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Review Article

Immunology primer for neurosurgeons and neurologists part 2: Innate brain immunity

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Abstract

Over the past several decades we have learned a great deal about microglia and innate brain immunity. While microglia are the principle innate immune cells, other cell types also play a role, including invading macrophages, astrocytes, neurons, and endothelial cells. The fastest reacting cell is the microglia and despite its name, resting microglia (also called ramified microglia) are in fact quite active. Motion photomicrographs demonstrate a constant movement of ramified microglial foot processes, which appear to be testing the microenvironment for dangerous alteration in extracellular fluid content. These foot processes, in particular, interact with synapses and play a role in synaptic function. In event of excitatory overactivity, these foot processes can strip selected synapses, thus reducing activation states as a neuroprotective mechanism. They can also clear extracellular glutamate so as to reduce the risk of excitotoxicity. Microglia also appear to have a number of activation phenotypes, such as: (1) phagocytic, (2) neuroprotective and growth promoting, or (3) primarily neurodestructive. These innate immune cells can migrate a great distance under pathological conditions and appear to have anatomic specificity, meaning they can accumulate in specifically selected areas of the brain. There is some evidence that there are several types of microglia. Macrophage infiltration into the embryonic brain is the source of resident microglia and in adulthood macrophages can infiltrate the brain and are for the most part pathologically indistinguishable from resident microglia, but may react differently. Activation itself does not imply a destructive phenotype and can be mostly neuroprotective via phagocytosis of debris, neuron parts and dying cells and by the release of neurotrophins such as nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF). Evidence is accumulating that microglia undergo dynamic fluctuations in phenotype as the neuropathology evolves. For example, in the early stages of neurotrauma and stroke, microglia play a mostly neuroprotective role and only later switch to a neurodestructive mode. A great number of biological systems alter microglia function, including neurohormones, cannabinoids, other neurotransmitters, adenosine triphosphate (ATP), adenosine, and corticosteroids. One can appreciate that with aging many of these systems are altered by the aging process itself or by disease thus changing the sensitivity of the innate immune system.

Key Words: Immune surface receptors, immunoexcitotoxicity, innate immunity, microglia, microglial priming

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SUMMARY OF PART I: IMMUNITY PRIMER

In part one, we explored the anatomy and functional activation of the various components of the systemic immune system. Basically, systemic immunity is divided into an innate system and an adaptive system. These two systems do not act independently, but rather exhibit intimate interactions so as to initiate a coordinated attack. The innate system reacts rapidly, acting as the first line of defense when the interior of the body is invaded, whereas the adaptive system reacts slower, but with far greater specificity – that is, it can identify individual molecular differences in invading organisms and other antigens.

Next to the central nervous system (CNS), the immune system is one of the most complex systems in the body. This complexity extends to its individual components, especially the various cells involved in immunity. The innate immune system contains a number of specialized immune cells located in tissues lining the respiratory air passages, the gastrointestinal (GI) tract's mucosa and the mucosal lining of the urogenital tract. It is here that most invasions take place. Combined with the physical barriers of the skin and mucosal cell layers, these innate immune cells provide a formable protective defense.

The adaptive immune system utilizes a system of presentation of invading antigens that involves specialized molecules such as complement, to aid in molecular identification for future reference. Macrophages within tissues play a major role in transporting the antigen to the regional lymph nodes so that they can be presented to lymphocytes where tagging for future reference occurs. Certain lymphocytes store memories of the antigen for later identification, should the same antigen reinvade the body. This then allows for very rapid responses, since the complex identification and recognition process can be avoided.

Of special importance to this discussion is the communication between the systemic immune system and the brain's own special innate immune system. Previous beliefs about the brain being an immunologically privileged site have now been modified once it was realized that this immune barrier was incomplete.

The brain does not contain its own adaptive immune system, but rather can transport antigens to regional cervical lymph nodes that can process the antigen and then direct activated lymphocytes and other immune cells back to the brain substance. Immune chemokines from microglia and astrocytes can direct systemic immune cells to the brain as well. In addition, the vagus nerve acts as a rapid response system to turn on brain innate immune cells when inflammation occurs within the GI tract or abdominal cavity. This two-way communication between the brain and the systemic immune system is critical. One can easily see that when the body is invaded, the brain must be rapidly informed of the invasion so that it can gear up its innate immune system in preparation of a possible invasion.

This gut – brain and systemic immune axis each play a major role in a number of neurological disorders, especially in the case of neurodegenerative diseases. We now know that when an infection or inflammatory condition occurs systemically, microglia are activated within specific brain areas within minutes. In fact, even minor surgical procedures have been shown to trigger microglial activation within the brain. This is especially important in treating patients with multiple injuries, since microglial activation worsens the neurological picture if prolonged or too intense.

Along this line, it should be appreciated that the immune system, both systemic and within the CNS, contains a great number of fail-safe systems and feedback controls to prevent over-activation of the immune process. In addition, complex cell signaling mechanisms are utilized to switch the brain's microglia so as to tailor the immune cell to the particular function that is needed. For example, with brain trauma microglia migrate to the site of the injury and are switched to a phagocytic phenotype so as to remove cellular debris. They can then be switched to a reparative phenotype to aid in repairing the damage. It is now apparent that dysfunction of this switching mechanism can play a major role in a number of neurological disorders should the microglia become stuck in a neurodestructive mode.

It is also important to appreciate that systemic immune cells, particularly macrophages, frequently enter the brain from the blood stream and once inside the brain take on the appearance and function of resident microglia. Under certain conditions, such as with brain trauma and CNS infections, large numbers of neutrophils, lymphocytes, mast cells, and macrophages enter the CNS. The functional state of these immune cells can make the difference between progressive brain destruction or a reversal of immunoexcitotoxicity. For example, macrophages can assume a primarily cytotoxic phenotype called M1 or an antiinflammatory phenotype called M2. Likewise, lymphocytes can be either cytotoxic or suppress brain cell inflammation and injury. The antiinflammatory lymphocytes, called regulatory T-cells or Tregs, are now recognized to play a critical role in protecting the brain. In fact, we now know that early assumptions concerning the pathological role of invading lymphocytes in experimental allergic encephalomyelitis (EAE), for example, were mistaken and that these were mostly Tregs attempting to combat the out of control inflammatory response.

Finally, several hormones and neurotransmitters also play a critical role in controlling immune reactions. One of the critical control systems is the immune–hypothalamo–pituitary axis, with corticosteroids playing a major role.

Links between systemic immune activation and microglia within the hypothalamus trigger a release of corticosteroids, which under most conditions, dampens the immune reaction, both systemically and within the brain – but not always. Under certain conditions, these hormones can worsen inflammation.

Frequently forgotten is the fact that norepinephrine, epinephrine, and acetylcholine play a major role in dampening inflammation, especially within the CNS. A loss of acetylcholine levels in the Alzheimer's brain, for example, would significantly enhance any inflammatory reaction that is ongoing and may explain in part why acetylcholine agonists improve symptoms. With this brief review, now let us examine more closely the innate immune reactions within the CNS.

