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Evidence for Synaptic Stripping by Cortical Microglia

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KEY WORDS
neuroprotection; microglial activation; chemokine CCL2; BCG; immune privilege

ABSTRACT
Recent studies have described significant demyelination and microglial activation in the cerebral cortex of brains from multiple sclerosis patients. To date, however, experimental models of cortical demyelination or cortical inflammation have not been extensively studied. In this report we describe focal cortical inflammation induced by stereotaxic injection of killed bacteria (BCG), followed 1 month later by subcutaneous injection of the same antigen, a protocol that overcomes the immune privilege of the cortex. Intracerebral BCG injection produced focal microglial activation at the injection site (termed acute lesion). Ten days after peripheral challenge (termed immune-mediated lesion), larger areas and higher densities of activated microglia were found near the injection site. In both paradigms, activated microglia and/or their processes closely apposed neuronal perikarya and apical dendrites. In the immune-mediated lesions, ~45% of the axosomatic synapses was displaced by activated microglia. Upon activation, therefore, cortical microglial migrate to and strip synapses from neuronal perikarya. Since neuronal pathology was not a feature of either the acute or immune-mediated lesion, synaptic stripping by activated microglia may have neuroprotective consequences.

INTRODUCTION

Microglia, the resident inflammatory cells of the CNS, comprise 5–20% of the total glial cell population in the mammalian brain (van Rossum and Hanisch, 2004). Collectively, microglia extend a network of nonoverlapping processes that covers most of the CNS parenchyma and monitor the CNS by process extension, retraction, and remodeling (Davalos et al., 2005). Microglia cells are of myeloid mononuclear phagocyte lineage and have diverse roles in the CNS, including immune surveillance, antigen presentation, phagocytosis, and neuroprotection (Kreutzberg, 1996). They become activated in response to a variety of subtle, severe, or noxious changes in their microenvironment (Streit et al., 1999). The transition from a resting to activated state is rapid and easily documented by increased expression of "activation" markers, changes in morphology, and defined pattern of physiological transformations (Farber and Kettenmann, 2005). In neurodegenerative diseases (e.g., Alzheimer's, HIV dementia, multiple sclerosis), cortical microglia become highly activated and are thought to play a role in the pathogenesis of neurological and cognitive dysfunction (Gonzalez-Scarano and Baltuch, 1999; Kim and de Vellis, 2005; McGeer et al., 1992; Minagar et al., 2002; Peterson et al., 2001). In vitro, activated microglia can produce substances that cause neuronal damage and destruction, such as free oxygen radicals (Thery et al., 1991), nitric oxide (Chao et al., 1992), *N*-methyl-D-aspartate (NMDA) receptor agonists (Giulian et al., 1993), proteases, and cytokines (Moore and Thanos, 1996). Microglia lacking the chemokine receptor, CX3CR1, can be neurotoxic in vivo (Cardona et al., 2006).

In addition to their role in immune surveillance and phagocytosis, microglia can have a neuroprotective role and enhance nerve repair (Streit, 2005). It is reasonable to suggest that a primary role of microglia is neuroprotection, since they secrete neurotrophins that support neuronal survival and physiological function. Activated microglia may also protect neurons by physically removing synaptic input. This process known as "synaptic stripping" is best documented following transection of the PNS portion of the facial nerve. Following facial axotomy, microglia in the ipsilateral facial nerve nucleus rapidly become activated and denervate facial nerve neurons by physically separating pre- and postsynaptic components (Blinzinger and Kreutzberg, 1968; Graeber et al., 1993). Synaptic stripping also occurs following autoimmune demyelination of the facial nerve (Gehrmann et al., 1992b). The degree to which synaptic stripping occurs in the CNS has not been extensively studied. Physical associations between cortical microglia and neurons have been described in varied CNS diseases including neurosyphilis (Kaneko et al., 1989), ischemic infarcts (Gehrmann et al., 1992a; Neumann et al., 2006), Alzheimer's disease (Haga et al., 1989; Itagaki et al., 1989), HIV dementia (Yoshioka et al., 1992), and MS (Peterson et al., 2001). Our interest in this phenomenon grew from investigating demyelinated lesions in the cerebral cortex of MS patients, where microglia closely apposed neuronal perikarya and proximal dendrites.

The objective of this study was to examine the consequences of focal microglial activation in the rodent

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cerebral cortex. Microglial activation was induced by intracerebral injection of heat-killed Bacillus Calmette–Guerin (BCG) bacteria. Animals were examined following a single intracortical injection of BCG. Peripheral challenge with BCG in Freund's adjuvant 30 days later (Matyszak and Perry, 1995) examined the possibility that increasing the extent and chronicity of the local inflammatory response would alter microglial activation. Both the injection of BCG and increased inflammation following peripheral induction of BCG immunity induced focal cortical microglial activation at the injection site. Based upon confocal and electron microscopic analyses, these activated microglia closely apposed neuronal cell bodies and removed ~45% of axosomatic synapses.

