Role of Microglia in Central Nervous System Infections

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INTRODUCTION

The early years of research on the nature of microglia, the resident macrophages of the nervous system, are noteworthy for the remarkable insights of many illustrious anatomists and neuropsychiatrists (reviewed in reference 300), including Gluge (who in 1841 identified phagocytic cells of mesodermal origin in the damaged brain), Virchow (who in 1846 observed phagocytes ["foam cells"] contributing to a disease process termed congenital encephalitis), His (who in 1890 described amoeboid mesodermal corpuscles which entered the developing brain of human embryos in the second month, colonized both grey and white matter, and emitted protoplasmic radiations), Nissl (who in 1899 suggested that glial cells in the brain have similar functions to macrophages in other tissues), Robertson (who in 1900 distinguished "neuroglia" and "mesoglia," the latter cells, derived from mesoderm, displaying phagocytic activity in pathological conditions such as chronic brain degeneration), Alzheimer (who in 1904 believed that glial cells became amoeboid in certain acute infections and were destined to combat the infection), and Cajal (who in 1913 recognized mesoglia as the "third element" of the central nervous system [CNS]). However, it was the Spanish neuroanatomist Pio del...
Rio-Hortega who in 1932 earned the title “father of microglia biology.” He was the first to demonstrate (in 1919 to 1922) that mesoglia were composed of microglia, which are of mesodermal origin, and oligodendroglia, which, along with astroglia and neurons, are of neuroectodermal lineage. In his classic treatise published in 1932 (97), Rio-Hortega framed a “modern conception of microglia” that remains relevant to this day.

Following this era of vigorous scientific inquiry, the field of research on microglia experienced an eclipse that lasted half a century. Over the past 15 years, however, a phenomenal reawakening of interest has erupted (a Medline search reveals more than 1,800 articles published during this period with the term “microglia” in the title). This rebirth of interest is due in no small part to the recognition of the role of microglia in neurodegenerative disorders, such as human immunodeficiency virus (HIV)-associated dementia (HAD) and, ironically, the disease named after one of the earliest researchers, i.e., Alzheimers disease. During this same period, a number of reviews have appeared on various aspects of microglia biology (10, 27, 122, 200, 224, 262, 264, 273, 278, 340, 341, 343, 350). With the exception of articles focusing on HIV, however, the literature on the role of microglia in defense against and pathogenesis of CNS infections has not been critically evaluated; such an evaluation is the principal aim of this review. It is also the intent of this review to highlight concepts that have been evolving in recent years, such as the pivotal role of microglia in innate immunity of the nervous system, the controversy over the protective versus destructive activities of activated microglia, and the tropism of certain microorganisms for microglia versus macroglia (astrocytes and oligodendrocytes) and neurons.

Definition, Derivation, and Distribution

The term “microglia” refers to cells that reside within the parenchyma of the nervous system, that share many if not all the properties of macrophages in other tissues, but that in their nonactivated or resting state have a characteristic “ramified” morphology not seen in resident macrophages of other organ systems. Although microglia are “brain macrophages,” they are distinguished by their parenchymal location and certain functional differences from other types of brain macrophages such as meningeal and perivascular macrophages (264, 290, 291) and perivascular cells or pericytes (351, 376), which are enclosed by a perivascular basement membrane within blood vessels and are not part of the CNS parenchyma.

The origin of microglia was a matter of intense controversy in Pio del Rio-Hortega’s day (97). Although it is still a somewhat contentious issue, most authorities now agree on the correctness of his concept of mesodermal glial cells invading the parenchyma during embryonic development followed by the ingress of bone marrow-derived blood monocytes in the postnatal period (89, 172, 224). Thus, microglia are currently regarded as members of the “mononuclear phagocyte system.” Another of his concepts that has withstood the test of time is that of three phases of microglia reflecting their plasticity: an amoeboid phase found in the fetus, a ramified (resting) phase found in the nervous system framework, and a third phase of recovery of amoeboid properties and motility “necessary for active discharge of their macrophagic function” (97). As Rio-Hortega recognized, the penetration and migration of microglia takes place very quickly, and postnatally, microglia are to be found in every location within the nervous system (97). Often not appreciated, however, is the fact that the brain is composed primarily of glial cells. While about 15% of the cells in the brain are neurons, it is estimated that microglia are found in roughly equivalent numbers (341). In a recent study of the local density of microglial cells in the normal adult brain, ramified microglia bearing markers such as CD68 and major histocompatibility complex (MHC) class II antigen were found to be more concentrated in white matter than in grey matter, and significant regional differences were observed, with microglia ranging from 0.5 to 16.6% of all the cells within various areas of the brain parenchyma (250). Grey matter of the cerebellum had the lowest density of microglia, while the highest level of CD68- and MHC class II-positive cells was found in the medulla.

Consistent with the concept of Rio-Hortega (97), amoeboid, ramified, and reactive microglia are currently viewed as different forms of a single cell type. Amoeboid microglia are active macrophages during development and are precursors of resting or ramified cells, which can, in response to a variety of insults such as infection, traumatic injury, or ischemia, reactivate in the postnatal brain, assume an amoeboid shape, and move to the site of injury (350). Early in human fetal brain development, microglia are mainly amoeboid in appearance, whereas by 18 weeks of gestation, a ramified morphology predominates (Fig. 1A). Consistent with the ability of these cells to assume an amoeboid morphology, upon isolation and culture, a homogenous population of amoeboid microglia can be obtained for in vitro studies (Fig. 1B and C).

Astrocytes are the predominant cell type within the CNS, and astroglia-microglia interactions appear to play an important role in microglial cell biology. For example, in vitro studies have shown that blood monocytes and amoeboid microglia develop branching processes when layered on astrocytes, suggesting that astrocytes induce the morphology of resting or ramified microglia (301, 307). Moreover, amoeboid and even fully ramified microglia have been shown to migrate rapidly when seeded on a confluent layer of astrocytes (307). Although astrocytes differ morphologically (Fig. 1D) and functionally from microglia, the two glial cell types appear to act in concert as the intrinsic immune system of the CNS (326).

FUNCTIONS OF MICROGLIA

Ramified (Resting) Microglia

The term “glia,” derived from the Greek word for “glue,” suggests that microglia share with astroglia and oligodendroglia the property of brain support and, more particularly, the support of neurons. However, such a supportive role in the healthy brain is better appreciated for astroglia, which make important contributions to neurotransmitter metabolism, and for oligodendroglia, which are the source of myelin, than for ramified (resting) microglia. While it seems likely that ramified microglia also contribute to the well-being of neurons, this “neuronocentric” view may underestimate the importance of neuronal support of microglia. Nonetheless, amoeboid microglia are thought to have a crucial scavenger function in the...
developing brain by removing the large number of cells in the neocortex that die in the course of normal remodeling of the fetal brain (364). Scavenger receptors have been identified on neonatal murine microglia, whereas this class of cell surface protein is not detected on microglia in postnatal mouse or normal human adult brain (163). Further evidence of a supportive role of microglia has been shown in the facial nerve axotomy paradigm, in which the recovery of injured neurons is dependent on the trophic function of activated microglia (264, 340).

**Activated Microglia**

As already mentioned, certain cell surface markers of importance in immune regulation, such as MHC class II molecules, are constitutively expressed on ramified microglia in the normal adult brain (250). However, in response to a variety of CNS insults such as microbial invasion, ramified microglia have the capacity not only to dramatically change their morphology to reactive or ameboid forms but also to rapidly up-regulate a large number of receptor types and produce a myriad of secretory products that are thought to contribute to the defense of and, potentially, damage to the infected brain.

The state of microglial activation represents a continuum that is reflected by in vitro studies, with relatively minor changes being observed just in the process of preparing and culturing ameboid microglia, which express CD14 (Fig. 1C), a marker not found in ramified microglia. At the far end of the activation spectrum, marked alterations are seen following stimulation with microbial products such as lipopolysaccharide (LPS). Because activated microglia are regarded as a pivotal cell in both defense against and immunopathogenesis of infections and inflammatory diseases of the CNS, numerous in vitro studies of the regulatory factors involved in microglial activation have been reported (reviewed in reference 260), and in recent years, techniques to identify activated microglia in vivo have been applied to studies of various pathological conditions (23, 24, 371).

**Cell membrane receptors.** As already mentioned, immune recognition molecules, such as MHC class II, can be identified on ramified (resting) microglia in the undisturbed brain (250). Relatively few studies of other cell membrane receptors involved in immune responses have been carried out with ramified microglia. However, activated microglia have been the focus of many studies, and in this functional phase they have been shown to express a number of such receptors, e.g., members of the immunoglobulin superfamily, complement receptors, cytokine/chemokine receptors, and Toll-like receptors (TLRs) (Table 1). In addition to MHC class I and II glycoproteins and costimulatory molecules (269, 385), a population of microglia that have properties of dendritic cells may arise during infectious and inflammatory conditions (110). It has been suggested that these dendritic cell-like microglia may present antigens to Th1 lymphocytes and thereby participate in chronic inflammation of the nervous system (110).