PART II: MICROGLIA

In 1932 Pio del Rio-Hortega introduced the concept of the microglia with incredible accuracy in the publication *Cytology and Cellular Pathology of the Nervous system*, citing many characteristics that would not be agreed upon until over half a century later. For instance, he accurately postulated that microglia entered the brain during early neurodevelopment and that these cells were of mesodermal origin. Further, that the guiding structures for the embryonic precursors included blood vessels and white matter tracts, which allowed these cells to migrate to all areas of the brain.

He was also the first to suggest that in the undisturbed brain microglia assumed a ramified (resting) morphology and upon disturbance or encountering specific brain pathology, they were morphed into an amoeboid phenotype. Del Rio-Hortega suggested that these cells could not only migrate within the brain toward areas of injury, but also could proliferate and carry out phagocytosis. The only error he made was to assume that microglia were evenly distributed throughout the brain. We now know that there is a heterogeneous distribution, with the highest concentrations in the gray matter and particularly within the limbic areas (hippocampus, amygdala, and entorhinal cortex) and substantia nigra, which has the highest microglial density of any area of the brain.[89,92]

Largely ignored until quite recently, microglia have now become the focus of a great deal of research. Newer studies suggest that microglia form a part of the tripartite synapse along with astrocyte foot processes and the synapse itself. Time lapse two-cell microphotography, extending up to 10 hours of observation, demonstrate that ramified microglia, the so-called resting phenotype, actually are quite active, with continuous extension and retraction of foot processes around the synaptic neuropile.[111] Active phagocytosis of cellular debris and chemicals was observed, suggesting a state of continuous surveillance of the microenvironment. This allows the microglia to maintain homeostasis and react rapidly when significant microenviromental threats exist. Microglia, along with astrocytes, also play a major role in maintaining low levels of extracellular glutamate, so as to prevent excitotoxicity and disturbed neural signals.

These newer studies also demonstrate that microglia are not uniform in their activity but exist as a number of subtypes, which offers an extensive program of activation states in response to a wide number of dynamic pathological conditions. In addition, microglia, as mentioned, are heterogenous in their distribution. Microglia found along brain–blood vessels are often found in an activated state and form a particular immunological barrier for the brain in conjunction with the blood–brain barrier (BBB).[88] Microglia are also concentrated at sites of incomplete BBB function, such as the circumventricular organs (CVOs), such as the organum vasculosum of the lamina terminalis, subcommissural organ, subfornical organ, area postrema, posterior pituitary, median eminence, pineal, and choroid plexus, as these are sites of entry of blood-borne invaders and even large molecules.[20]

Under pathological conditions, resident microglia may not only proliferate, but also can migrate great distances and accumulate near the sites of pathologic damage. Activation stages of microglia are not all or none, rather they are capable of assuming gradations of activation, which also includes priming phenotypes [Figure 1]. Likewise, we see phenotypes that are in various states of activity, including primarily phagocytic and neuroprotective; combined cytotoxic and neuroprotective or predominantly cytotoxic and destructive.[98,125] It is not clear whether these phenotypes are based purely on individual subtypes of microglia, or various activation

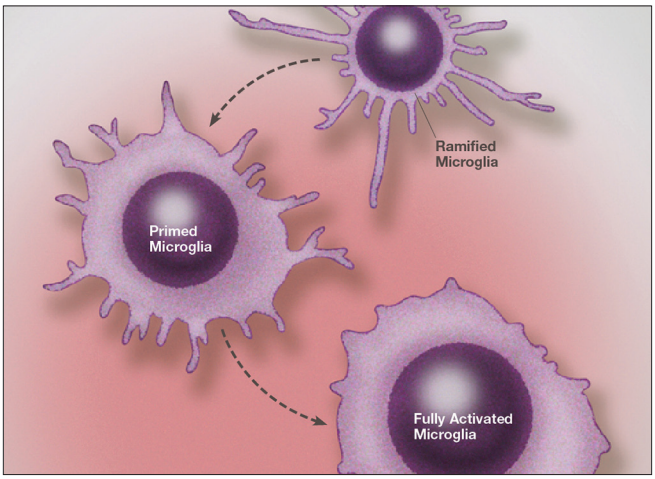

Figure 1: Illustration of the stages of microglial activation from a resting (ramified) state, to a primed state and finally to a fully activated phenotype. The exact phenotype and physiology of each stage of activation is determined by a number of extraneuronal molecules and environmental conditions

states of a uniform type of microglia in response to the microenvironmental cues. Also likely are dynamic fluctuations in these activation phenotypes during the evolving pathological process. A number of stimulatory molecules within the microenvironment seem to determine the activation state, and one can see that as the microenvironment changes, the activation state of the microglia changes as well.

We are learning that much like the peripheral immune system, the brain's innate immune system is enormously complex. Further complexity is added when we consider the interaction between the innate immune system and the adaptive immune system composed of invading immune cells and immune factors from the periphery.

Developmental anatomy of microglia and of the innate CNS immune system

It is now accepted that microglia are derived from mesodermal/mesenchymal tissues, primarily myeloid cells from the bone marrow. These cells migrate to the CNS during the first trimester and throughout the early part of the second trimester in the human. There is evidence that during these stages microglia consist of two populations, which include (a) myeloid/mesenchymal cells and (b) a developmental and transitory forms of fetal macrophages.[35] These cells enter the fetal brain, traveling along blood vessels and nerve tracts and once within the parenchyma of the brain transform into resting microglial phenotypes. A population of amoeboid microglia is observed during later embryogenesis that appears to play an important role in synaptic pruning. For example, a recent study by Paolicelli *et al*. demonstrated a central role for microglia in synaptic pruning and circuit development in the developing embryonic brain.[116] Previous evidence suggested that resting microglia constantly monitor synaptic function and play a role in neurotransmission, but also were capable of synaptic stripping, even in the adult brain.

Clinical Correlation: Fractalkine, a cytokine modulator of microglial proinflammatory activation, and its receptor, located on microglial cell membranes, in general suppress inflammatory signaling. In the Paolicelli et al. study they found that microglia via stimulation by fractalkines, modulated synaptic pruning during neural development and that deficiencies in fractalkine or its receptor led to excessive synaptic connectivity and abnormal brain development. This condition also produced excessive sensitivity to excitatory neurotransmission and excitotoxicity-related disorders, such as seizures. While other factors are playing a role in embryonic brain pruning, such as the compliment cascade components C1q and C3, during the early stages of neurodevelopment, fractalkine is the primary factor.

While macrophages enter the developing brain in significant numbers; in the adult brain such an invasion is quite rare in the undisturbed brain. Under pathological conditions invasion of leukocytes is dramatically increased.

Molecular pathophysiology of microglia

Microglia membrane receptors, second messengers, and cell signaling

There many cell signaling pathways from receptors on the cell surface connecting to the genes in the nucleus. These signaling pathways are called second messengers, a collective term referring to a number of molecules in the signaling pathway. When the cell surface receptor is engaged by its specific stimulating extracellular activator, the intracellular signaling pathway is activated leading directly to the nuclear genes. These genes or gene, when activated, lead to the production of proteins that are made in the cytoplasm directed by mRNA, which copies the sequence of the gene in the nucleus and then travels to the cytoplasm to participate in the manufacture of the gene-specific protein on the ribosomes. These proteins then direct certain activities of the cell. These signaling pathways exist in all cells but there are some that are very important in microglia, which are discussed as follows.