MATERIALS AND METHODS

Intracortical Injections of BCG and Peripheral Challenge

Adult male Lewis rats (Charles River Labs; Wilmington, MA) were anesthetized by intramuscular injection of Ketamine (80 mg/kg) and Xylazine (10 mg/kg). One microliter of heat-killed BCG (1×10^5 organisms) in phosphate buffered saline (PBS) was stereotactically injected with a 30-gauge needle into the cerebral cortex (Matyszak and Perry, 1995). Control rats received an identical injection of 1 μL of saline only. Stereotaxic coordinates (Paxinos and Watson, 1986) were AP = −1.7 and ML = +1.7, relative to Bregma, and DV = −1.6, relative to the brain surface. This corresponds to a site 1.7 mm lateral to the midline, midway along the cerebrum (sensorimotor cortex), with the needle tip at a depth of 1.6 mm into the cortex, which is ~2/3 the distance between the pia and the corpus callosum. Adult C57BL/6 mice null for the Monocyte Chemoattractant Protein-1 (MCP-1) gene (Lu et al., 1998) and wild-type littermates were anesthetized and 0.5 μL of BCG was injected into the cerebral cortex (AP = −0.9, ML = +1.4, and DV = −1.0).

For control (saline) and focal, "unchallenged" lesions (BCG), animals were anesthetized and sacrificed at 3 days after intracranial injection and the brains processed for immunohistochemistry, as described later. To induce a "delayed-type" anti-BCG immune response lesion in the CNS, animals were peripherally challenged by a subcutaneous injection of BCG or saline. They received a 0.2 mL subcutaneous injection, divided between the hind legs, containing 1×10^7 heat-killed BCG in an emulsion of Complete Freund's Adjuvant (CFA, Sigma, St Louis, MO), 1 month after intracerebral BCG injection. Ten days after peripheral immunization, animals were anesthetized, perfused with 4% paraformaldehyde, and brains were removed for histological analysis. A total of 42 rats, 5 MCP-null mice, and 4 WT littermate mice were injected intracranially and peripherally challenged with BCG. As controls, 8 rats were PBS-injected and challenged with BCG, and 9 rats and 8 WT mice were BCG-injected then PBS-challenged. All protocols were approved by the Cleveland Clinic Animal Research Committee, in compliance with the Public Health Service policy on humane care and use of laboratory animals.

Immunohistochemistry

Animals were anesthetized and perfused through the heart with 4% paraformaldehyde in 0.08 M phosphate buffer (pH 7.4). Brains were removed, postfixed overnight, cryoprotected in 20% glycerol, and sectioned on a sliding microtome. Free-floating coronal brain sections (30 μm thick) were pretreated with 3% H_2O_2, 10% Triton X-100 in PBS, and 3% normal goat serum. Sections were placed in primary antibodies overnight and then stained by the avidin–biotin complex (Vector Laboratories, Burlingame, CA) and diaminobenzidine (DAB; Sigma) as described previously (Wujek et al., 2002). The DAB reaction product was enhanced with 0.04% osmium tetroxide. Sections were mounted on glass slides in glycerol and cover slipped for microscopic analysis.

Microglia were identified with mouse Mabs, ED1 (Serotec, Raleigh, NC, MCA341R), Iba-1 and F 4/80 (Sigma), or with a rabbit polyclonal antibody, Iba-1, (a generous gift from Dr.Shinichi Kohsaka, Tokyo, Japan, or generated at the Cleveland Clinic according to the method of Imai et al., 1996). Iba-1 immunostaining is a well-established method of staining both activated and resting microglia (Imai et al., 1996), and provides consistent staining. To raise the latter antibody (CCF-Iba-1), a 17 amino acid peptide (HQKPTGPPAKKAISELC) of the Iba-1 protein was synthesized, then conjugated to Keyhole Limpet Hemocyanin, and injected into New Zealand white rabbits. The rabbits were bled, and the antiserum was affinity purified, using the same peptide. This antibody recognized a single band of ~18 kDa and immunostained microglia in rat, mouse, and human brain sections.

Neurons were visualized with mouse monoclonal antibodies specific for phosphorylated (SMI-31) and nonphosphorylated (SMI-32) neurofilaments (Sternberger Monoclonals, Lutherville, MD) or with a rabbit polyclonal antibody against the neurofilament heavy chain (NF-H; Serotec, AHP245), and MAP2 (Chemicon, Temecula, CA).

Immunofluorescence and Confocal Microscopy

Associations between microglia and neurons were examined by confocal microscopy. Brain sections (30 μm thick free-floating) were incubated with two primary antibodies for 3 days at 4°C and then stained with fluorescently conjugated secondary antibodies (Vector Laboratories), mounted on glass slides, and examined in a Leica TCS confocal microscope.

Preembedding Immunohistochemistry for Electron Microscopy

Selected free-floating tissue sections were pretreated with 0.1% Triton X-100 and immunostained with ED1 antibodies and DAB as described earlier, postfixed in

2.5% glutaraldehyde, placed in osmium tetroxide, and embedded in Epon. Ultrathin sections were cut, mounted on Formvar-coated grids, and photographed in a Philips CM100 electron microscope.

Electron Microscopy

Rats were injected intracranially with BCG and peripherally challenged with BCG (five rats) or PBS (five rats) as described earlier. They were anesthetized and perfused with 2.5% glutaraldehyde and 4% paraformaldehyde in 0.08 M phosphate buffer (pH 7.4). Brains were removed, postfixed overnight, and cortical areas containing the injection sites were dissected out and embedded in Epon. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and viewed as described earlier.