Not only do microglia contribute to acquired immune responses through their interactions with CD4+ and CD8+ lymphocytes which enter the nervous system during infection or inflammation, but also they are a key cell in the innate immunity of the nervous system (264). In this regard, activated microglia have been demonstrated to express TLRs (54, 184, 264), CD14 (280), and mannose receptors (228), all of which play a role in recognition of so-called pathogen-associated molecular patterns, such as LPS of the gram-negative bacterial cell wall and peptidoglycan and teichoic acid of the gram-positive bacterial cell wall.

Little is known about the signals that induce the transformation of microglia from an ameboid to a ramified form, although cytokines such as granulocyte-macrophage colony-stimulating factor and interferon-β, and TNF-α, IL-1, IL-6, IL-10, IL-12, IFN-γ, IFN-α, IFN-β, IFN-γ, IL-1, IL-6, IL-10, IL-12, IL-16, TNF-α, M-CSF, GM-CSF, CCR, CXCR, CX3CR, Toll-like receptors, CD14 receptors, Mannose receptors, Purinergic receptors, Opioid receptors (μ, κ), Cannabinoid receptors, Benzodiazepine receptors (mitochondrial membrane) (Table 1).

**FIG. 1.** Human microglia. (A) Microglial cells in fetal brain tissue at 11 weeks’ gestation are predominantly ameboid in shape (left panel), whereas by 18 weeks they have assumed a ramified morphology (right panel) (stained with anti-CD68 antibodies, a macrophage marker). (B and C) Microglia in cell cultures isolated from 18-week fetal brain tissue have assumed an ameboid morphology (CD68 antibody positive) (B) and up-regulate CD14 antigen (a marker not seen in nonactivated ramified microglia) (C). (D) A double-stained mixed culture of microglia (anti-CD68 antibody positive, dark blue) and astrocytes (anti-GFAP antibody positive, red) from 18-week fetal brain tissue shows differences in morphology and size. (E) Microglial cell cultures infected for 14 days with HIV-1 assume a multinucleated giant cell morphology (stained with anti-p24 antigen antibodies, green). (F and G) LPS (100 ng/ml)-stimulated microglial cell cultures express intracellular CXCL8/IL-8 (green) (F) and intracellular CXCL10/IP10 (green) (G). (H) Microglial cell cultures are shown after 18 h of incubation with nonopsonized M. tuberculosis H37Rv (tuberculeto-cell ratio, 10:1) (auramine-rhodamine stain).

### Table 1. Microglial cell membrane receptors

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>Receptors Reported in the Literature</th>
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<tr>
<td>Scavenger receptors</td>
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<td>Cell adhesion molecules</td>
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<td>Immunoglobulin (Ig) superfamily</td>
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<td>1G2F receptors (FcγRII, RII, RIH)</td>
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<tr>
<td>MHC class I glycoproteins</td>
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<td>MHC class II glycoproteins</td>
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<td>CD4 receptors</td>
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<td>Intercellular adhesion molecule 1 (ICAM-1)</td>
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<tr>
<td>Integins</td>
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<tr>
<td>Leukocyte function-associated antigen 1 (LFA-1; CD11a/CD18; CR1)</td>
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<tr>
<td>Mac-1 (CD11b/CD18; CR3)</td>
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<tr>
<td>p150, p95 (CD11c/CD18; CR4)</td>
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<tr>
<td>Complement receptors: C1q, C5a</td>
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<tr>
<td>Cytokine/chemokine receptors</td>
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<tr>
<td>IFN-α, IFN-β, IFN-γ</td>
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<tr>
<td>IL-1, IL-6, IL-10, IL-12, IL-16</td>
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<td>TNF-α</td>
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<td>M-CSF, GM-CSF</td>
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<td>CCR, CXCR, CX3CR</td>
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<tr>
<td>Toll-like receptors</td>
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<td>CD14 receptors</td>
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<td>Mannose receptors</td>
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<td>Purinergic receptors</td>
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<tr>
<td>Opioid receptors (μ, κ)</td>
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<tr>
<td>Cannabinoid receptors</td>
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<tr>
<td>Benzodiazepine receptors</td>
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<td>(mitochondrial membrane)</td>
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*Receptors reported in the literature, whose expression may be influenced by the state of activation as well as by the anatomic location, age, and animal species from which the microglia are derived.*
stimulating factor (GM-CSF) can do so in vitro (278). Similarly, the mechanism underlying the change in morphology from a ramified to an activated or amoeboid form is poorly understood. However, Rio-Hortega had determined more than half a century ago that amoeboid microglia were capable of migrating in a directed fashion toward areas of brain injury and, once there, “discharge their macrophagic function” (97). A major contribution of the recent renaissance of research on microglia has been an understanding of the critical role of cytokine/chemokine receptors (Table 1) and their cognate ligands (Table 2) in directing the migration and the activation of microglial cells, topics that have been reviewed elsewhere (17, 19, 86, 94, 112, 143, 171, 173, 196, 338).

Insights into the functional consequences, both physiological and pathophysiological, of up-regulation in activated microglia of cytokine/chemokine receptors and their ligands have emerged from a variety of in vitro and in vivo paradigms of infection, as well as neuroinflammatory and neurodegenerative disorders. Although much remains to be learned, it appears that these receptors and their ligands not only contribute to shaping the development of the normal fetal brain (84, 187, 292) and IL-10 (190, 211, 377), classes of cytokines (Tables 1 and 2). Of these, matrix metalloproteinases (MMP-2, MMP-3, MMP-9) Free radicals: superoxide, nitric oxide Eicosanoids: PGD2, leukotriene C4 Growth factors: nerve growth factor, fibroblast growth factor Proteases: elastase, plasminogen Cathepsins B and L Quinolonic acid, glutamate Amyloid precursor protein Complement factors: C1, C3, C4

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**TABLE 2. Secretory products of microglia**

| Cytokines (IL-1α, IL-1β, IL-6, IL-10, IL-12, IL-16, IL-23, TNF-α, TGF-β) | Chemokines | CXC: CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES | CXC: CXCL8/IL-8, CXCL9/MIG, CXCL10/IP-10, CXCL12/SDF-1α | CX3C: CX3CL1/fractalkine | Matrix metalloproteinases (MMP-2, MMP-3, MMP-9) | Free radicals: superoxide, nitric oxide | Eicosanoids: PGD2, leukotriene C4 | Growth factors: nerve growth factor, fibroblast growth factor | Proteases: elastase, plasminogen | Cathepsins B and L | Quinolonic acid, glutamate | Amyloid precursor protein | Complement factors: C1, C3, C4 |

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* **Secretory products reported in the literature, whose generation is influenced by the state of activation as well as by the anatomic location, age, and animal species from which the microglia are derived.**
studies of murine (161), swine (158), and human (280) cells have demonstrated that activated microglia from all three of these species share the capacity to produce superoxide. Animal species differences, however, appear to prevail in the generation of the RNI nitric oxide (NO) by microglia. While microglia isolated from rat and mouse (167, 256) brains yield substantial amounts of NO when activated, the picture is less clear for human microglial cells. On stimulation with cytokines and LPS, human microglia release little or no appreciable NO (47, 99, 168, 282), a finding which is consistent with the hyporesponsiveness of the human inducible NO synthase (iNOS) gene reported for other populations of human mononuclear phagocytes.

MICROGLIA-MICROBE INTERACTIONS

Early observations demonstrating a poor alloreactive response to tissue engraftment within the CNS led to the concept of the brain as an “immunologically privileged” organ. Characteristics of the healthy brain that support this notion include a BBB composed of highly specialized endothelial cells and astrocytes that limit the passage of leukocytes and immune mediators from the circulation into the CNS, a relatively low level of expression of MHC class I and II molecules, and the absence of resident lymphocytes within the brain parenchyma. Nonetheless, the brain is routinely and effectively surveyed by the immune system (151), and when one considers the number of microglia within the parenchyma of the nervous system, which function as intrinsic immune effector cells, the CNS may be more properly regarded as a specialized immune organ.

Despite what appears to be a marvelous strategy for keeping microbes out of the brain parenchyma, when looked at from the perspective of the large number of viruses, bacteria, fungi, parasites, and proteinacious pathogens that are “neuropathic,” i.e., that have a predilection for infecting the nervous system (Table 3), the brain could be considered an “immunologically underprivileged” organ. Thus, an evolving concept of microglia is that when it comes to defense of the CNS against invading microorganisms, they do not function on their own but rely on their ability to “call in the troops,” i.e. lymphocytes, monocytes, and neutrophils. In the sections that follow, literature is reviewed which highlights what is currently viewed to be the role of microglia and their allies in defense against and pathogenesis of infectious disease agents.