- Signaling Pathways and MyD88, a major coordinating signaling protein: Immune cell receptors, like most cell membrane receptors, are linked to intracellular signaling molecules that carry the messages to effector systems and nuclear gene activators. This process is called cell signaling and is central to the activation and suppression of molecular pathways in all cells. Cells contain thousands of cell signaling molecules, which are linked into functional pathways and when stimulated cause the cell to perform specific physiologic roles. Central to immune activation responses is the cell signaling adaptor molecule, *myeloid differentiation primary-response protein 88 (MyD88)*, which acts like a coordinating molecule (called an adaptor molecule) linking surface receptors to immune cell signaling pathways within the cytoplasm. Mice lacking MyD88 cannot produce tumor necrosis factor-alpha (TNF-α) or Interleukin 12 (IL-12) when exposed to bacterial antigens.[138] *Individuals with a genetically determined deficiency in MyD88 are subject to recurrent life-threatening infections of viral, bacterial and fungal types*. Not all immune receptors are linked to MyD88.
- Signaling Pathways containing Janus kinases: Another cell signaling pathway in immune cells and microglia involve the Janus kinases. Critical to immune function are the Janus family of kinases, Jak1, Jak 2, Jak3, and Tyk2, which are involved in cell growth, survival, development, and differentiation of immune cells.[66] These are special enzymes that transduce cytokine-mediated signals from the immune cell membrane to internal cell signaling pathways, such as JAK-STAT pathway, which in turn impart immune competence to the cell. The critical nature of these

cell signaling molecules is seen with defects in Jak3, which leads to *combined immunodeficiency syndrome*.

- Signaling Pathway From Cell Membranes to a gene through NFkB: One essential cell signaling link from the cell membrane is directed to Nuclear Factor kappa B (NFkB), which activates genes controlling the production and secretion of proinflammatory cytokines such as TNF-α. Activation of toll-like receptors (TLRs) and other immune receptors on the surface of microglia, macrophages, and other immune cells are linked to NFkB activation. This cell signaling molecule represents a *major controlling mechanism of inflammation*.
- Signaling Pathways that reduce the inflammatory response of microglia and other immune cells:
  - Other cell signaling mechanisms play an important role in reducing inflammation and potentially dangerous states of immune activation. For instance, MAPK phosphatase 1 (MKP1), is a major negative modulator of TLR-induced inflammation. You will recall the TLRs are a family of receptors on the cell membrane of immune cells that interact with antigens and other immune chemicals to initiate immune activation. By modulating these receptors, MAPK phosphatase 1 can fine-tune the immune reaction.
  - Another immune response regulator is called a suppressor of cytokine signaling 1 (SOCS1), which is an encoded gene. When the SOCS1 gene is activated by extracellular produced cytokines, such as IL-2, IL-3, interferon-gamma (INF-γ), or GM-CSF, the microglial SOCS1 gene reduces the activation of proinflammatory cytokine cell signaling. That is, it also dampens the immune response. Another such immune inhibitory molecule TREM2, is found on the surface of myeloid cells (primarily monocytes and macrophages) from the bone marrow. This protein interacts with another protein produced by the TYROBP gene, which in turn reduces the proinflammatory signaling of microglia, macrophages and dendritic cells. Mutations of this gene, which have been found to be common in Alzheimer's cases, impairs this immune dampening effect, thus leading to chronic brain inflammation, as seen in many neurodegenerative diseases.[72]

There are a growing number of second messenger cell signaling molecules essential for modulating immune reactions. For a detailed discussion of immune cells signaling, see reference.[2]

Molecular pathophysiology of microglia

Immune receptors and microglia reactions

- Immune Receptors leading to molecular activation: A great number of surface membrane receptors are seen on microglia, which not only act as "on/off" signals, but also modulate their responses to the microenvironment. For example, proinflammatory cytokines and glutamate can activate microglia toward a cytotoxic role and lead to destruction of synapses, dendrites, and whole neurons if not interrupted [Figure 2]. Other signals, acting through immune suppressing molecules, such as fractalkines, can switch the microglia to a resting and reparative state [Figure 3]. Pattern recognition receptors (PRRs) respond to pathogen associated molecular pattern (PAMPs) and damage associated molecular patterns (DAMPs), the last two of which are produced by pathogenic organisms and products of neuronal damage, respectively. What this means

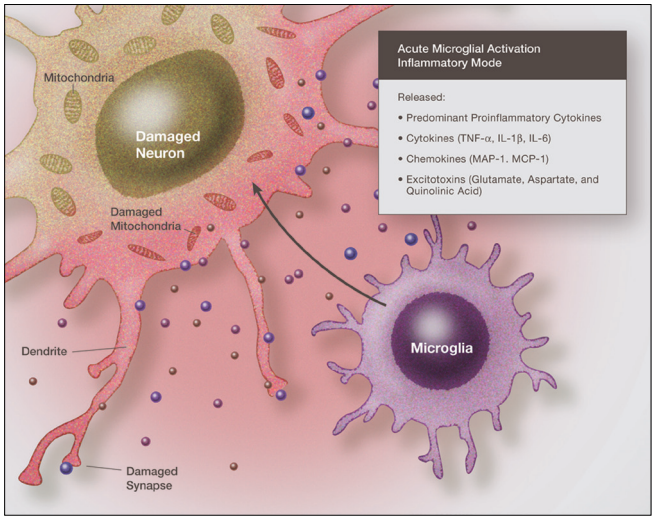

Figure 2: Illustration of fully activated microglia in a neurodestructive phenotype, with the release of proinflammatory cytokines, chemokines and excitatory amino acids, all acting in concert to damage the surrounding neurons

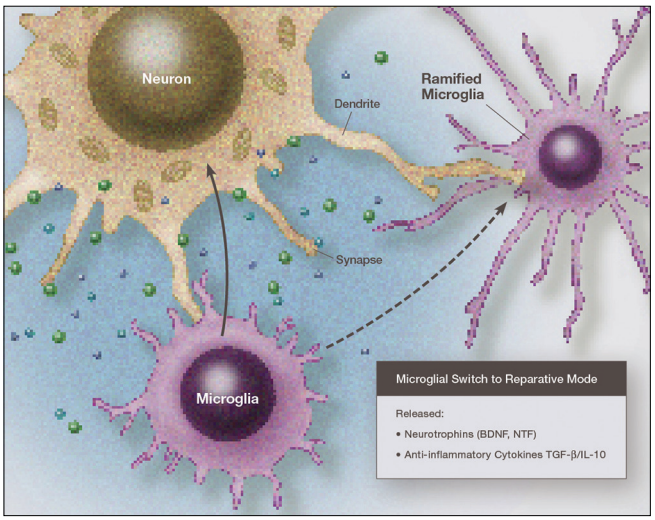

Figure 3: Illustration of a microglia in a primary reparative phenotype, which can be in either the resting mode or an activated-reparative mode, both of which release neurotrophic factors and antiinflammatory cytokines. This repairs the damage done during neurodestructive microglial activation

is that the particular molecular pattern of either the microorganism (PAMPs) or molecular signature of the debris of damaged cells (DAMPs) can react with specific activating receptors (PRRs) on the cell surface of microglia and lead to activation of the microglia's immune function. PAMPs activate various receptors and include TLRs, which trigger the release of cell surface activation molecules such as major histocompatibility complex class II (MHC class II), B7-1, B7-2, and CD40[5] [Figure 4]. These released molecules in turn stimulate the release of innate proinflammatory cytokines such as TNF-α, IL-1ß, IL-6, IL-12, IL-17, IL-23 as well as antiinflammatory cytokines IL-4, IL-10, and TGF-ß from microglia and invading macrophages.