Morphometric Analysis

The extent of neuronal cell body area occupied by synapses was measured in the cerebral cortex of unoperated or BCG-injected rats, using electron microscopy. High magnification montages of neuronal perikarya were used to identify axosomatic synapses. The circumference of neuronal perikarya and the length of synaptic contacts were measured using Photoshop 7.0 software (Adobe Systems, San Jose, CA). Synaptic innervation per neuron was determined and expressed as a percentage of neuronal cell body circumference occupied by presynaptic terminals. Data were analyzed with the Student *t* test.

To determine the extent of microglial activation in saline- and BCG-injected cerebral cortex, the area occupied by Iba-1 immunoreactivity was quantified. Images from areas surrounding the injection sites were digitally photographed using a Leica DMR microscope fitted with an Optronics Magna Fire CCD color video camera and image acquisition system. Digital images (six areas per cerebral cortex, from three control and three experimental animals) were captured at 100× magnification using Adobe Photoshop 7.0 software. Cortical area occupied by Iba-1 immunoreactivity was determined by measuring number of pixels above a set threshold value and expressed as a percent of total pixels within the microscope field. Data were analyzed with the Student *t* test.

RESULTS

Microglial Activation

We utilized two antibodies to identify and quantify microglia in three different paradigms. The ED1 antibody stains activated microglia, while Iba-1 antibody stains both resting and activated microglia. Three days following intracortical injection of saline into the rat, microglial activation was restricted to the needle track (Fig. 1A); this represents the control lesion. Three days after intracortical injection of BCG (focal, unchallenged lesion), ED1-positive activated microglia were more abundant near the injection site when compared with saline-injected rats (Fig. 1B). When intracerebral injection of BCG was followed 30 days later by subcutaneous immunization with BCG in CFA, the subsequent immu-

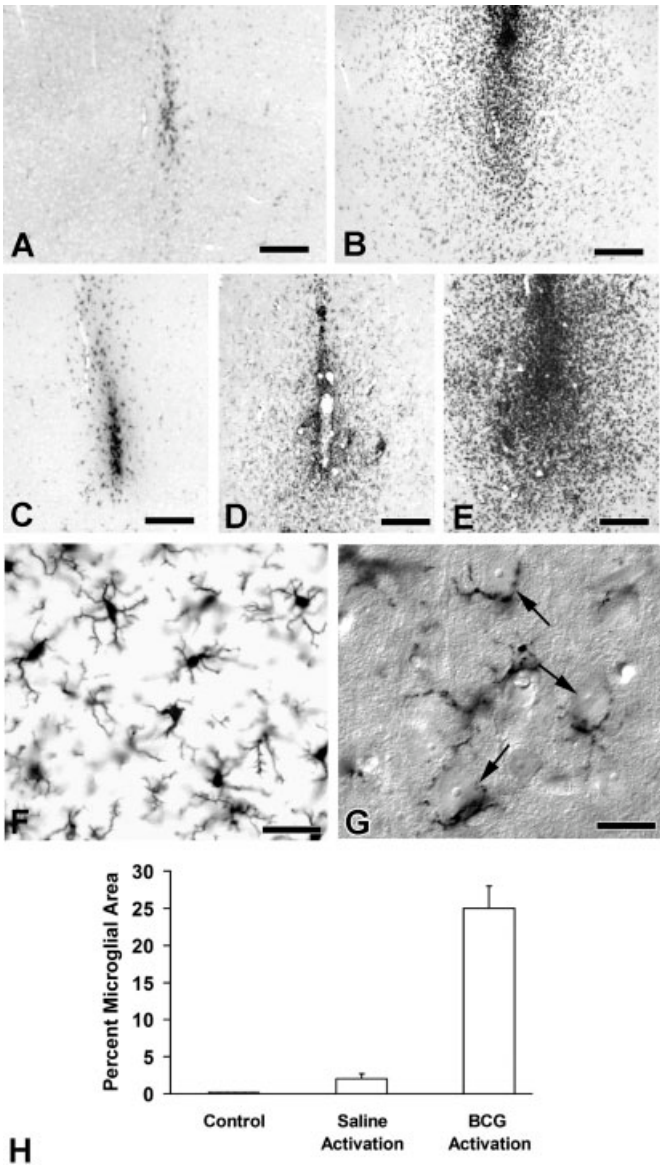

Fig. 1. Microglial cell activation in rat cerebral cortex. (**A**–**E**) Microglial activation was visualized by ED1 immunostaining at 3 days after saline (A) and BCG (B) injections, and in saline-injected (C) and BCG-injected (D,E) mice after peripheral challenge with BCG (C,E) or saline (D) (described in detail in Materials and Methods). In saline control lesions (A,C), microglial activation was restricted to the needle track. In BCG lesions (B,D,E), the density and number of activated ED1 positive microglia is significantly increased at 3 days after injection (B), but had attenuated at 40 days after injection when challenged with saline (D). In contrast, ED1-positive microglia were greatly increased around the injection site in brains injected and peripherally challenged with BCG (E). Iba-1-positive resting microglial cells in control lesions have many thin and branched processes (**F**). In immune response lesions, ED1-positive microglial cells are abundant and have shorter processes, which are asymmetrically distributed, and frequently apposed neuronal cell bodies (**G**, arrows). Quantification of cortical area occupied by activated microglial cells (**H**). In immune response lesions (i.e., BCG injected and BCG challenged), cortical area occupied by activated microglia was increased 10-fold when compared with control lesions (saline injected and BCG challenged). Scale bars: A–E = 200 μm; F,G = 20 μm.

