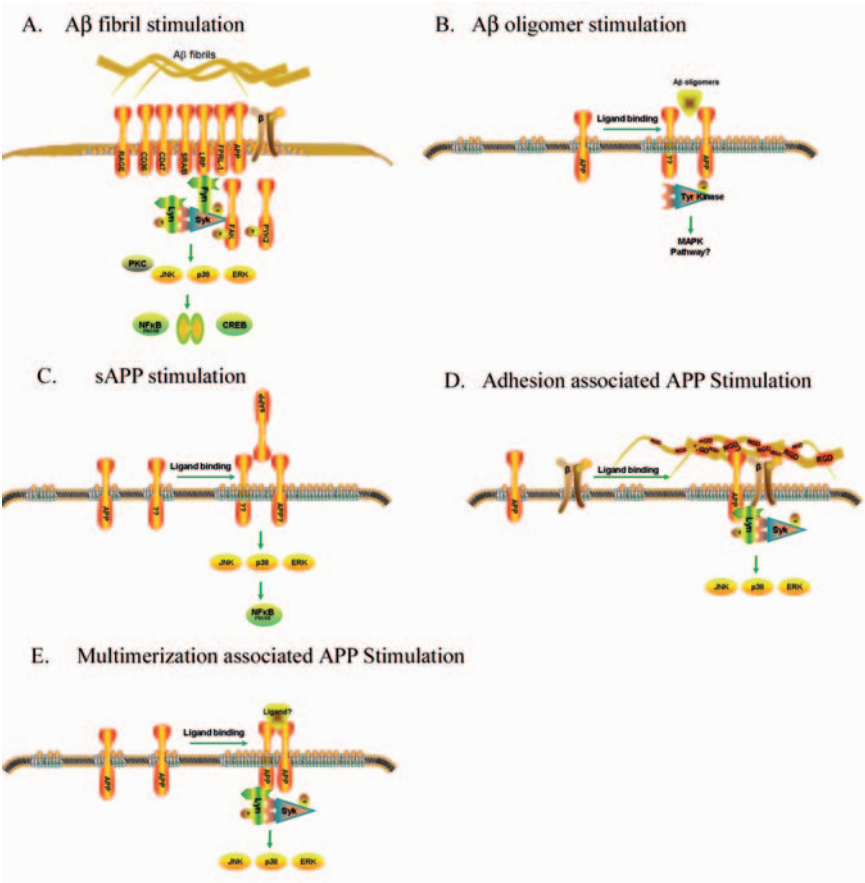
Not surprisingly, adhesion-dependent activation of THP-1 cells stimulates an increase in protein levels of a plethora of proinflammatory markers including COX-2, CD36, iNOS, and IL-1β. However, the more interesting observation is that these changes in protein levels were dependent upon expression of APP and the subsequent increase in tyrosine and MAP kinase activities induced upon ligand binding (Sondag and Combs, 2004). We extended this observation to define the behavior of THP-1 cells and microglia following antibody cross-linking of APP. As with the adhesion studies, the stimulated increase in proinflammatory protein levels was dependent upon recruited tyrosine and MAP kinase activities. Moreover, APP cross-linking increased cytokine secretion by the THP-1 cells and microglia. Most notably, cross-linked cells increased secretion of IL-1β and IL-6 in a tyrosine kinase dependent manner (Sondag and Combs, 2006). Because antibody-mediated receptor cross-linking is expected to influence endocytic events we also determined whether APP was

cleaved into Aβ peptides following stimulation. Cross-linking stimulated a selective release of Aβ1–42 compared to Aβ1–40 from the monocytes. However, Aβ1–42 secretion was independent of the increase in tyrosine and MAP kinase activities we had observed since inhibition had no effect on stimulated Aβ1–42 secretion. Therefore, secretase control of APP metabolism was independent of the tyrosine kinase based activation pathway. Our results thus far have suggested that APP has a common function in monocytes and microglia that is important in acquisition of a reactive phenotype. More importantly, it appears that the protein can act as an independent receptor, as in the case of antibody cross-linking, or it can be recruited into a multi-receptor signaling complex, as in the case of adhesion dependent activation. This novel signaling mechanism by which monocytes and microglia generate Aβ peptides could be a relevant contribution to plaque pathology in AD and vascular amyloidosis. Collectively, these results strengthen existing data that suggest microglial-derived APP can contribute to amyloid production in AD (Bauer et al., 1991).

Although we have demonstrated a rather robust role for APP in monocyte/microglial activation as a single receptor or within a multi-receptor complex, it is not clear how APP is involved in activating these cells in vivo. While it is easy to imagine APP participating in adhesion-mediated activation of microglia adhering to extracellular matrix, it is more difficult to envision how APP can behave as an independent proinflammatory receptor. This is largely due the fact that an agonist ligand for the extracellular domain of APP is not yet known. One interesting possibility is that the Aβ peptide itself can behave in an autocrine fashion to interact with APP to mediate clustering and subsequent signal transduction. Interestingly, Aβ has already been demonstrated to bind to the extracellular region of APP (Chung et al., 1999; Lorenzo et al., 2000; Shaked et al., 2006; Van Nostrand et al., 2002; Wagner et al., 2000) offering the possibility of a proinflammatory feed-forward pathway in which Aβ-APP interaction leads to increased APP-dependent proinflammatory signaling that results in further Aβ production. Alternatively, in vitro studies have shown that membrane-bound APP can form homodimers leading to the speculation that full-length APP can be its own ligand acting in a cis (same cell) or trans (opposing cell) fashion (Lu et al., 2003; Rossjohn et al., 1999; Scheuermann et al., 2001; Wang and Ha, 2004). Therefore, although it is well accepted that APP processing to Aβ peptide is an important contribution to plaque formation in AD (Citron et al., 1992, 1994; Jarrett et al., 1993; Masters et al., 1985; Scheuner et al., 1996), it is possible that APP has a multi-faceted role in the progression of this disease particularly as a proinflammatory receptor on microglia.

In conclusion, although reactive microglia are a histological hallmark in the AD brain, their contribution to neuron death and cognitive decline remains unclear. In addition, the stimulus for their reactivity is also not defined. A large collection of data demonstrates that proinflammatory changes occur in not only AD brains but also its animal models. These data offer hope that attenuating microgliosis will offer benefit against disease conditions. However, before this can be approached in a specific fashion it is important to define not only the source of reactivity but also the subtle differences in activation phenotype that surely must exist in vivo. For example, there is a well recognized association of a certain reactive microglial phenotype with mature, dense core plaques and fibrillar Aβ peptides are activating ligands for microglia. However, as illustrated above, it is also clear that nonfibrillar

forms of the peptide as well as the secreted N-terminus and full length APP itself, all have the capacity to stimulate microglia to acquire unique, reactive phenotypes. It remains to be seen which of these species, if any, has the most significant role in promoting microglial activation in AD.



Comparison of Modes of Microglial Actication by APP and its Proteolytic Fragments.

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