One can easily see that either chronic activation of the brain's innate immune cells or even a condition where the cells are unable to switch off their immune activation, can lead to progressive destruction of synapses, dendrites or even the entire neurons or complex networks of neurons, as we see in neurodegenerative diseases. Examples of chronic microglial activation would include long-term systemic immune activation, as with autoimmune diseases and chronic infections (periodontal infections, chronic viral infections, Lyme disease, etc.). Neurotoxic metal accumulation within microglia or astrocytes, as seen with aluminum, lead, triethyl tin, and mercury exposure, also leads to continuous activation of microglia/astrocytes and macrophages.

- Immune receptors leading to microglia phagocytosis: Stimulation of PRRs by PAMPs and DAMPs can also activate microglia phagocytosis.[88] When microglia are in the phagocytic phenotype they can either do so in a mode that releases neurodestructive cytokines or other such molecules or one that is not inflammatory. During phagocytic activity one sees greatly increased upregulation of cell-surface costimulatory molecules, which aid in engulfing the invaders or debris. The engulfed bacteria or viruses are then destroyed by a "respiratory burst" through upregulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a producer of high levels of superoxide.[152] The respiratory burst of highly concentrated free radicals within the immune cells (macrophages, microglia, and polymorphonuclear white blood cells [WBCs]) kills the microorganisms and then allows the proteosomal enzymes to eliminate the debris.
- Upregulation of Fc receptors by pathogens: Exposure to pathogenic organisms also upregulates Fc receptors on microglia and macrophages, which aid phagocytosis by opsonizing the antigen. Opsionization is a process whereby antigens are attached to larger molecules (opsonins) that make phagocytosis easier by giving the phagocytic microglia a larger target to grasp. Microglial responses to cellular debris from dying cells either mounts a phagocytic response without a release of proinflammatory cytokines or combines phagocytosis with proinflammatory cytokine release.[70,71,132]
- Toll-Like Receptors as Antigen-Specific Receptors: Human microglia recognize bacteria, fungi, viruses, parasites, and the host itself, by utilizing specific toll-like receptors 1-9 (TLRs 1-9) located on the microglia cell membrane. Like peripheral macrophages, brain microglia demonstrate specific ligand reactions to TLR subtypes. For example, lipopolysaccharides (LPS), which make up the outer wall of Gram-negative bacteria, react specifically with TLR4, petidoglycan (PGN) (from cell walls of Gram-positive bacteria) reacts with TLR2, unmethylated CpG-DNA (viruses) reacts with TLR9, polyI: C (synthetic double-strand RNA) responds with TLR3 and West Nile virus RNA signals via TLR3[113,169] [Figure 5].

In experimental studies, vaccinations and in human infections, TLR4 is of vital importance. In conjunction with CD14, TLR4 makes up the primary receptor for LPS the cell wall component of Gram-negative organisms [Figure 6]. LPS is also found in many human vaccines. This receptor is upregulated when the brain is inflamed, as in the case of endotoxemia.[94] CD14 is a helper protein that aids the immune TLR in reacting to the particular antigen. Once the TLR activates the microglia, the exact reaction phenotype (phagocytic, reparative or cytotoxin/inflammatory) depends on the signaling message traveling through the cell signaling pathways. That is, the particular molecular pattern of the antigen (via PRRs, such as TLRs) determines the reaction state of the immune cell.

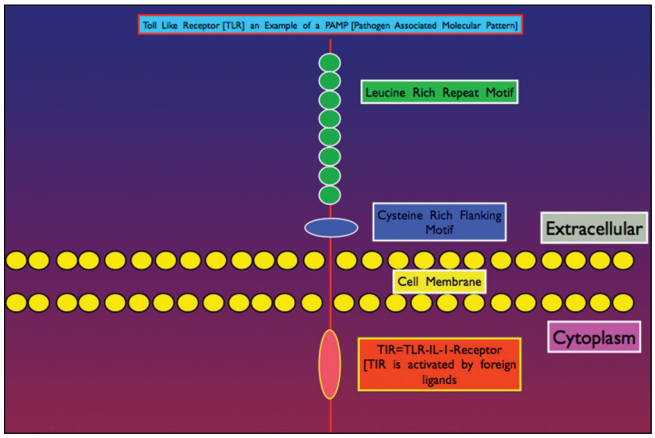

Figure 4: Illustration of general cellular anatomy of a TLR type Pattern Recognition Receptor (PRR). The external receptor interacts with specific Pathogen Associated Molecular Patterns (PAMPs) that translates signals to the intracellular signaling pathways for gene activation of defense mechanisms

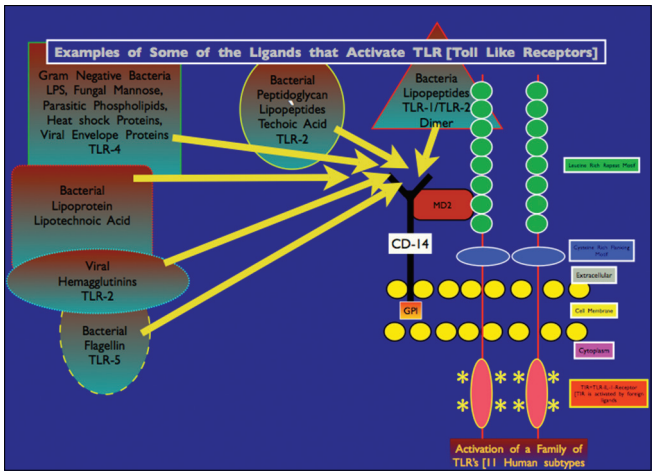

Figure 5: Illustration of various microorganism-related molecular ligands that can interact with specific TLRs leading to intracellular activation of defensive cell signaling mechanisms

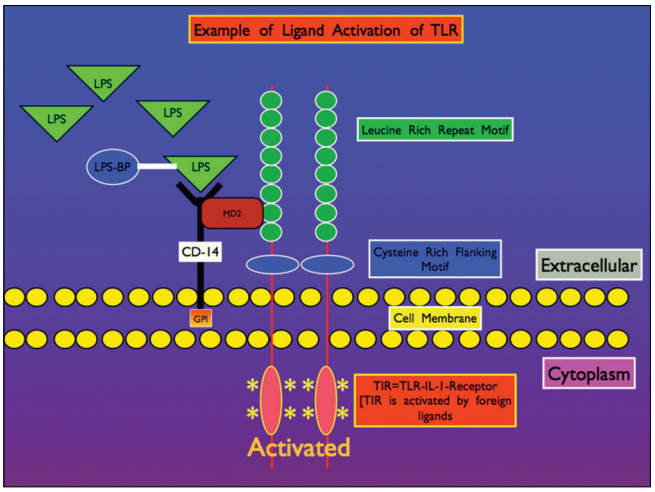

Figure 6: Illustration of lipopolysaccharide (LPS) Gram-negative cell wall molecular component interacting with TLR on microglial membrane surface, which along with co-stimulatory molecule CD14, activates defensive cell signaling. The IL-1 type pro-inflammatory cytokine receptor TIR, plays a major role in microglial activation

Cellular debris has a different molecular pattern and operates through Damage Associated Recognition Receptors (DAMPs). What activates the DAMPs is that the debris contains either internal cellular components that normally would not be in contact with the immune cell surface receptor or a mutated molecule is formed from the damaging process that is then recognized as a foreign molecule. Again, enzymes within the proteasome and lysomes digest the foreign protein and debris and either remove it from the body or utilize the proteins for cell repair.