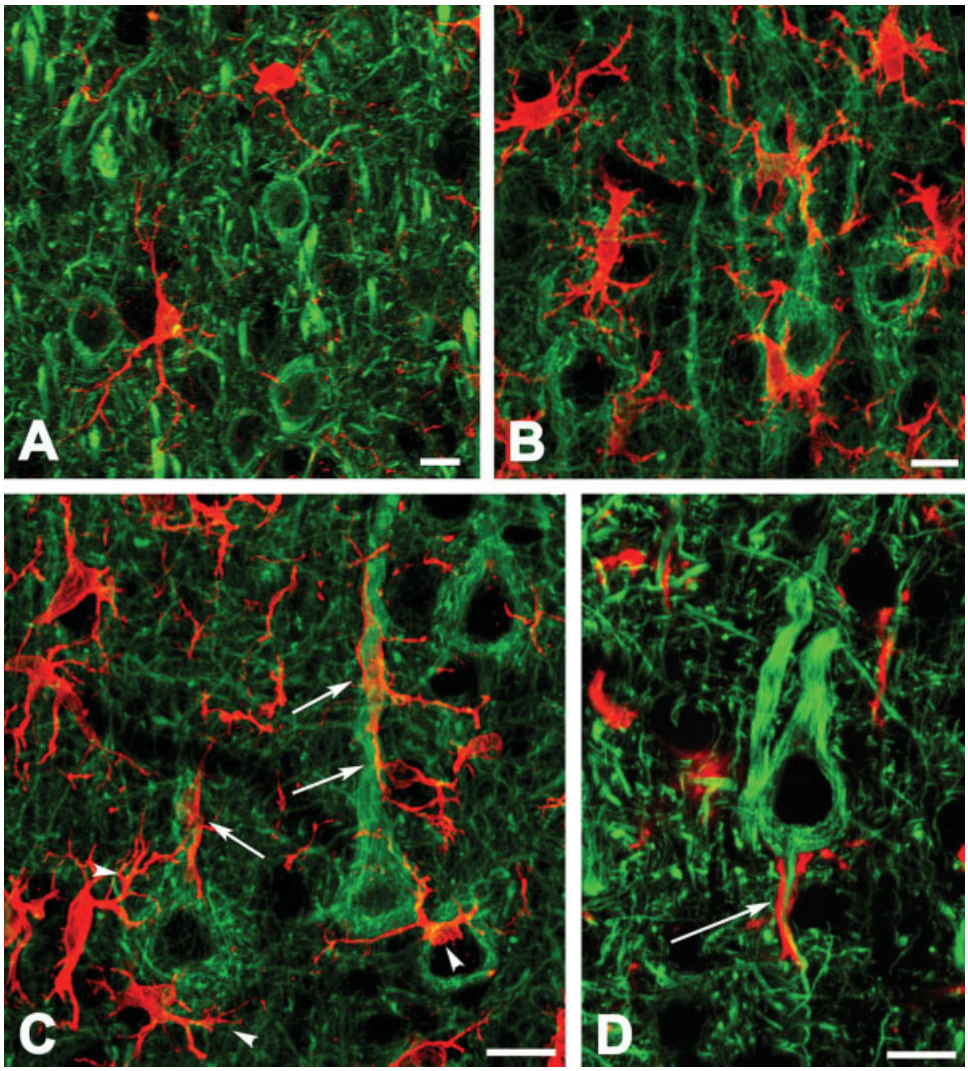

Fig. 2. Activated microglial cells associate with neurons. Sections were double labeled for microglia and neurons and examined by confocal microscopy. Resting microglia (**A**, red) had extensive fine processes and their cell bodies rarely associated with cortical neurons (green) in control lesions. (**B**–**D**) Activated microglial cells target neurons in BCG immune lesions. Compared with controls, activated microglia (red) were increased in BCG immune lesions after peripheral challenge and frequently extended processes to and along the neuronal cell bodies and proximal dendrites (green). Some microglia cell bodies closely appose neuronal perikarya (B, C), while others (C, arrowheads) extend processes to adjacent neurons, dendrites (C, arrows), and axons (D, arrow). All images are projections of multiple optical confocal sections. Scale bars = 10 μm.

nological reaction, evaluated 10 days later, produced larger areas and higher densities of activated microglia that were concentrated around the injection site (Fig. 1E), compared with saline-injected or saline-challenged controls (Figs. 1C,D). When viewed at higher magnification, Iba1-positive resting cortical microglia from saline-injected rats had small cell bodies and numerous fine branching processes (Fig. 1F). In the control lesions, ED1 antibodies stained occasional activated microglia near the needle tract (Figs. 1A,C), with resting microglia remaining unstained. Typically, reactive microglial cells have more asymmetrically oriented processes. In the focal, challenged lesions, ED1 immunohistochemistry revealed numerous activated microglia near the injection site (Fig. 1G). The density of activated microglial cells was quantified by anti-ED1 immunostaining (Fig. 1H). In saline-injected control lesions, activated microglia occupied 2% of the cortical neuropil, whereas in the immune response lesion, there was a 10-fold increase in the cortical area occupied by activated microglia (24% neuropil area). For comparison, microglia in uninjected brains occupied 12% of the area, as determined by Iba-1 immunostaining. Therefore, the extent of ED1 immunoreactivity (24%) suggests proliferation and/or hypertrophy of microglia or the recruitment of peripheral macrophages.