Viruses

Human immunodeficiency virus. HAD is characterized by cognitive, behavioral, and motor deficits ranging from mild disease to profound dementia. Since the introduction of highly active anti-retroviral therapy, the incidence and prevalence of HAD have decreased dramatically (229). A considerable amount of attention has been focused on the factors involved in the development of HAD over the last 20 years, and the neuropathogenesis of HIV has been extensively reviewed elsewhere (120, 132, 262, 274, 379). A key element in the development of HAD appears to be infection of mononuclear phagocytes with HIV-1, including infection of microglial cells, which are the only brain cell type that is productively infected with this virus. It has also become clear that neurotoxic mediators released from brain macrophages/microglia play a pivotal role in HIV-1 neuropathogenesis.

HIV-1 enters the CNS early after infection (90), and productive replication and macrophage invasion occur years later and only in certain individuals (274). Microglia are the principal target for HIV-1 (82, 178, 373) and HIV-2 (254) in the brain, although there is limited evidence of infection in neurons, oligodendrocytes, and astrocytes (9, 267, 310, 355). This low level infection in astrocytes may serve as a reservoir of HIV (245, 353). Although parenchymal microglia are actively infected (8, 82), some have suggested that the more specific targets for HIV-1 are perivascular macrophages or infected monocytes infiltrating the CNS (90, 111, 285, 378, 379), and these cells have been implicated as a possible mechanism for HIV-1 entry into the CNS (90, 185). HIV-1-infected microglia are primed by HIV-1 (266), demonstrate cytopathic features and form multinucleated giant cells, but are not necessarily killed by the virus (205). Infected microglia harbor viral particles intracellularly, reflecting their potential as a reservoir (379). When histopathological features of HAD have been examined, the number of activated microglia and macrophages in the CNS is a better correlate with HAD than is the presence and amount of HIV-1-infected cells in the brain (131), and microglial activation is a better correlate of neuronal damage than is productive HIV-1 infection in the CNS (2). These observations demonstrate the importance of activated microglia/macrophages in HIV-1 neuropathogenesis.

HAD is associated pathologically with HIV-1 encephalitis. HIV-1 encephalitis is characterized by multinucleated giant

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<th>TABLE 3. Neurotropic infectious agents</th>
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<td><strong>Viruses</strong></td>
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<tr>
<td>Retroviruses</td>
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<td>HIV</td>
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<td>Human T lymphotropic virus type 1</td>
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<td>Herpes group</td>
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<td>HSV</td>
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<td>CMV</td>
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<td>Epstein-Barr virus</td>
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<td>Human herpesvirus 6</td>
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<td>B virus</td>
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<td>Enteroviruses</td>
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<td>Polioviruses</td>
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<td>Coxsackieviruses</td>
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<td>Echoviruses</td>
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<td>Arboviruses</td>
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<td>Rabies virus</td>
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<td>Mumps virus</td>
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<td><strong>Lymphocytic choriomeningitis virus</strong></td>
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<td>Measles virus</td>
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<td>Rubella virus</td>
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<td>Nipah virus</td>
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<td>Hendra virus</td>
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<td>JC virus</td>
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<tr>
<td><strong>Bacteria</strong></td>
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<tr>
<td>Mycobacterium tuberculosis</td>
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<td>Treponema pallidum</td>
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<tr>
<td><strong>Protozoa</strong></td>
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<td>Borrelia burgdorferi</td>
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<td>Nocardia asteroides</td>
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<td>Leptospira</td>
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<td>Brucella</td>
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<td>Rickettsia</td>
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<td>Ehliichia</td>
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<td>Parasites</td>
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<td>Cysticercus</td>
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<td>Entamoeba histolytica</td>
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<td>Free-living amebas</td>
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<td>Gnathostoma spiniger</td>
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<td><strong>Fungi</strong></td>
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<td>Cryptococcus neofor mans</td>
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<td>Coccioides immitis</td>
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<td>Histoplasma capsulatum</td>
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<td>Blastomyces dermatitidis</td>
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<td>Candida</td>
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<td>Zygomycetes</td>
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<td>Aspergillus</td>
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<tr>
<td>Sporothrix schenckii</td>
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<td><strong>Prions</strong></td>
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*Human pathogens that have the capacity to invade, multiply, and elicit a pathologic response within the brain parenchyma. This list does not include the bacteria that most commonly cause meningitis and brain abscesses or the parasite Plasmodium falciparum, which is the cause of CM.*
cell formation, microglial nodules, and macrophage infiltration into the CNS (274). Multinucleated, giant cell formation is mirrored by HIV-1 infection of microglia in vitro (Fig. 1E). Active infection of microglia is ultimately associated with astrogliosis, myelin pallor, and neuronal loss (274).

HIV-1 can enter the microglial cell via CD4 receptors and chemokine coreceptors such as CCR3, CCR5, and CXCR4 (147, 186), with CCR5 being the most important of these (7, 323). Interestingly, humans with double allelic loss of CCR5 are virtually immune to HIV (311). IL-4 and IL-10 enhance the entry and replication of HIV-1 in microglia through up-regulation of CD4 and CCR5 expression, respectively (368). The chemokines CCL5/RANTES, CCL3/MIP-1α, CCL4/MIP-1β, all of which bind to CCR5, are inhibitory to HIV-1 replication in microglial cells, apparently by their ability to block viral entry (177, 327). GM-CSF and macrophage-colony stimulating factor (M-CSF) stimulate the production of these β-chemokines but actually inhibit the antiviral properties of these chemokines when added to microglial cell cultures (327).

One interesting discrepancy noted in HIV-1 encephalitis is the limited number and localization of HIV-1-infected microglia in comparison to the diffuse CNS abnormalities that occur in HAD (205). This paradox suggests that diffusible factors that are released from macrophages and microglial cells are contributing to neuronal loss. It has become more and more apparent that HIV-1-infected microglia and macrophages actively secrete both endogenous neurotoxins such as TNF-α (266), IL-1β (18), CXCL8/IL-8 (382), glutamate (170), quinolinic acid (150), platelet-activating factor (123), eicosanoids (266), and NO (1), as well as the neurotoxic viral proteins Tat (263), gp120 (46), and gp41 (1). In addition to inducing neurotoxicity, these viral proteins can affect microglial cell function (148, 322). The chemokine CX₃CL1/fractaline appears to be neuroprotective against gp120 neurotoxicity (246). At least one of these neurotoxic mediators, TNF-α, can be inhibited in vitro by pentoxifylline, dexamethasone, or thiadomide (67, 276, 284), and insulin-like growth factor 1 protects neurons from TNF-α-induced damage (383). Infected microglia can also enhance the recruitment of additional microglia and macrophages to the site of infection by inducing endothelial cells to produce adhesion molecules and by release of CCL2/MCP-1, CCL3/MIP-1α, and CCL4/MIP-1β (205, 262, 275). Although the initial stimulus for secretion of neurotoxins appears to be microglial activation by HIV-1 or viral proteins, interactions with astrocytes, neurons, and monocytes may regulate this secretion (38, 205). Furthermore, microglia may require a secondary trigger, such as opportunistic organisms, neoplasms, cytokines, and CNS-specific regulatory elements, to bolster secretion of these neurotoxic factors (205). Ultimately, the complex interactions among activated microglia/macrophages, astrocytes, and neurons trigger the onset and progression of CNS damage. Despite a large body of evidence pointing to a neuropathogenic role of activated microglia in the development of HAD, recent data have suggested some neuroprotective capabilities of microglia, at least in early-stage HIV disease (137, 358).

Overall, microglia are the principal target of HIV-1 in the brain parenchyma, and when activated by HIV-1 or viral proteins, they secrete or induce other cells to secrete neurotoxic factors; this process is accompanied by neuronal dysfunction or apoptosis. Although HIV-1-associated CNS injury is a complex process and probably involves numerous pathways and neurotoxic agents, it is clear that activated microglia contribute greatly to this neuropathogenic process.

Cytopathic Virus. Human cytomegalovirus (HCMV) causes congenital encephalitis and encephalitis in patients with AIDS, but is rare in other immunocompromised individuals (15). Sequela of HCMV encephalitis include cognitive deficits, delirium, cranial nerve palsies, ataxia, and death (330). In patients with advanced AIDS, CNS infection due to HCMV results in two distinct neuropathological patterns: microglial nodular encephalitis and ventriculoencephalitis (138), which in turn have distinct clinical manifestations. Micronodular encephalitis consists of diffuse microglial cell synctia, aggregates of astrocytes, and cytomegalic cells. Although the clinical importance of HCMV-related micronodular formation is unknown, it was initially implicated as an important cause of HCMV-associated dementia in AIDS patients. The second major type of HCMV encephalitis, ventriculoencephalitis, is a necrotizing infection of the ependyma and subependymal layers, but focal necrosis may also be found deep in the brain parenchyma. Most cases of rapidly progressing HCMV brain disease appear to manifest as ventriculoencephalitis. The current state of knowledge regarding the pathogenesis of HCMV encephalitis is based largely on clinical features and postmortem studies, which reflect the final stages of disease progression. More recent work has evaluated the role of glial cell-mediated defenses against HCMV.