In some instances, as with persistent viruses and neurotoxic metal accumulation, the offending antigen cannot be removed and thus acts as a constant source of immune stimulation. We see this with aluminum accumulation, where the highest concentration is within microglia. This would keep the microglia in an activated proinflammatory state, which could lead to a chronic state of immunoexcitotoxicity and subsequent neurodegeneration.

Clinical Correlation: There is growing evidence that excessive or overreactive responses to certain vaccines or groups of vaccines are responsible for many of the complications associated with vaccinations, including encephalomyelitis, seizures, autism spectrum disorders, and the recently described macrophagic myofascitiis.[7,14,16,65] The mechanism for these untoward reactions could be based both on the intense, prolonged peripheral stimulation by the aluminum-containing adjuvants (even lasting several years) or localized microglial priming/activation by immune adjuvant molecules accumulating within astrocytes and microglia within affected brain areas. Studies have shown that aluminum and mercury both accumulate within the brain following vaccination.[61,126] Both metals can cause prolonged microglial activation and mercury has been shown to kill astrocytes, its primary site of accumulation.[13,36,105] A loss of astrocytes can result in not only a massive release of stored glutamate upon astrocyte cell death, but also a long-term loss of one of the brain's major regulators of extracellular glutamate. The use of aluminum adjuvants in vaccines should be discouraged and in my opinion, such vaccines should be used only in cases where no alternative is available and in the prevention of very high-risk, communicable diseases of childhood. A discussion of the vaccine issue is beyond the scope of this paper, but growing evidence suggest we should reevaluate the number of and manner in which these vaccines are given.

- Neuropathic Pain: Clinical Correlation: TLR4 has also been implicated with neuropathic pain, for example, as that associated with transection of the L5 nerve root, thus suggesting microglial reactions to DAMPs. A number of studies have linked the production of chronic pain to a triggering of immune-excitotoxic pathways, which are initiated by activated microglia.[103,134,151] Microglial activation in spinal ganglion and central pain pathways trigger the release of proinflammatory cytokines and this increases glutamate receptor sensitivity with prolonged activation of these receptors. This immunoexcitotoxic reaction increases nociceptive signaling, resulting in chronic, unrelenting pain syndromes.[83]
- Microglia and TLR Molecular specificity: Free gangliosides and sialic acid-containing glycosphingolipids found in neural membranes, are known to activate microglia via TLR4, offering another way cellular debris can activate microglia.[85] This explains the frequent finding of reactive antibodies to these neuronal components with head injuries and strokes. It is possible that this produces a state of

microglial priming, which would mean that subsequent systemic immune activation from any cause, trauma, surgery, autoimmune disease, etc., would increase immunoexcitotoxic reactions in the damaged areas.

TLR7 is vital for microglial reactions to viruses such as the herpes simplex virus[60] [Figure 7]. Besides being integral players in the release of proinflammatory cytokines, chemokines and interferons, TLR2, TLR4 and TLR9 on the microglial surface induce microglial production of IL-6 and IL-10, IL-6 and TNF-α and TNF-α and nitric oxide (NO), respectively.[88] The proinflammatory cytokines increase the production of a number of free radicals, especially the super oxide radical, which in itself is a rather mild radical. Yet, when it combines with NO, it forms the very powerful peroxynitrite radical, which is found in high concentration is a number of neurodegenerative diseases. So, the increase in NO seen with activation of these immune receptors becomes very important.

- Other Microglial actions: Microglia also contain a number of scavenger receptors, which can identify modified lipoproteins and various polyanionic ligands. These include scavenger receptors of class A1 (SR-A1), SR-B1, and CD36 that are differentially regulated by microglia in response to various pathologies.[88] These receptors play a major role in defense against bacteria and can be upregulated in response to injury and cytokines as well. That is, they respond to a wide assortment of pathological insults.
- Scavenger receptors.
  - Scavenger receptors are upregulated in Alzheimer's disease (AD) in reaction to amyloid beta (Aß).[3] In this way, as the amyloid progressively accumulates and is deposited in the extracellular space it acts as a source of continued microglial stimulation, with resulting release of a number of toxic molecules. Activated microglia are known to accumulate within amyloid plague in the Alzheimer brain.

    Another important scavenger receptor in neurodegenerative diseases, receptor for advanced glycation end-products (RAGE), recognizes advanced glycation end products produced as a reaction to elevated glucose levels in the brain. The RAGE receptors on microglia are upregulated in the presence of Aß, which enhances plaque phagocytosis.[174]
- MAC1 Receptor (The Integrin or Compliment Receptor)
  - The MAC1 receptor (macrophage antigen complex 1), also called integrin CD11b/CD18 or complement receptor 3 (CR3), functions both as an adhesion molecule and a PRR capable of recognizing diverse ligands.[117] It also plays a critical role in phagocytosis activation in response to a number of compounds. Activation of this microglial receptor can trigger a respiratory burst and activation of neutrophils and macrophages.

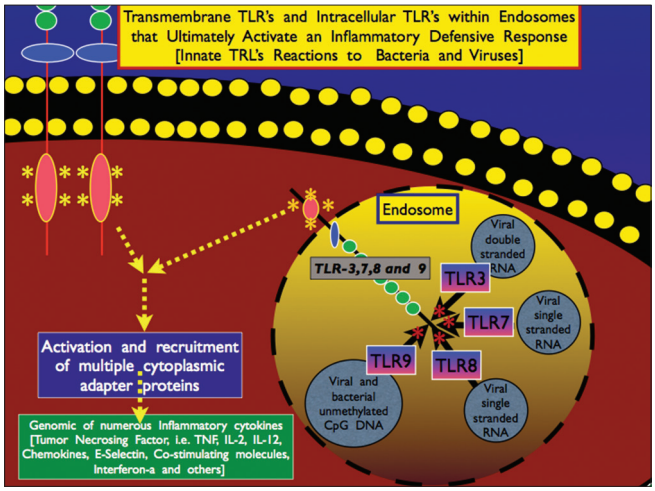

Figure 7: Illustration of intracellular TLRs found on the membrane of endosomes, which play a major role in neutralizing viruses I within the cell itself

How microglia are activated: Molecular mechanisms behind "microglial priming"

Through receptors

Cell signaling pathways can set in motion a series of molecular reactions that activate microglia. When an antigen is recognized by a receptor on the surface of the microglia cell, the receptor, through a series of conformational changes which requires energy, sets off a chain of molecular reactions that signal the genes in the microglia to produce proteins that activate the microglial cells to respond by producing proteins that react with the antigen and or initiate phagocytosis. For example, when PRRs in the microglial membrane recognize an antigen, NADPH oxidase is activated in response to antigen stimulation of the immune receptor. NADPH oxidase generates high levels of superoxide, which as discussed previously, combines with free NO to produce the powerful radical peroxynitrite. Superoxide can also break down into other powerful radicals, such as the hydroxyl radical.