Activated Microglia Associate with Neurons and Strip Synapses

To investigate the association of activated microglia with neurons, brain sections from rats 10 days post

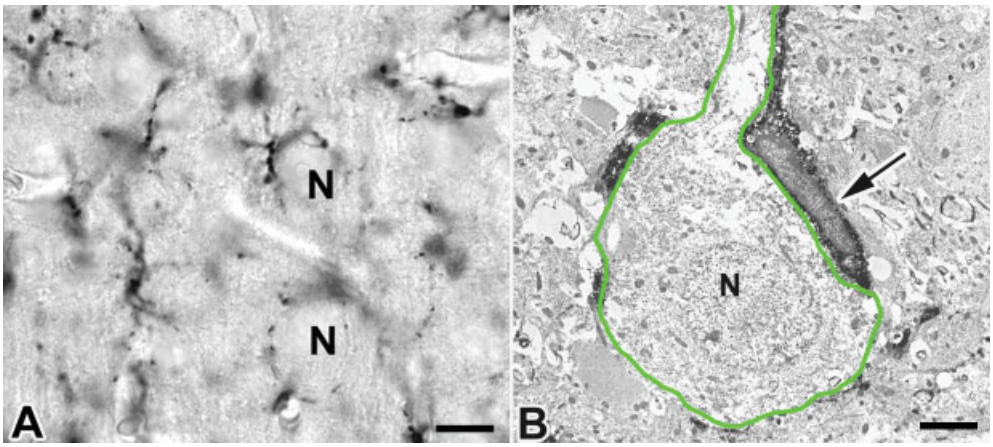

Fig. 3. Activated microglial cells and their processes directly apposed neuronal cell bodies. (**A**) In phase contrast images of sections immunostained with ED1 antibodies, activated microglial surround neurons (N) in BCG immune response lesions. (**B**) ED1 immunohistochemistry of free-floating sections was followed by Epon embedding and electron microscopy. ED1-positive microglial cells (arrow) directly appose a neuronal cell body (N, green outline indicates neuronal plasma membrane). Scale bars: A = 25 μm, B = 5 μm.

peripheral BCG immunization were double-labeled with neurofilament and ED1 antibodies and examined by confocal microscopy. Resting microglial cells in control cortex (Fig. 2A) have a stellate appearance and their cell bodies rarely apposed neuronal cell bodies. In the cerebral cortex of rats with immune response lesions, reactive microglial cells were closely apposed to neuronal cell bodies and their processes. Often, microglial cell bodies were wrapped around neuronal perikarya (Fig. 2B). Other activated microglia located close to neuronal cell bodies extended several processes, which appeared to contact and partially ensheath the neuronal surface (Fig. 2C). Microglial processes were also seen associated with proximal dendrites and axons (Figs. 2C,D). Thus, following induction of an immune response lesion, activated microglia closely associated with neurons, and particularly with the neuronal perikarya and proximal dendrites.

One consequence of this close apposition between microglia and neurons may be interruption of synaptic contacts. This phenomenon, termed synaptic stripping, has been observed in the facial nucleus axotomy model (Blinzinger and Kreutzberg, 1968). We investigated the relationship between microglia and neuronal cell bodies in the rat cortex 10 days after induction of immune response lesions. By phase contrast microscopy, ED1-positive microglial cells and their processes closely apposed neuronal cell bodies (Fig. 3A). To confirm and extend these results, we examined microglia and neurons in the immune response lesion at the ultrastructural level. Preembedding electron microscopic immunohistochemistry using ED1 antibodies was employed to identify microglia unequivocally. Neuronal cell bodies were identified by virtue of their larger cell bodies and prominent nuclei (Fig. 3B). Many neurons were surrounded and closely apposed by microglia, which were intensely stained by the electron-dense DAB reaction product. ED1-immunoreactive cells exhibited ultrastructural features of microglia including elongated nuclei with condensed chromatin immediately underneath the nuclear membrane (Fig. 3B; arrow). Microglia did not closely appose neuronal cell bodies in control lesions. The close apposition between microglia and neurons suggested the possibility that presynaptic terminals might be lost from the neuronal cell bodies.