Key cells in the defense against HCMV include microglia and T lymphocytes (76, 297). There is evidence that murine CMV (MCMV) productively infects murine microglia and that IFN-γ suppresses this infection (319). In the human CNS, however, HCMV appears to infect primarily astrocytes and ultimately leads to cell destruction (153, 213, 235), although neuroepithelial precursor cells and differentiating neurons are permissive to HCMV infection, suggesting that the fetal CNS is especially vulnerable to HCMV-induced injury (233, 289). HCMV-infected astrocytes in turn secrete chemokines, primarily CCL2/MCP-1, that recruit microglial cells to the area of infection (76). Microglia, however, do not show signs of productive infection or cytopathic changes (213). Nonetheless, microglial cells, but not astrocytes, produce the antiviral cytokine TNF-α, which suppresses HCMV replication in astrocytes (76). Nonproductive infection of human microglial cells also elicits the production of the T-lymphocyte chemoattractant CXCL10/IP-10, which then recruits T lymphocytes to the area of infection (75). These activated T lymphocytes can then secrete IFN-γ, which also inhibits HCMV replication in astrocytes (74). The anti-inflammatory cytokines IL-10 and IL-4 inhibit this T-lymphocyte recruitment process, as does the HCMV-derived cmvIL-10 (180), which is a viral analogue of human IL-10. This viral analogue therefore demonstrates a mechanism for HCMV to negatively impact the antiviral capabilities of human microglia.

Infection with HCMV may impact coinfection with other agents, such as HIV. Early studies explored the relationship of HCMV to AIDS-related dementia. From these earlier evaluations, HCMV infection does not appear to contribute significantly to HAD (294, 366). However, the HIV entry coreceptor CCR5 is suppressed in astrocytes, microglia, and monocyte-
derived macrophages infected with HCMV (189). In monocyte-derived macrophages this process impaired HIV infection, although in astrocytes and microglia this was not apparent. In contrast, HCMV superinfection in astrocytes infected with certain strains of HIV actually increased p24 production, suggesting a stimulatory effect on HIV expression (234).

In summary, productive infection of astrocytes by HCMV appears to initiate a cascade of events that ultimately facilitates the antiviral activities of both microglial cells and activated T lymphocytes. This process can be diminished by anti-inflammatory cytokines as well as by HCMV gene products.

**Herpes simplex virus.** Herpes simplex virus (HSV) causes a devastating CNS infection in neonates and immunocompetent adults, which results in acute focal necrotizing encephalitis with severe neuroinflammation and swelling of the brain (214). Despite reductions in mortality with the use of acyclovir or vidarabine therapy, fewer than 20% of patients with herpes encephalitis recover without significant long-term neuropathologic manifestations (237, 331). The mechanisms responsible for the sequelae following herpes encephalitis appear to involve both direct virus-mediated damage and indirect immunemediated processes. During herpes encephalitis in both humans and experimental animal models, the virus load in the cerebrospinal fluid or brain tissue does not correlate well with the severity of structural damage or clinical findings (372). Studies have shown that long-term neuroimmune activation and cytokine production persist after HSV infection in patients (16) and after experimental infections in mice (59, 141, 247, 324, 325). These studies suggest that herpes encephalitis and its neuropathologic sequelae are related at least in part to an inflammatory process within the CNS.

Human HSV encephalitis has been modeled in rats experimentally by inoculation of virus in peripheral nerves (105, 370). HSV moves along peripheral neurons, achieving widespread distribution in the brain by days 8 to 10 postinfection. Granulocytes, T lymphocytes, and monocytes/microglia infiltrate the sites of infection early. Microglial cells express increased MHC class I and class II glycoproteins and have a wide distribution throughout the brain, including areas separate from the productive infection. In some instances, this focal microglial cell reaction remains for several weeks (105).

Productive viral infection by HSV is observed in cultures of purified primary human astrocytes as well as neurons and is ultimately cytopathic in both cell types (217). Despite a productive infection, unlike HCMV infection, neither of these cell populations produces chemokines or cytokines in response to HSV. In contrast, human microglial cells infected with HSV provide only limited replication followed by a rapid decline in infectious virus. This is associated with high levels of both the immediate-early antigen ICP4 and reporter gene expression (LacZ) from recombinant viruses, and a minority of infected microglial cells display late viral antigen (nucleocapsid) expression (217). Despite limited viral replication, cytopathic effects are evident in HSV-infected microglia, with death mediated through an apoptotic pathway. Additionally, microglia produce considerable amounts of TNF-α, IL-1β, CXCL10/IP-10, and CCL5/RANTES, together with smaller amounts of IL-6, CXCL8/IL-8, and CCL3/MIP-1α, in response to nonproductive infection. TNF-α inhibits HSV replication in astrocytes (217), and CXCL10/IP-10 inhibits replication in vivo (223) and in neurons (217).

Cytokine-induced neurotoxicity may be a mechanism underlying HSV-related CNS damage. Cytokines produced by microglia are toxic to neurons (61, 66, 68, 71). Although microglia-derived cytokine toxicity has not been elucidated completely as a mechanism for CNS damage as a result of HSV infection, activated microglial cells in HSV encephalitis patients do persist for more than 12 months after antiviral treatment (58).

Overall, productive HSV infection occurs primarily in astrocytes and neurons, and activated microglia appear to be involved both in inhibition of viral replication and in neurotoxicity. The contrasting forms of viral encephalitis produced by the herpesviruses CMV and HSV demonstrate the dual nature of microglia: they contribute to the defense of the CNS but may also bear responsibility for CNS damage.

**Bacteria**

**Lipopoly saccharide.** Neisseria meningitidis and Haemophilus influenzae are the most important causes of gram-negative bacterial meningitis. The major component of the outer membrane of the gram-negative bacterial cell wall, LPS (302), is a potent stimulus of many secretory products of microglia including cytokines (TNF-α, IL-1β, and IL-6), chemokines, and prostaglandins (4, 67, 260) and often has been used for activation of microglia in vitro (260). Examples of microglial cell stimulation by LPS include the induction of the chemokines CXCL8/IL-8 (Fig. 1F) and CXCL10/IP-10 (Fig. 1G). Although astrocytes are capable of cytokine production (278), microglial cells are substantially more responsive to LPS than are astrocytes (195). In contrast to microglial cells from adult brain tissue (357), amoeboid microglial cells express CD14 receptors, which, along with TLR4 (183), are the main plasma membrane binding sites for LPS-induced cytokine expression (117). Although LPS has been used as a classic activating agent, a recent study of rat microglia demonstrated that prolonged LPS exposure induces a distinctly different activated state from that in microglia acutely exposed to LPS (4). The microglial cells demonstrated a degree of adaptation to repetitive exposure to LPS, with diminishing TNF-α and NO, but persisting prostaglandin E2 (PGE2) production. Interaction between TLR4 and TLR2 may play a role in this adaptive response (183). These observations imply that microglia possess a level of plasticity when faced with bacterial products, which may ultimately affect the resolution of brain inflammation.

**Streptococcus pneumoniae.** Streptococcus pneumoniae is the most common and most serious cause of bacterial meningitis, with a mortality rate of 30% and neurologic sequelae in 30 to 50% of survivors (101, 287). S. pneumoniae meningitis is localized primarily to the subarachnoid space, but cytokines and chemokines are produced by cells lining the brain side of the BBB (313), most probably by microglia and astrocytes. Also, intracerebral edema, which is a major cause of death and sequelae in S. pneumoniae meningitis, may result from inflammatory processes triggered by intraparenchymal glia (313). Investigations into the role of microglial cells as the source of neuronal damage in pneumococcal meningitis have centered on murine and rat models.
The contribution of activated microglia to defense against the pneumococcus is probably similar to that for other infectious agents, i.e., functioning in the initial immune response and recruitment of cells of the peripheral immune system (neutrophils, monocytes, and T lymphocytes) to the site of infection. Since pneumococci can cross the BBB (303), microglia may respond directly to intact bacteria or to pneumococcal cell wall. In the murine model, the pneumococcal cell wall induces microglia to produce TNF-α, IL-6, IL-12, keratinocyte-derived chemokine, CCL2/MCP-1, CCL3/MIP-1α, CXCL2/MIP-2, and CCL5/RANTES, as well as soluble TNF receptor II, a TNF-α antagonist (144). The induction of these inflammatory mediators involves activation of the extracellular signal-regulated protein kinases 1 and 2 (ERK-1 and ERK-2) mitogen-activated protein kinase intracellular signaling pathway (144). The implications of this profile of cytokine release by microglia include recruitment of leukocytes into the CNS for the purpose of defense, as well as inflammatory mediator-induced neuronal damage. With the influence of IFN-γ produced by T lymphocytes that have entered the brain, the chemotactic profile shifts from favoring neutrophils to a preferential recruitment of monocytes and T lymphocytes (146), which is also seen during the clinical course of bacterial meningitis.