Several PRRs can activate NADPH oxidase, the major source of microglial reactive oxygen species (ROS), especially the superoxide radical. NADPH oxidase is composed of several subunits that must be assembled for activity. These subunits are distributed between the cytosol and the membrane of intracellular vesicles. Once activated by complexing, they are translocated to the cell membrane. MAC1 appears to be crucial for assembly and activation of NADPH oxidase.[186] Intense activation of NADPH oxidase is seen in both Alzheimer's dementia and Parkinson's disease brains.[140]

Through elevated levels of ROS

The morphology and proliferation of microglia are to a large degree regulated by H_20_2 generated by NADPH

oxidase and, as a result, higher levels of ROS, in most instances, amplifies inflammatory responses.[9,101] By altering the level of intracellular ROS, one can prime microglia to respond in an exaggerated way to subsequent stimuli. In fact, NADPH oxidase is essential for microglial priming.[40] NADPH oxidase activation alone has been shown to be relatively benign, since the superoxide radical itself is rather weak. Cell killing requires the simultaneous activation of inducible nitric oxide synthase (iNOS).[100] This is because NADPH oxidase primarily produces oxygen radicals and iNOS generates nitrogen radicals, which when combined forms the very powerful reactive nitrogen species (RNS) peroxynitrite. High levels of peroxynitrite are found in most of the neurodegenerative diseases as well as strokes and neurotrauma. Several antibiotics, peptides, and small molecules known to inhibit NADPH oxidase activation demonstrate neuroprotective effects.[41]

Through chemokines and cytokines

Chemokines are a family of molecules released from microglia that attract other microglia or immune cells, even at great distances. Microglia contain a variety of receptors for chemokines and cytokines.[88] Chemokines released by neurons can signal microglia of impending danger, causing them to migrate toward the zone of injury. Damage to the CNS, including neurotrauma, ischemia/hypoxia, and infectious inflammation, trigger the release of the various chemokines not only from microglia and astrocytes, but also neurons.[12,50,51] One of the most potent triggers for chemokine release is a high level of glutamate. Chemokines, along with adenosine triphosphate (ATP) and glutamate, act as gradients for macrophage and microglia migration. Alteration of chemokine receptors is common with a number of neurodegenerative disorders such as AD and multiple sclerosis.[45,173] Chemokines, such as CXCL10 and CXCL12, by reacting with their respective microglial and astrocytic receptors, are linked through cell signaling pathways to excessive glutamate release and excitotoxicity under a number of pathological conditions.[39]

Clinical Correlation: Elevated levels of chemokines within the CNS are seen with a number of neurological conditions, including AD, amyotrophic lateral sclerosis (ALS), human immunodeficiency virus (HIV) dementia, ischemia, viral encephalitis, multiple sclerosis, and Parkinson's disease (PD).[31,82,115,121,178] Sometimes, chemokines can be neuroprotective by reducing inflammatory cytokine expression. For example, Donohue et al. found that patients with severe strokes had lower CX3CL1(fractalkine) levels at day one as compared with those with less severe strokes.[53] CX3CL1 levels were also lower at 180 days in patients with a poor stroke outcome. Interestingly, the association of fractalkine levels to outcome was independent of the severity of the stroke. High fractalkine levels were also associated with lower inflammatory markers, such as high-sensitivity C-reactive protein (CRP). Fractalkines suppress microglial activation.

Microglia contain receptors for a number of cytokines, both proinflammatory and antiinflammatory. One of the critical types of cytokine receptors for microglia activation is the IL-1ß receptors, which includes the subtypes IL1RI, IL-1RII, and IL-RIII.[93] Systemic macrophage surface receptor reaction to LPS, a substance found within the cell wall of Gram-negative bacteria, triggers the release of IL-1ß and this cytokine in turn activates brain microglia. While under resting conditions, with resting microglial, IL-1RIII has the strongest expression and upon LPS stimulation, one sees a significant increase in expression of IL-1RI and IL-1RII in activated microglia. Human microglia have been shown to have IL-1RI, IL-1RII, IL-5R, IL-6R, IL-8R, IL-9R, IL-10R, IL-12R, IL-13R, and IL-15R type receptors.[93] Systemic activation of IL-1Rs by any pathological process, rapidly activates brain microglia, and while being a major activator, it is not the only one. IL-1ß activation occurs systemically with LPS exposure, infections, trauma, and with autoimmune disorders.

How glutamate is produced by activated microglia

Another proinflammatory cytokine, TNF-α not only plays a critical role in inflammatory brain pathology, but is integral to immunoexcitotoxicity itself as it links the immune response to glutamate excitotoxicity[15,18] [Figure 8]. A number of immune cells produce TNF-α, including macrophages, microglia and to a lesser extent, astrocytes and neurons. TNF-α acts through two main receptors, TNFR1 and TNFR2. Like IL-1ß, TNF-α plays a significant role in microglial activation.[179] In general activation of the TNF-α subtype receptor TNFR1, triggers cell-signaling pathways that are predominantly neurodestructive.[102] These receptors are found mostly on neurons. TNFR2 subtype receptor is neuroprotective when stimulated and is found mostly on glia cells, which makes sense as high levels of TNF-α released during innate immune activation would otherwise damage the brain's principle immune cell, the microglia.

Clinical Correlation: The concept of immunoexcitotoxicity is based on the interaction between inflammatory cytokines and glutamate excitotoxicity. In a previous paper, it was demonstrated that TNF-α had a number of effects on glutamate neurotransmission and excitotoxicity, including upregulation of astrocyte glutaminase (which converts glutamine into glutamate), increased trafficking of calcium-sensitive AMPA receptors to the synaptic membrane, inhibition of glutamate transport proteins (excitatory amino acid transporters [EAATs]), suppression of glutamate removal by Kreb's cycle enzymes and conversion of glutamate to glutamine (glutamate dehydrogenase and glutamine synthase) and increased trafficking of NMDA receptors.[18]

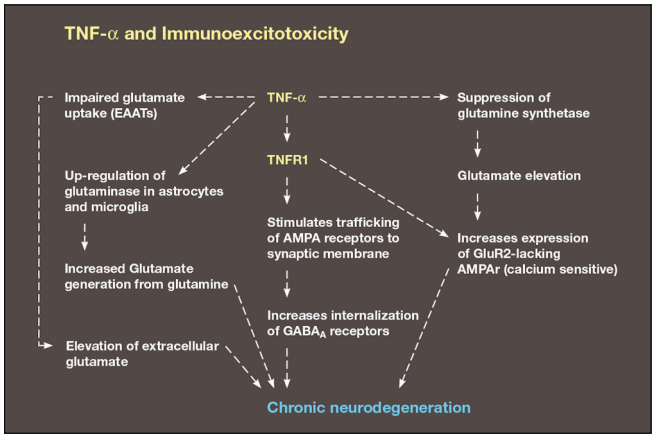

Figure 8: Schematic demonstrating the link between TNF-α and an enhancement of excitotoxicity via its interaction with glutamate transport proteins, upregulation of glutaminase, suppression of glutamine synthetase, increased trafficking of AMPA calcium-permeable receptors to the synaptic membrane and endocytosis of GABA receptors

Based on this central effect of TNF-α, Tobinick *et al.* demonstrated dramatic, rapid improvement among 629 consecutive stroke patients following injection of the TNF-α-blocking drug etenercept given perispinally (Batson's plexus).[158] Statistically significant improvements were seen in a wide range of neurological deficits and symptoms, including motor impairment, spasticity, sensory impairment, cognition, behavioral function, aphasia, and pain. Incredibly, they noted a strong effect even in patients having strokes as long as 10 years previously. They found similar improvements among 12 patients with traumatic brain injury.