Conventional EM was used to investigate the consequences of activated microglia–neuron interactions. To determine if synapse density decreased following microglial association, we quantified axosomatic synapses present in electron micrographs from control and immune response lesions. In saline-injected control lesions, microglial cell bodies were rarely found in contact with neuronal perikarya (Fig. 4B). In immune response lesions, microglia directly contacted neuronal perikarya (Fig. 4A), with no intervening cellular processes. While neuronal surfaces apposed by microglia were clearly devoid of presynaptic terminals, axosomatic synapses were observed on the remaining neuronal surface (Fig. 4A; highlighted profiles). Our analysis indicated that fewer synapses remained on cortical neurons in immune response lesions when compared with control lesions. To quantify synaptic stripping, synaptic density was expressed as a percentage of neuronal cell body circumference occupied by presynaptic terminals. As shown in Fig. 4C, the percentage of neuronal cell surface apposed by presynaptic terminals was decreased from 29% in control lesions to 16% in the immune response lesions ($P < 0.008$), a reduction of 45%. As microglia preferentially wrap around neuronal cell bodies, inhibitory axosomatic synapses are stripped. Neuronal pathology, neurite transection, and demyelination were not apparent in electron micrographs of focal or immune response lesions. There was no evidence of neuronal cell death by EM. Based on this and a paucity of activated caspase-3 staining, neuronal apoptotic pathways were not activated in the focal or immune response lesions (data not shown).

MCP-1/CCL2 is Not Required for Microglial Activation

The chemokine, monocyte chemoattractant protein-1 (MCP-1; CCL2 in the systematic nomenclature), plays a role in migration and attraction of monocytes/macrophages to injured tissue (Charo and Ransohoff, 2006).

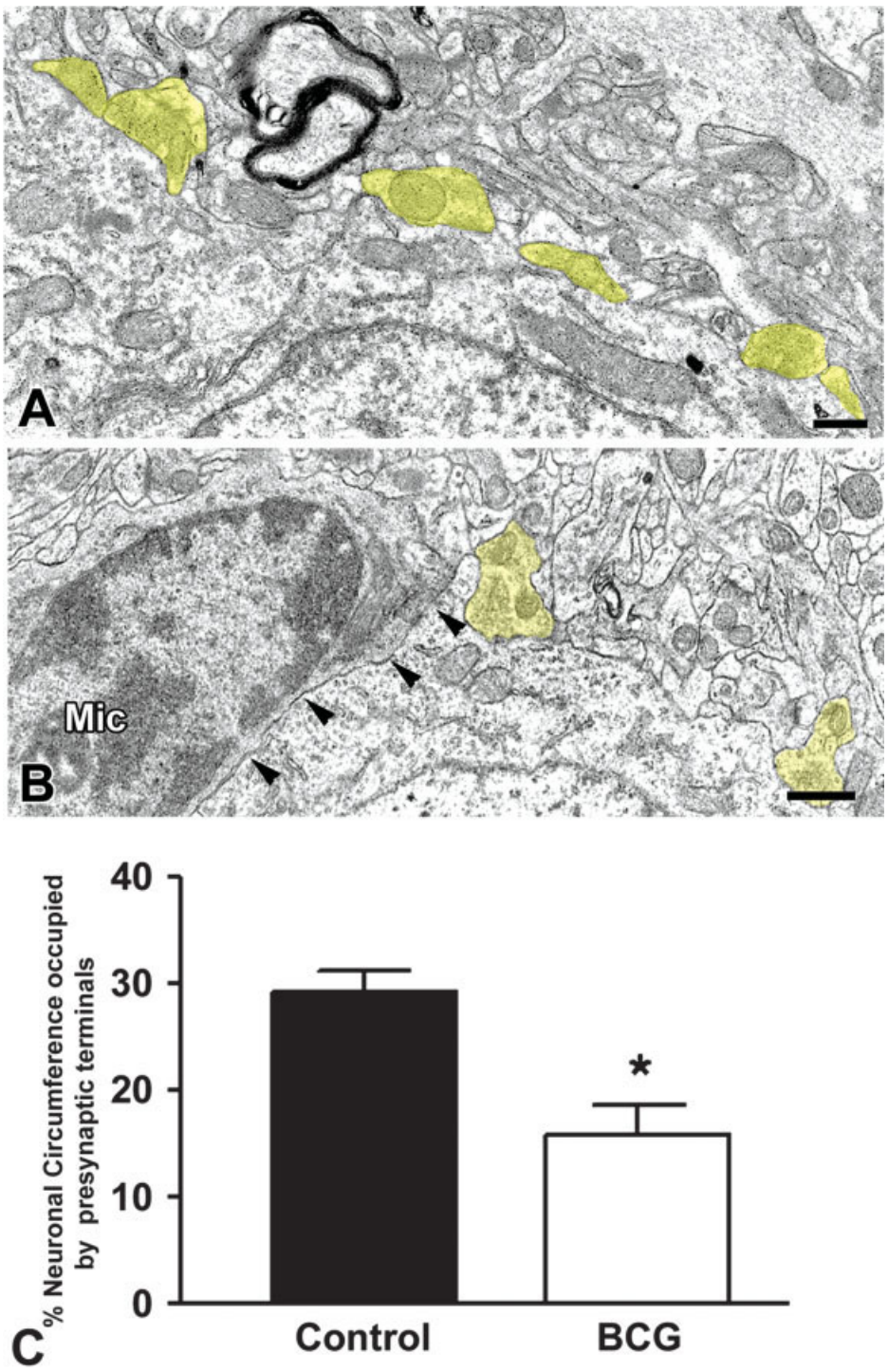

Fig. 4. Synapses are lost from neuronal surface areas occupied by activated microglia. (**A**) Synapses terminating on a neuron in control lesion identified by transmission electron microscopy (presynaptic terminals are highlighted in yellow). (**B**) In immune response lesions, activated microglial cells directly contact the plasma membrane of neuron cell bodies. These regions (arrowheads) lack synapses, unlike adjacent plasma membrane, suggesting that microglia have displaced presynaptic terminals. (**C**) Quantification of percent area of neuronal perikaryal surface occupied by presynaptic terminals. In control lesions, 29% of the neuronal cell body surface was occupied by presynaptic terminals. In immune response lesions, presynaptic terminals occupy 16% of perikaryal surface. Unpaired Student t test detected a 45% decrease in neuronal perikaryal innervation in immune response lesions ($P < 0.008$; $n = 26$ neurons (BCG injected) and 30 neurons (control) from five rats in each group). Mic, microglia; N, neuron. Scale bars = 0.5 μm.