Neuronal damage in the rat model is caused at least partially by the production of NO by activated microglia and astrocytes, which is greatly attenuated by dexamethasone in vitro (176). More recent work suggests that permanent loss of neurons by the induction of apoptosis in the dentate gyrus of the hippocampus probably contributes to the poor outcome of pneumococcal meningitis (43, 386). Although neuronal apoptosis is triggered in part by the inflammatory process via caspase activation, pneumococci can also directly induce apoptosis in primary rat hippocampal and cortical neurons and in human microglial and neuronal cell lines (44). The proposed mechanism involves the release of apoptosis-inducing factor, leading to rapid and massive damage to neuronal mitochondria (44). Additional studies identified several pathogenic factors unique to S. pneumoniae that are involved in mediation of this apoptotic process. These include the exotoxins hydrogen peroxide (H2O2) and the pore-forming molecule pneumolysin, which induces apoptosis via translocation of intracellular calcium and apoptosis-inducing factor (45). Either pneumolysin or H2O2 is sufficient to trigger mitochondrial damage and apoptosis in vitro, and inactivation of these toxic mediators effectively prevents this damage (45).

**Staphylococcus aureus.** Brain abscesses are a serious CNS infection, accounting for 1 in every 10,000 hospital admissions in the United States (354). The most common etiologic agents of brain abscesses in humans are Streptococcus milleri and Staphylococcus aureus (230). A limited number of studies have addressed the factors involved in the acute CNS response to these organisms. An in vivo murine model of experimental brain abscess demonstrated that S. aureus leads to the rapid and sustained expression of numerous proinflammatory cytokines and chemokines (174). It also underscored the importance of both the neutrophil-attracting chemokine CXCL1/Gro-α and neutrophils in the acute host response to S. aureus in the CNS (174).

As immune effector cells of the brain, microglia facilitate neutrophil recruitment into the CNS. This was demonstrated by recent work of Kielian et al. (175) showing that murine microglia have bactericidal activity against S. aureus and that S. aureus is a potent inducer of TNF-α, IL-1β, and CXCL1/Gro-α gene expression in microglia. Also, a number of microglial cell genes were suppressed, including those for CXCR4, mannose receptor, and tissue inhibitor of metalloproteinase 2 (175). The findings by these authors also suggest that intact S. aureus and peptidoglycan boost TLR1, TLR2, TLR6, and CD14 expression in murine microglia, which may serve to augment microglial activation during CNS infection (175).

**Mycobacterium tuberculosis.** CNS tuberculosis accounts for 1 to 10% of all cases of tuberculosis and clinically manifests as meningeitis or intraparenchymal infection (tuberculoma); it carries a high mortality (87, 197). The causative agent in most cases is Mycobacterium tuberculosis, which appears to enter the subarachnoid space via rupture of an adjacent parenchymal tubercle rather than by hematogenous spread (197) as is seen in acute bacterial meningitis.

The limited research on microglial interactions with M. tuberculosis has focused on the mechanisms of ingestion and the factors that influence this process. A distinctive characteristic of M. tuberculosis is its capacity to enter and replicate within macrophages. Human microglial cells are productively infected with M. tuberculosis and may in fact be the principal cell target in the CNS (87, 280). In our laboratory we have found that challenge of purified human microglia and astrocytes is associated with selective infection of microglia by M. tuberculosis (Fig. 1H). We also have found that ingestion of nonopsonized M. tuberculosis by human microglia is facilitated by the CD14 receptor (280), although this appears not to be the case with human monocyte-derived macrophages (321). This receptor, along with the β2-integrin CD18 and TNF-α, is also involved in the formation of histologically characteristic multinucleated giant cells by porcine microglia infected with M. bovis (277).

A recent study demonstrates that human microglia are more efficient at ingesting M. tuberculosis than virulent and avirulent strains of M. avium and that following infection with M. tuberculosis, there is a significant, lasting inhibition of both IL-1 and IL-10 production (87). The authors suggested that mycobacterial infection induces immunosuppressive effects on microglial cells, which is more evident with more virulent species.

Examination of external influences on M. tuberculosis uptake by microglial cells has been limited. Although opiate addiction has been identified as a risk factor for clinical tuberculosis, treatment of human fetal microglial cell cultures with morphine stimulates the phagocytosis of nonopsonized M. tuberculosis through an interaction with G-protein-coupled receptors on microglial cells (281). It was suggested that such a phenomenon could favor the intracellular growth of tubercle bacilli.

**Borrelia burgdorferi.** Lyme disease has been associated with inflammatory damage within the CNS. However, little work has been done on the role of microglial cells in neuroborreliosis. Rasley et al. demonstrated that Borrelia burgdorferi stimulates the production of IL-6, TNF-α, and PGE2 by murine microglia (296). This effect was also shown to be associated with an increased expression of TLR2 and CD14, which are receptors known to underlie spirochete activation of other immune cell types. Their conclusion was that microglia are a source of inflammatory mediators following challenge with B.
*Anaplasma phagocytophilum* and that this phenomenon may play an important role during the development of neuroborreliosis.

**Nocardia asteroides.** An in vitro study examining murine microglia and astrocytes showed that *Nocardia asteroides* productively infects astrocytes but not microglia after phagocytosing the organism (30). In the course of a lethal infection, there appears to be a propensity for growth within the soma of neurons and their axonal extensions, and there is evidence of demyelination and axonal degeneration. It is postulated that this compartmentalization of nocardiae within neurons could contribute to their failure to induce an inflammatory response or a cytopathic effect (29). Overall, the role of microglia may simply be in clearing the organism in nonlethal infections.

**Fungi**

*Cryptococcus neoformans*. *Cryptococcus neoformans* is a ubiquitous, opportunistic fungus that has a pronounced predilection for the CNS, which is less likely to be the result of tissue tropism than the result of the ability of cryptococci to grow unimpeded in the CNS. Defending the CNS against this microbe requires both innate and adaptive immunity. CD4+ T lymphocytes are critical to the anticytotoxic capability of the murine CNS (152), and perivascular macrophages and microglial cells appear to cooperate in this defense against *C. neoformans*. Murine studies support this hypothesis by demonstrating that lymphocyte-mediated resistance to *C. neoformans* brain infection occurs through interactions with CD4+ T lymphocytes and replenishable perivascular macrophages that lie in close proximity to cerebral vasculature (3). Although T lymphocytes may augment the antifungal activity of parenchymal microglia via stimulation with inflammatory cytokines such as IFN-γ, expression of MHC class II on parenchymal microglia does not appear necessary for effective anticytotoxic activity. This observation argues that direct lymphocyte-yeast interactions, independent of MHC class II restriction, are not an important means of host resistance to *C. neoformans* in vivo.

In addition to the interaction of perivascular macrophages with T lymphocytes, the phagocytic role of microglia has been established. Microglia can ingest and inhibit the growth of *C. neoformans* (39, 192). Porcine (201) and murine (39) microglia ingest nonopsonized cryptococci, but opsonization is required for human microglia to ingest cryptococci (192, 203), and the ingested yeast can eventually lyse the microglial cell (193). Enhanced phagocytosis and killing of cryptococci by IFN-γ-stimulated microglia has been established in vitro in experiments with murine cells (39), but even though cryptococcal growth is arrested, actual killing of the cryptococcus does not occur in human microglia, even with the addition of IFN-γ (204). This animal species-related difference appears to be associated with the relatively inefficient generation of NO by cytokine-activated human microglia compared to that by murine microglia (64, 282).

In the absence of specific antibody, *C. neoformans* fails to elicit a chemokine response, but in the presence of specific antibody, human microglia produce CCL3/MIP-1α, CCL4/MIP-1β, CCL2/MCP-1, CXCL8/IL-8, and low levels of CCL5/RANTES via Fc-receptor activation (133). The cryptococcal polysaccharide component glucuronoxylomannan (GXM) does not induce a chemokine response even when specific antibody is present, and it actually inhibits CCL3/MIP-1α induction associated with antibody-mediated phagocytosis of *C. neoformans* (133). The authors of this study hypothesize that GXM-specific antibody complexes elicit different signals from those due to *C. neoformans*-specific antibody complexes and that soluble GXM found in the brain and cerebrospinal fluid of patients with cryptococcal meningoencephalitis may elicit an immunosuppressive effect by inhibition of chemokine production by microglia. Interestingly, GXM also elicits CXCL8/IL-8 production by human microglia but effectively blocks migration of neutrophils into the CNS (202).