In a second group of patients, Tobinick and Gross, utilizing the same technique found that blocking of CNS TNF-α in a single case of severe AD produced rapid and sustained improvements in speech, agitation, and tests of spatial function.[157] Less impressive improvements were seen in memory function, but being a late stage case, one would expect severe loss of hippocampal neurons. Others have had similar results.[162]

The final effect of TNF-α depends on which TNF receptor is activated. Microglia contain predominantly TNFR2 type receptors that when stimulated by high levels of TNF-α, are protective, thus protecting the microglial cell from its own released cytokine. Neurons, in contrast, contain mostly TNFR1 subtype, which when stimulated by high levels of TNF-α triggers a series of cell signaling pathways that are neurodestructive. Very low, constitutive concentrations of TNF-α are considered neurotrophic.

Neurotransmitter and neuroregulatory receptors on microglia

Microglia contain a number of receptors for neurotransmitters and other neuroregulatory molecules, including those for glutamate, acetylcholine, dopamine, gamma-aminobutyric acid (GABA), adrenergic compounds, cannabinoids, opioids, substance P, vasoactive intestinal peptide, histamine, glucorticoid, somatostatin, angiotensin II, platelet activating factor (PAF), and neurotrophin.[88]

Ionotropic glutamate receptors

Glutamate is the most abundant neurotransmitter in the brain, contributing 90% of cortical neurotransmission and 50% of all brain neurotransmission. These receptors include three subtypes named NMDA receptors, AMPA receptors, and kainite receptors [Figure 9]. Each has its own particular physiological response to glutamate stimulation, providing the brain with a wide range of reaction options to a single neurotransmitter, glutamate. Interestingly, glutamate receptors reside on most neurons, even those utilizing other neurotransmitters, such as dopamine, serotonin, norepinephrine, and acetylcholine. There exists an interaction between these receptors so that each modulate the others functions. Being the most abundant receptor type, glutamate receptors play a major role in controlling the other neurotransmitter receptors.

Glutamate receptors on neuronal dendrites and synapses play a major role controlling the strength of the synaptic impulse and does so by a variety of mechanisms. Of special importance is the role glutamate neurotransmission plays in learning and memory and behavioral control.

Interestingly, glutamate receptors are not limited to neuronal dendrites and synapses. Over the past decade, researchers have demonstrated a significant role for glutamate receptors in regulating microglial behavior. One role for microglia as a member of the tripartite synapse is to modulate extraneuronal concentrations of glutamate, especially in the vicinity of the synapse. This helps to sharpen the signal and prevent interfering "noise" caused by excess diffused glutamate from surrounding synapses. Cell surface receptors for glutamate are divided into inotropic receptors and metabotropic receptors. The ionotropic receptors regulate opening of various ionic channels, principally those for sodium and calcium and the metabotropic receptors act through G-protein-coupled receptors to modulate the inotropic receptors. Microglial inotropic glutamate receptors play a role in microglial migration as well as microglial activation. When sites of injury or pathology result in the release of high levels of glutamate, the diffused extracellular gradient attracts surrounding microglia to the site of injury.

Of the ionotropic glutamate receptors, AMPA receptors appear to be the most abundant and critically important for microglial reactivity. There is some evidence that NMDA receptors exist on microglia, most of which is indirect evidence. For example, blocking NMDA receptors in experimental models has been found to reduce microglial activation associated with ischemic insult or exposure to HIV protein.[148] Blocking inotropic glutamate receptors has been shown to reduce the size of the infarct and improve

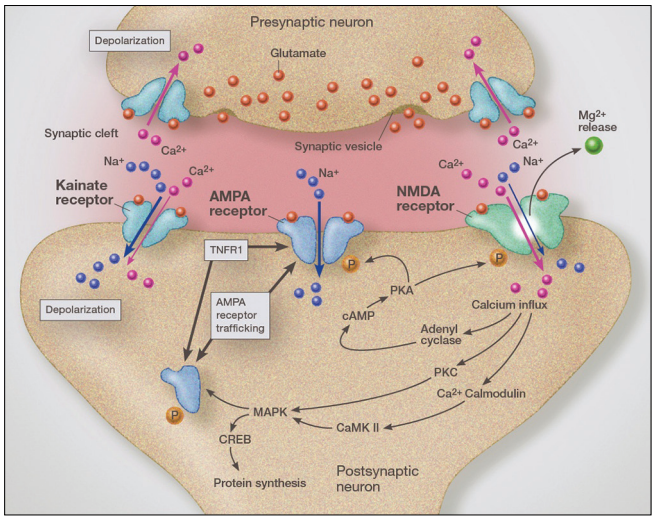

Figure 9: Illustration demonstrating the trafficking of AMPA calcium-permeable receptors from the endoplasmic reticulum to the synaptic lipid raft, thus increasing glutamate sensitivity and increasing the internal flow of calcium. We see a condition of crosstalk between TNFR1 receptors and AMPA receptor trafficking mechanisms

neurological outcomes in animal stroke models but has had little success in human studies, mainly because of side effects of the blocking drugs.[80] Recent studies may have further demonstrated the reason for failure of these drugs. Chen *et al*. found that the subtype of NMDA receptor was critical, with the NR2B subtype NMDA receptor triggering neuronal death and NR2A subtype being neuroprotective when activated.[37] One can see that blocking both receptors could be detrimental, as it would not only block the neurodestructive NR2B-containing NMDA receptor subtype, but also the NR2A-containing NMDA receptor neuroprotective subtype.

Stimulation of microglial AMPA receptors induces the release of TNF-α, which in a paracrine manner stimulates further microglial activation and migration. Glutamate receptors, in particular AMPA receptors, play a major role in microglial chemotaxis. By following gradients of extraneuronal glutamate, microglial cells migrate toward sites of pathological injury or damage.[95] With brain injury, massive amounts of glutamate are released from astrocyte stores. Also of interest is the finding that stimulation of AMPA receptors triggered rapid and extensive remodeling of the cytoskeleton (actin filaments) within microglia suggesting a role in regulation of microglial motility and phagocytosis when activated.[42]

Also of interest is the finding that under certain conditions, AMPA receptor stimulation can be neuroprotective and promote brain repair after a stroke.[43] Recovery after a stroke was improved by AMPAR stimulation, but only if done after 5-days from the stroke. At this stage, AMPAR stimulation mediated the release of brain derived neurotrophic factor (BDNF). Blocking AMPA receptors after the onset of this later period worsened the pathological damage. Prolonged activation of AMPA receptors, that is, after several weeks to months, again switches the microglia to a neurodestructive phenotype. Likewise, stimulating AMPA receptors during the first 5 days worsened the outcome and blocking these receptors during this time period improved brain injury, indicating that time of intervention is critical.