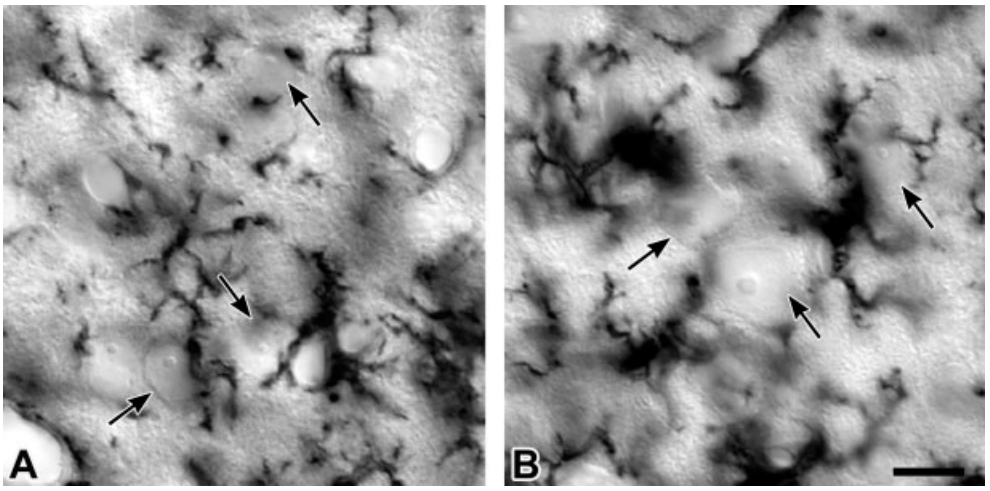

Fig. 5. MCP-1 is not required for neuronal targeting by activated microglia. Images of Iba-1-positive microglia in wild-type (**A**) and MCP-$1^{-/-}$ (**B**) mice. In immune response lesions produced in both MCP-$1^{-/-}$ and wild-type mice, activated microglia closely appose neuronal cell bodies (arrows). Scale bars = 25 μm.

MCP-1 is expressed by facial nerve neurons 6 h after facial nerve axotomy and has been proposed to mediate neuronal/microglial interactions (Flugel et al., 2001). To investigate whether this chemokine is responsible for attracting of microglia to neurons, cortical BCG-induced immune response lesions were induced in MCP-1 null and wild type mice. Microglial activation did not differ between MCP-1 expressing (Fig. 5A) and MCP-1 null mice (Fig. 5B). As in the rat cerebral cortex, examination at higher magnification indicated that activated microglial processes surrounded neuronal cell bodies. These data demonstrate that MCP-1 is not required for activation of microglia or their subsequent targeting of neurons in these lesions, and is consistent with prior demonstration that the immune response to mycobacterial infection is unaltered in the absence of CCL2 (Lu et al., 1998).

DISCUSSION

The present study investigated the consequence of cortical microglial activation following induction of an immune response in the cerebral cortex. BCG injection into the cortex produced an acute lesion, characterized by modest microglial activation and synaptic stripping at the injection site. Cortical BCG injection does not result in a primary immune response (Matyszak and Perry, 1995), but provides a local antigen depot which can be targeted following activation of adaptive immunity by peripheral antigenic challenge. This failure to activate an adaptive immune response to the BCG depot contrasts sharply with consequences of injecting BCG into peripheral organs and has been termed "immune privilege." The explanation for immune privilege lies with inability of the privileged organ to generate afferent signals to lymphoid organs. In the case of the cerebral cortex, lack of local dendritic cells accounts for this deficit. Following cutaneous challenge with BCG, a systemic adaptive immune response occurs, and the intracortical BCG depot is readily localized by effector cells, which mount an attack on the "invader." Ten days following induction of such cortical immune response lesions mediated by peripheral BCG challenge, microglial activation and synaptic stripping were significantly increased at the initial cortical injection site. Thus, introduction of pathogen-associated molecular components into the cortex and induction of a peripheral immune response to these components causes microglia to remove synapses from neurons. In this context, microglial-mediated synaptic stripping was not detrimental to neuronal survival. Based upon these observations, we hypothesize that synaptic stripping is a neuroprotective function of activated cortical microglia. In support of this hypothesis, recent studies have demonstrated that microglia ensheath and protect neurons from death caused by ischemic insult (Neumann et al., 2006).