*C. neoformans* elicits a wide range of tissue inflammatory responses, and there is evidence that this variability is due to both host immune status (191) and attributes of fungal cells, including the polysaccharide capsule and phenotypic switching (134). Granulomatous inflammation is the tissue response usually associated with control of infection (191), although in most AIDS patients, cryptococcal meningoencephalitis is associated with minimal inflammation (13).

In addition to antifungal therapy, other agents have demonstrable effects on cryptococcal-microglial cell interactions. Uptake of cryptococci by human microglia is enhanced by morphine via both μ-opioid receptors and complement receptors (203), whereas morphine suppresses porcine microglial uptake of cryptococci (335). Also, chloroquine enhances the anticytotoxic activity of the murine microglia-derived cell line BV2 in vitro (232).

**Parasites**

*Toxoplasma gondii*. Toxoplasmic encephalitis (TE) is a condition that affects AIDS patients as well as other immunocompromised individuals with defective cell-mediated immunity. In AIDS patients, TE is most often due to reactivation of latent infection, resulting in disruption of tissue cysts followed by proliferation of tachyzoites. This breakdown in the containment of latent cysts probably stems from impaired T-cell immunity as well as impaired IFN-γ production (258). A comprehensive review of host resistance to *Toxoplasma gondii* infections of the brain has been provided by Suzuki (344). Briefly, CD8+ T cells, CD4+ T cells, and NK cells work in concert with resident cell populations of the CNS, including microglia, astrocytes, and neurons, to suppress the proliferation of *T. gondii* tachyzoites in the CNS, primarily through the actions of IFN-γ. Furthermore, both host genetic factors and *T. gondii* strain differences play a role in the development of TE. Murine microglia, as well as astrocytes, neurons, and oligodendrocytes, are susceptible to infection with tachyzoites, and all but oligodendrocytes produce latent cysts after infection with bradyzoites (109). In the rat model, intracerebral replication of *T. gondii*, as well as spontaneous conversion of tachyzoites to bradyzoites, occurs primarily within neurons and astrocytes. Activated microglia appear to effectively inhibit growth (219).

Microglia are major effector cells in the prevention of *T. gondii* tachyzoite proliferation in the brain. Previous studies by our laboratory have shown that both murine (62) and human (64) microglia inhibit the proliferation of tachyzoites following treatment with IFN-γ plus LPS. NO mediates the inhibitory effect of activated murine microglia on intracellular replication
of tachyzoites. Simultaneous treatment of microglia with IFN-γ plus LPS and NG-n-monomethyl-l-arginine, which blocks the production of NO, abrogates their antitoxoplasmic activity (62). Furthermore, IFN-γ plus TNF-α inhibits T. gondii multiplication in a dose-dependent manner and TGF-β suppresses this antitoxoplasmic activity of murine microglia by interfering with NO generation (70, 278). Freund et al. (115) reported that murine microglia stimulated by IFN-γ and TNF-α inhibited T. gondii replication via both an NO-dependent and a separate IFN-γ-dependent mechanism. This separate mechanism was not associated with the creation of ROIs or degradation of tryptophan. Deckert-Schüler et al. (91) reported that murine microglia are activated primarily through IFN-γ. IFN-γ activates microglia in different ways (155, 345).

In contrast to the observations with murine microglia, our laboratory reported that NO is not involved in the antitoxoplasmic activity of activated human microglia (64). Rather, the host defense activity of human microglia against T. gondii is dependent primarily on the activating properties of IFN-γ, TNF-α, and IL-6, which reduced the entry of T. gondii into microglia. Once tachyzoites gain entry into human microglia, however, cytokine treatment has little or no effect on tachyzoite replication (64). In humans, therefore, CD4+ and CD8+ T cells may play an even more prominent role in the defense against T. gondii.

Plasmodium falciparum. One of the most serious complications of Plasmodium falciparum infection is cerebral malaria (CM), with approximately 1 million deaths annually (95). Pathological features of CM include cerebral venules filled with infected erythrocytes, microhemorrhages, local and global ischemia, and glial proliferation, which culminates in the formation of astrocyte and microglial aggregates called Dürck’s granulomas (95). CM is primarily a hematogenously derived infection, which places endothelial cells and the BBB as the first line of defense. It is the interplay of the parasitized erythrocytes with endothelial cells, disruption of the BBB, microhemorrhage, and formation of glial aggregates that is thought to promote clinical CM (95).

The pathogenesis of human CM is still debated and has been reviewed thoroughly elsewhere (95). One hypothesis is that CM is the result of an excessively vigorous immune response. CD4+ T cells, cytokines, and ROI are involved in the development of the cerebral complications of malaria (243). Within the murine CNS parenchyma, astrogliosis, degeneration of astrocytes, activation of microglia, an increase in the amount of c-fos, and an increase in TNF-α expression have all occurred in CM and may be important in the initiation and perpetuation of the cerebral complications associated with this disease (241). Additionally, examination of the cerebrospinal fluid of Kenyan children with CM demonstrated increased levels of excitotoxins (100).

Recent investigations have implicated microglia in the pathogenesis of CM. A prominent feature of human CM is widespread activation of microglial cells (315). The most widely utilized murine model of CM is a “fatal” model involving CBA/T6 mice infected with P. berghei ANKA (CBA-PbA). This fatal murine cerebral malaria (FCM) model exhibits neurological and histological changes similar to those in human CM (242). Essential components of the immunopathogenesis associated with FCM are T lymphocytes, monocytes, and cytokines, especially TNF-α. Using a retinal whole-mount technique, microglia were found to have morphologic changes very early in FCM (242). These changes included a decrease in process length, an increase in soma size, an increasingly amoeboid appearance, and vacuolation. Redistribution of the microglia to the venous side of the vascular endothelium, with compromised barrier properties, was also noted (242). These less ramified microglia increased in numbers until the terminal stage of the disease (242). TNF-α production by microglia, astrocytes, peripheral blood monocytes adherent to the meningeal vessels, and cerebrovascular endothelial cells prior to onset of cerebral symptoms was also detected (243). Other investigators have also found TNF-α production in human CM cases at autopsy, along with IL-1β (53). More specifically,
human microglia were also found to express the immunosuppressive cytokine TGF-β1 in Dürck’s granulomas (95).

An experimental increase in BBB permeability in the FMCM model was sufficient to elicit thickening of microglial processes and redistribution of microglia toward the vasculature, but microglia with amoeboid and vacuolated morphology were not observed (241). Microglia are not activated by circulating malaria parasites in the absence of an immune response, however. This was demonstrated by early treatment with dexamethasone, which resulted in fewer activated microglia, and this coincided with less severe neurological symptoms without affecting parasite growth (241). This led the authors to conclude that a likely course of events in FMCM involves the malaria parasite producing a vasoactive factor that increases BBB permeability, which initiates a redistribution of microglia and astrocytes toward the blood vessels. Concurrently, dexamethasone-sensitive immunopathological events further activate microglia, which release TNF-α and eventually lead to irreversible cerebral complications (241). The serine protease urokinase-type plasminogen activator receptor, which is important in cell adhesion and spreading, was isolated to macrophages and microglia in the same sites (106), as well as endothelial cells (95). These results suggest that urokinase-type plasminogen activator receptor may be important in microglial migration and/or the breakdown in the BBB by acting as an adhesion molecule for parasitized erythrocytes.

Both β-hematin and hematin, which are synthetic products that mimic hemozoin (malarial pigments) normally produced by the malarial parasite, induce a dose-dependent inhibition of murine macrophage production of TNF-α and NO, but not IL-1 (347). These malarial products also trigger the production of NO (347). The production of TNF-α and NO was not altered in murine microglia, however, and β-hematin had less of an oxidative stress (347, 348).

Heme oxygenases function as antioxidants by degrading heme to carbon dioxide, iron, and biliverdin. In human CM, the inducible antioxidant heme oxygenase 1 was isolated to macrophages and ramified microglia located around Dürck’s granulomas and petechial hemorrhages induced by *P. falciparum* (314). However, Taramelli et al. (348) found that β-hematin is resistant to heme oxygenase 1 and that heme-iron-mediated oxidative stress may contribute to malaria-induced immunosuppression. In essence, microglia appear less susceptible than macrophages to the immunomodulatory properties of malarial pigments.

Overall, investigation of the pathogenesis of CM largely revolves around understanding the entry of parasitized erythrocytes through the BBB and the subsequent proinflammatory response that occurs. Evidence to date supports the notion that microglia are involved in the neuropathogenesis of CM.

*Trypanosoma brucei*. Human African trypanosomiasis (HAT) is caused by *Trypanosoma brucei gambiense* or *T. brucei rhodesiense*. Infection with these trypanosomes is associated with severe neurological complications, including sleep disturbances, which are eventually fatal if untreated. Unique factors associated with HAT include a pronounced antigen variation associated with its variable surface glycoprotein, its ability to penetrate into the CNS and take advantage of its immune-privileged status, the toxicity of therapy, and the occurrence of a fatal posttreatment reactive encephalitis. Initial infection is associated with a hematolymphatic first stage, followed by a second stage, which is characterized by CNS invasion.

In the rat model, early infiltration of the brain occurs in areas in which the BBB is not well developed: the sensory ganglia, area postrema, pineal gland, and median eminence (162). Later, the BBB is disrupted more diffusely (288). Astrocyte activation is one of the first signs of neurological involvement (162). Late-stage HAT and the development of PTRE are histologically characterized by perivascular cuffing, nonspecific lymphoplasmacytic meningoencephalitis, microglial hyperplasia, reactive astrocytes, and infrequent demyelination (162).

Although early activation of astrocytes occurs diffusely, marked activation of microglial cells occurs in a discrete distribution in advanced disease (79). Areas of activation include the cerebral cortex, septum, and hypothalamus and are not associated with neuronal damage histologically (79). The onset and progression of microglial cell activation also correlates with the onset and progression of sleep disturbances, leading to speculation about the role of microglia in this prominent clinical manifestation of HAT (79).

Cerebrospinal fluid from patients with African trypanosomiasis induces apoptosis in both human microglial and endothelial cells (128), and cerebrospinal fluid from late-stage disease induces apoptosis at higher levels in microglial cells than does that from early-stage disease. Also, the levels of soluble Fas ligand and anti-Fas antibodies, both potent inducers of the Fas (CD95) apoptotic signaling pathway, are higher than levels found in cerebrospinal fluid from uninfected subjects (128).

Experimental infection with *T. brucei brucei* in rats shows early production of CXCL2/MIP-2, CCL5/RANTES, CCL3/MIP-1α, and, to a lesser extent, CCL2/MCP-1 from both microglia and astrocytes. Production of these chemokines is probably involved in recruitment of T lymphocytes from the circulation into the CNS. Interestingly, *T. brucei brucei* releases a lymphocyte-triggering factor that stimulates CD8+ T cells to secrete IFN-γ, which favors the growth and proliferation of the trypanosomes (270). *T. brucei brucei* also induces iNOS in both astrocytes and microglia in the murine model, and the generation of NO is potentiated by IFN-γ treatment (127).

In essence, microglia appear to be advantageous in combatting HAT through their T-lymphocyte-recruiting capabilities, but they are potentially deleterious, as demonstrated in their temporal relationship between activation and progression of clinical disease.

*Acanthamoeba castellani*. *Acanthamoeba castellani* species are opportunistic parasites typically associated with keratitis. Cerebral acanthamebiasis (granulomatous amebic encephalitis) occurs primarily in immunocompromised patients, including those with AIDS, and is pathologically characterized by focal granulomatous lesions in the CNS as a result of an immunological response by the host to the presence of trophozoites and cysts. Very few studies have actually investigated the role of microglia in this relatively rare clinical disease.

Murine microglia activated with either recombinant prolactin or LPS plus IFN-γ produce synergistic amebastatic activity; infection with *Acanthamoeba castellani* in vitro stimulates TNF-α, IL-6, and IL-1β production (33, 34). More specifically, TNF-α and IFN-γ-activated microglia have amebicidal activity, whereas those activated by IL-6 and IL-1β with or without...
IFN-γ have only amebastatic activity (32). The NO-dependent pathway does not appear to be involved in this amebastatic activity. It appears that activators of microglia such as LPS, prolactin-prolactin receptor complex, and IFN-γ/IFN-γ receptor complex, through the IFN-γ receptor on microglia, lead to induction of inflammatory cytokines, which in turn trigger antimicrobial activity against A. castellani infection in the brain.

Prions

The predominant form of human prion disease, Creutzfeldt-Jakob disease (CJD), occurs spontaneously with no known cause, although there are also inherited and iatrogenic forms of CJD (293). Additional human prion diseases include Kuru, Gerstmann-Straussler-Scheinker syndrome, and fatal familial insomnia, as well as scrapie in goats and sheep and bovine spongiform encephalopathy in cattle (299). The recently described variant CJD (374), which is clinically distinct from classic CJD, has similarities to A. castellani, although a causal link has been elusive (48). Excellent and detailed reviews of prion disease pathogenesis can be found elsewhere (42, 48, 78, 299).

Pathologically, prion disease exhibits spongiform degeneration, prion plaques, astrogliosis, microglial activation, and neuronal apoptosis, which have been linked to clinical disease (360). The pathogenesis of prion diseases largely remains an enigma, with supporters of a protein-only hypothesis and a viral hypothesis continuing to pursue investigative work (78). Despite the controversy, microglia are emerging as a potential mediator of neurodegeneration in prion disease (37, 48).

Prion protein (PrP<sup>sc</sup>), an abnormal derivative of the normal extracellular glycoprotein (PrP<sup>res</sup>) with specific conformational changes, is proposed as the infectious agent in prion disease, and specifically the cause of neurodegeneration in prion disease (42). This conformationally abnormal protein has become the most important diagnostic marker in prion disease (55). Normal PrP<sup>res</sup> is expressed in the CNS by neurons (182), astrocytes (255), and microglia (49), although it is neuronal expression that appears to be important for accumulation of PrP<sup>res</sup> in the CNS (48). In the scrapie model, PrP<sup>res</sup> plaques colocalize with microglia (375). This plaque formation precedes microglial activation, which in turn precedes neurodegeneration (375). At the microscopic level, neuronal apoptosis and neuronal death in general are preceded by microglial activation both in vitro and in vivo (37, 126). Recently, murine microglia demonstrated migration toward PrP<sup>res</sup> aggregates in vivo in a manner that at least partially relied on neuronal and astrocyte chemokine production (CCL5/RANTES and CCL4/MIP-1β) and the activation of microglial cell chemokine receptor CCR5 (227). Alternatively, in the viral hypothesis, murine models also show that microglia have a high level of infectivity by the so-called CJD agent, which may be a means by which this agent is dispersed and possibly replicated within the CNS (21).

Several experimental studies of PrP<sup>res</sup> have focused on its neurotoxic portion, called PrP106-126 (113), which is taken up by neurons, astrocytes, and microglia (238). In the mouse model, neurodegeneration by PrP<sup>res</sup> or PrP106-126 appears to require the presence of specific components: neuronal PrP<sup>res</sup>, microglial PrP<sup>res</sup>, the presence of activated microglia, and possibly astrocyte PrP<sup>res</sup> (42, 52).

Neurons expressing PrP<sup>res</sup> become more sensitive to microglial neurotoxins when exposed to PrP106-126 activity (52). PrP106–126 interacts with neuronal PrP<sup>res</sup>, which effectively inhibits it and subsequently increases neuronal sensitivity to oxidative stress (48). It has been suggested that PrP<sup>res</sup> expression by microglia increases their sensitivity to various activating stimuli and also to proliferation induced by astrocyte-derived cytokines (48). Once activated, microglia are capable of either directly or indirectly affecting neurons (48).

Astrogliosis is a hallmark of prion disease and typically precedes neurodegeneration (48). In fact, it is thought that astrocytes are the cells in which PrP<sup>res</sup> first replicates in the CNS (98) and contribute to PrP106–126-induced neurotoxicity via decreased glutamate uptake by astrocytes (50, 52). Both microglia (51) and astrocytes (114) proliferate in response to PrP106–126, although astrocyte proliferation is microglia-dependent and requires PrP<sup>res</sup> (51). PrP106–126 induces microglial production of IL-1 and IL-6 (286), which in turn contributes to the multifactorial process of astrogliosis (140). Another component of PrP<sup>res</sup>, fibril lar mouse PrP106–132, induces IL-6 and TNF-α, but not IL-1, in adult human microglia (361), suggesting species- and/or age-related differences with regard to cytokine profiles. Thus, one could envision the role of microglia in neuronal damage as occurring indirectly through the proliferation and activation of affected astrocytes (48). In addition to its contribution to astrogliosis, however, a temporal relationship was recently established between activation of microglia, IL-6 production, and microglia-related killing of PrP-treated neurons (28).

Finally, CJD-infected murine microglia display a unique gene expression profile linked to IFN-γ signaling, complement cascade components, lipid and cholesterol metabolism, and several proteases compared to other activating agents (20). This uniqueness held up even compared to expression profiles of microglia exposed to PrP<sup>res</sup> (20). This provides some insight into what may be considered early microglial changes in CJD.