Although the functional significance of microglial reaction to the stimuli in this paradigm remains to be fully elucidated, we propose that it may be neuron-supportive. Electron microscopy revealed normal-appearing neurons, with no signs of morbidity or necrosis, and immunocytochemistry did not detect activated caspase-3-positive neurons. The absence of neuronal death, and the lack of phagocytosis by microglia, despite the intimate association between neurons and microglia, suggest that the microglia/neuron associations described here are neurotrophic rather than neurotoxic. It should be noted, however, that peripheral challenge with BCG can induce necrotic lesions caused by infiltration of immune cells at the injection site (Matyszak and Perry, 1995). Several variables can regulate the immune response at the injection site including the amount of BCG injected into the brain and/or during the peripheral immunization. We restricted our analysis to time points and conditions where only microglial activation occurred.

What may be the mechanisms for this potential neuroprotective effect? An important clue comes from the classic peripheral axotomy paradigm, which involves transection of the peripheral axon of a CNS neuron. Activation and subsequent targeting by the microglia in those models are premised on the presence of stress

signals originating from the injured neuron (Streit et al., 1999). It is proposed that the ensheathment of motor neurons by microglial cells following axotomy could serve a dual purpose. Insertion of microglial processes could deafferent the injured neurons and at the same time, place microglial cells into very close proximity to the injured cells. This proximity could facilitate contact-dependent neuronal–microglial interactions, such as delivery of microglia-derived trophic factors (Streit et al., 1999). This concept is supported by studies which show that axotomizing intrinsic CNS rubrospinal neurons, which exhibit poor regenerative potential, are also associated with minimal microgliosis without ensheathment of neuronal cell bodies by microglia cell processes (Barron et al., 1990; Tseng et al., 1996).

An intriguing question concerns the molecular mechanism that guides the migration of activated microglia to neurons following neuronal injury. Chemokines are small protein molecules that are involved in immune cell attraction (Laskowski et al., 2000; Ward et al., 1998). One chemokine in particular, MCP-1 was a strong candidate to be a molecule that would attract microglial cells to neurons. This chemokine is expressed in both neurons and astrocytes (Che et al., 2001; Flugel et al., 2001; Ransohoff et al., 1993; Schreiber et al., 2001) and is involved in immune cell adhesion, migration, and activation (Charo and Ransohoff, 2006). Our analysis of mice deficient in MCP-1 (Lu et al., 1998) demonstrated strong microglial activation in cortical immune response lesions. In addition, microglial cells surrounded neuronal cell bodies and processes. No apparent difference was detected in the extent of microglial activation comparing wild-type and MCP-1 deficient mice. These results suggest that MCP-1 is not directly involved in the activation of microglia or their migration and adhesion to neurons induced via the BGC immune paradigm. ATP was identified as a novel mediator of microglial reactions to acute injury and constitutes a potential candidate for this function (Davalos et al., 2005). Recent studies indicate that CD11a may mediate microglial migration to neurons following ischemic insult (Neumann et al., 2006). Signaling through CXCR3 is involved in microglial recruitment to denervated dendrites in entorhinal cortex lesions (Rappert et al., 2004), but loss of CXCR3 did not change microglial responses in the facial axotomy model (Rappert et al., 2004). Precisely what factors trigger microglial activation and neuronal targeting remains unresolved. Microglia are extraordinarily sensitive to changes in the brain microenvironment, whatever the exciting mechanism or substance be (Barron, 1995), and it seems plausible that chemotactic signals will be used in differing pathophysiological contexts to promote microglial/neuronal interactions.

In addition to allowing for a more specific and effective cellular interplay, close interaction between microglia and neuronal cell bodies in these lesions also disrupts synaptic inputs to the neurons (Blinzinger and Kreutzberg, 1968). In immune response lesions, neuronal surfaces occupied by activated microglia were devoid of synapses (Fig. 4). Removal of axosomatic synapses by activated microglia constitutes a potential neuroprotective mechanism. The majority of synapses that terminate on cortical neuronal perikarya use γ-amino butyric acid (GABA) as their neurotransmitter (Mallat and Chamak, 1994; Streit et al., 1999). GABA-ergic axosomatic synapses are inhibitory, so that transient loss of axosomatic synapses preferentially reduces inhibitory inputs. Inhibition or temporary disruption of inhibitory synapses decreases the threshold for firing synaptic NMDA receptors. Neuronal survival depends upon synaptic NMDA receptor activity (Hardingham and Bading, 2003; Ikonomidou and Turski, 2002). Increased synaptic NMDA receptor activation occurs following inhibition of GABAergic input by bicuculline and promotes neuronal survival or renders neurons more resistant to injury (Papadia et al., 2005). Firing synaptic NMDA receptors stimulates an anti-apoptotic and/or anti-necrotic signaling pathways through cAMP-response-element-binding-protein (CREB) (Hardingham and Bading, 2003). By analogy to bicuculline treatment, removal of inhibitory axosomatic synapses by activated microglia may promote survival of neurons by increasing the firing of synaptic NMDA receptors and CREB activation

Our data highlight one aspect of microglia–neuronal interaction that may have important implications in synaptic plasticity and neuroprotection. Although microglial activation can be viewed as a double-edged sword, with a documented potential to cause harm when its activity is protracted and exaggerated (Cardona et al., 2006), our data suggest that microglial activation and subsequent neuronal targeting during inflammatory and immune reactions primarily support neuronal survival.

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