In summary, activation of microglia by abnormal PrP<sup>res</sup> may precipitate neuronal damage either directly or indirectly (48). Alternatively, microglia may be a carrier or replicative source of the CJD agent (21, 226). As described previously, evidence for this is drawn predominantly from in vitro models. Evidence of microglial cell involvement in human disease, unfortunately, is limited. Despite this, continued focused research on this cell may elicit more conclusive evidence of its role and may in the end provide a target for therapy.

MICROGLIA IN NEUROINFLAMMATORY AND NEURODEGENERATIVE DISEASES

Although researchers in the early 1900s had a remarkable appreciation of the scavenger and host defense functions of microglia, the concept of activated microglia contributing to brain damage did not emerge until the end of the 20th century (10, 25, 121, 129, 199, 308, 336). As has been mentioned in several sections of this review, substantial support for this concept has come from studies of neurotoxic mediators released from microglia in response to microorganisms or microbial products. A parallel literature has incriminated activated microglia in the neuropathogenesis of inflammatory and degenerative diseases that have been suggested to be triggered.
by infectious agents, such as multiple sclerosis (80, 107, 139, 244, 248, 262, 332), Alzheimer disease (22, 35, 57, 104, 142, 236, 248, 252, 262, 271, 304, 318, 346, 367, 380, 381), Parkinson disease (118, 119, 181, 188, 207, 208, 363), amyotrophic lateral sclerosis (181), and Huntington disease (312), as well as in brain injury due to ischemia and trauma (130, 166). In recent studies, activated microglia were demonstrated to manifest pathological effects on neural stem cells (104, 251), the progenitors of neurons both in the developing brain and in the adult brain, where they play an important role in new-memory formation.

Despite considerable evidence that activated microglia can damage or induce apoptotic death of neurons, either directly through the release of toxic mediators such as cytokines and free radicals or indirectly by attracting activated T cells, monocytes, and neutrophils into the CNS, controversy exists over the neurodegenerative versus neuroregenerative roles of microglia (225, 249, 340, 342). The finding of activated microglia in areas of neuronal loss, such as in amyloid plaque deposits seen in the brains of patients with Alzheimer disease, does not necessarily indicate a causal role in the associated neurodegeneration. As pointed out earlier, the facial nerve anomaly model points to a trophic role of activated microglia in neuroregeneration (262, 340). A countervailing concept of how microglia may be involved in Alzheimer disease has also been proposed, i.e., the loss of such a restorative or supportive function by senescent microglia (340). A vaccine trial with amyloid beta-peptide, a putative neurotoxic moiety in Alzheimer disease, has provided conflicting interpretations of the neuroprotective versus neurotoxic roles of microglia (253). In support of the notion that activated microglia contribute to neuronal support, a majority of patients showed clinical improvement, suggesting that microglia were removing amyloid beta-peptide from areas of diseased brain. However, the trial was stopped early after several patients developed severe encephalitis, which hypothetically could be attributable to activation of microglia by amyloid beta-peptide. Thus, the evidence to date suggests that activated microglia function as a “double-edged sword,” with neuroprotective features predominating in the healthy nervous system and neurodestructive properties observed in various disease states.

MICROGLIA AS A PHARMACOLOGICAL TARGET

Based on observations that microglia can facilitate the growth of certain intracellular microorganisms such as, HIV-1, M. tuberculosis, and T. gondii, more attention should be paid to the development of immunotherapeutic and chemotherapeutic strategies that would enhance the intracellular killing of such pathogens. In addition to assessments of the efficacy of conventional antibiotics and cytokines performed in vitro using microglial cell cultures, consideration should be given to the development of drugs that would alter the microglial cell in ways that make it less susceptible to infection or less hospitable for intracellular microbial growth. An example of this approach has been demonstrated in studies of benzodiazepines which suppress HIV-1 replication in microglia by inhibiting NF-κB activation (215).

In disease states in which activated microglia appear to contribute to inflammation-induced injury of the nervous system, anti-inflammatory agents should be investigated. So far, dexamethasone, a glucocorticoid which potently inhibits the generation of many inflammatory mediators by activated microglia, has been demonstrated to be effective as adjunctive therapy for pneumococcal meningitis (359) and tuberculous meningitis (56). Trials of dexamethasone and other anti-inflammatory agents should be considered for other CNS infections where activated microglia appear to contribute to brain damage. Preliminary data suggest that drugs such as minocycline (104, 352), naloxone (60, 206), dextromethorphan (209), and agents that up-regulate glutamate receptors (137, 349) have neuroprotective properties through their effects on microglial cells. Challenges in designing studies to test the therapeutic benefit of agents such as these are substantial and include pharmacological issues (drug penetration into the CNS, optimal dose, and timing of drug administration) and biological considerations (modulating the deleterious aspects of activated microglia without seriously hampering their critical role in host defense and their neuroregenerative properties).

MICROGLIA: THE KNOWN UNKNOWN

At the dawn of the 21st century, the physiological role and pathophysiological importance of the mesodermal element of the brain parenchyma—microglial cells—are topics of intense research interest. The field of microglia biology has benefited greatly by the large number of keen minds preoccupied by these cells in the early years of the 20th century and by a dramatic renaissance of interest in microglia at the turn of the century, fostered by technological advances such as the development of reliable techniques for studies of homogenous populations of microglia in culture, monoclonal antibodies for many cell receptors, and assays for a large number of cytokines and chemokines. Although a clearer understanding of the role of microglia in defense against and neuropathogenesis of CNS infections has emerged from this prodigious research effort, much remains unknown about critical aspects of microglia-microbe interactions.

Given the large number of neurotropic pathogens (Table 3), the involvement of microglia in defense and neuropathogenesis has been elucidated for a surprisingly limited number of microorganisms, as has been made evident in this review. Not surprising, however, is the amount of attention paid to HIV-1, but even in this case much remains to be discovered. The tropism of HIV-1 for microglia appears to be shared by some but not all intracellular microorganisms, and many of the neurotoxic mediators produced by HIV-1-infected or viral protein-stimulated microglia have been implicated in the neuropathogenesis of other neurotropic pathogens. Unlike HIV-1, the intracellular replication of some microorganisms is halted in activated microglia. Nevertheless, relatively little is known about the intracellular localization of these infectious agents, and the contribution of oxygen-dependent and oxygen-independent antimicrobial mechanisms of microglia is poorly understood. Studies of the iNOS system of rodent versus human microglia points to the potential importance of animal species differences:

Micronodular lesions, composed of aggregates of microglial cells, are often identified on examination of the CNS at autopsy, as a result of infection from a variety of bacteria, pro-
toxoa, and, especially, viruses. This phenomenon is particularly prominent in patients with AIDS, where microglial nodules are considered one of the histopathological hallmarks of HIV-related brain disease, but may also be seen as a consequence of opportunistic infections by other intracellular pathogens. Fusion of microglia (syncytia) is also observed in vitro following infection with HIV-1 (Fig. 1E). A recent pathologic series of specimens from AIDS patients with microglial nodules found that productive HIV infection was found in 55.1% of microglial nodules, T. gondii was found in 34.1%, HCMV was found in 29.1%, and multiple opportunistic agents were found in 9% (261). While the formation of these nodules is often observed, the mechanism behind the formation of these multinucleated giant cells is not completely understood. In our laboratory, CD14, the β2-integrin CD-18, and TNF-α were found to be involved in the formation of multinucleated giant cells by porcine microglia in response to infection with M. bovis (277).

The role of microglia in directing T-cell trafficking into the brain is just beginning to be appreciated. The relative importance of activated T cells, as well as other cells of the somatic immune system, in defense of the brain has only recently been considered. Also, the biological and pathophysiological significance of the anatomical heterogeneity of microglia is unknown. While in vitro studies of isolated microglia have contributed many insights into microglia-microbe interactions, these observations need to be interpreted with caution, since in the nervous system microglia are in close proximity to neurons and are surrounded and greatly outnumbered by astrocytes. An area of gaping ignorance is the nature of the complex influence of neurons, astrocytes, and lymphocytes on microglia and vice versa in vivo.

If the escalation of knowledge witnessed in the past decade is any indication of what is to come, we can anticipate answers to many of the questions about the role of microglia in the healthy brain and in disease states. It seems likely that future studies of the roles of microglia in infectious diseases of the CNS will also shed light on the pathogenesis of neuroinflammatory diseases and neurodegenerative disorders which afflict a growing number of elderly individuals. It is also plausible that future studies will yield some surprises regarding the role of microglia and their involvement in pathologic processes such as chronic pain (356, 369), drug dependence, and neuropsychiatric disorders.

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