Metabotropic glutamate receptors

Metabotropic glutamate receptors (mGLuRs) are classed as noniontropic receptors and rather than utilizing ionic pores, such as the calcium pore seen with ionotropic glutamate receptors, this group of receptors is coupled to membrane G-proteins that act through cytoplasmic second messengers. Cloning studies have grouped them into three basic types. Group I mGLuRs, composed of metabotropic glutamate receptor subtypes 1 and 5 (mGluR1 and mGluR5), operate through activation of phospholipase C. In general, these enhance the activity of the iontropic glutamate receptors (NMDAR, AMPAR, and kainite receptors).

Group II metabotropic receptors include mGluR2 and mGluR3 and through their G-protein receptors inhibit adenylate cyclase. Most often they are inhibitory of the ionotropic receptors, but not always. Group III contains mGluR4, mGluR6, mGluR7, and mGluR8. They also inhibit adenyl cyclase and are considered to be inhibitory of ionotropic glutamate receptors. They are linked to the ionotropic receptors though cell-signaling molecules.

From a potential therapeutic viewpoint, mGLuRs exhibit a number of interesting properties. Group I mGluRs regulate LPS-induced microglial activation in primary cultures.[59] This links microglial activation by Gram-negative organisms as well as vaccines containing LPS as an adjuvant. Activation of group II mGLuRs triggers microglial activation along with cytotoxic release of TNF-α.[156] A more recent study clearly indicates that mGluR II and III stimulation suppresses the release of glutamate from microglia and prevents neurotoxicity associated with neuroinflammation.[106] In this instance microglia are moderating their own release of glutamate via these mGLuRs. Blocking these two receptors, Group II and Group III, under conditions of LPS immune stimulation greatly magnify associated glutamate excitotoxicity (immunoexcitotoxicity). In this study, group I mGLuRs played a very minor role in regulating glutamate release from microglia.

In essence, the mGLuRs are acting as modulators of inotropic receptors either enhancing or dampening their response. This adds an important layer in controlling the excitatory response to glutamate, so as to prevent excessive brain excitation, which can lead to neurodegeneration or seizures. In addition, they also aid in sharpening the signals during glutamate neurotransmission. As with

the ionotropic glutamate receptors, the metabotropic receptors play an important role in controlling other neurotransmitter function and levels of activity.

Cholinergic receptors

Frequently overlooked in discussion on neurodegeneration is the critical role played by cholinergic systems in controlling inflammation, especially the α7-nicotinic receptors (α7nAchRs). Cholinergic pathways are impaired in both AD and PD, most likely as a result of the immunoexcitotoxic reactions in Ach-controlling areas of the brain, such as the nucleus basalis of Meynart.[109] This small nucleus contains a great number of glutamate receptors and when overstimulated can lead to destruction of the nucleus and a loss of brain cholinergic input. The basal nucleus of Meynart is the central source for the brain's cholinergic fiber system.

Clinical Correlation: Any condition that reduces brain acetylcholine, such as brain trauma, dietary restriction of choline, advanced aging or chronic neurodegenerative diseases that lowers brain acetylcholine, could worsen brain inflammation. One can see that a combination of poor diet and advanced aging in particular can worsen Alzheimer's dementia by removing or suppressing this antiinflammatory system. Likewise, improvements in some AD patients taking acetylcholine-enhancing medications, such as donepezil (Aricept) may be as a result of Ach's antiinflammatory effect. Chronic activation of these receptors, as occurs with smoking or nicotine patches, could impair microglial function in cases of cerebral infections, strokes, and other inflammatory pathologies. It would also explain how smoking reduces one's risk of PD and why nicotine patches may reduce risk.[27,87,124] Microglia containing α7nAcRs (acetylcholine nicotinic receptors), when activated generally suppress immune responses as part of the "cholinergic anti-inflammatory pathway."[141]

Adrenergic receptors

Norepinephrine is known to suppress the release of NO, TNF-α, and IL-6 following immune stimulation with LPS and plays a critical role in protecting the brain from neuroinflammation.[58] Stimulation of $ß_2$ adrenergic receptors (ARs) ($ß_2$ARs) suppresses microglial proliferation. Norepinephrine acting through $ß_{1/2}$ receptors inhibits cyclic adenosine monophosphate (cAMP)-induced release of TNF-α.[108] ARs are instrumental in regulation of microglial migration and phagocytosis. In essence, like acetylcholine, norepinephrine and epinephrine play an important role in dampening immune activation and associated brain inflammation.

Adrenergic receptors and the locus ceruleus

This small collection of neurons, located in the posterior area of the rostral pons along the floor of the fourth ventricle, sends adrenergic (norepinephrine and epinephrine) projections widely throughout the CNS, thus making it the central controlling adrenergic nucleus (referred to as the locus ceruleus–noradrenergic [LC-NA] system). Stimulation of these neurons is generally excitatory for the brain, producing an arousal effect. Degeneration of the LC is an early event with AD and PD.[171,187] Recent studies have shown that norepinephrine suppresses microglial activation and migration, explaining how a loss of LC-NA system plays a significant role in these diseases.

Dopamine receptors

Functional dopamine receptors on microglial membranes have been identified in both human brain tissue and mouse brain. Chronic stimulation of microglial dopamine receptors enhanced migratory activity, and like ARs, attenuated LPS-induced NO release.[58] Cultures from elderly human microglia demonstrate dopamine receptor-induced chemotaxis. Interestingly, the substantia nigra has the highest concentration of microglia in the brain, which puts it at risk when they are activated.[92] We see this activation of substantia nigra microglia with exposure to certain agricultural chemicals such as rotenone and maneb, common chemical exposures linked to PD.[63,153] Activation of substantia nigra microglia either by rotenone or other agents used to produce experimental models of PD, increase the secretion of proinflammatory cytokines and excitotoxins (immunoexcitotoxicity) and this results in a selective degeneration of these pigmented neurons, as well as degeneration in other striatal neurons. Of interest has been the finding that inhibition of D_4 dopamine receptors suppresses microglial activation in the spinal cord of transgenic models of ALS, slowing down disease progression.[154]

Clinical Correlation: ALS: A number of new studies considerably strengthen the case for an interaction between inflammation and excitotoxicity as the central mechanism in ALS. It has been determined that in experimental models of the disease microglial-triggered inflammation occurs in the presymptomatic stage of the disease, and microglial number increase as the disease progresses.[75] Interestingly, one also sees increased systemic inflammation with elevations in CD16+ monocytes in the peripheral blood as well as elevated levels of LPS (endotoxin).[183,184] This is important, as several studies have shown that stimulation of systemic immunity in ALS cases can worsen symptoms and cause the disease to advance more rapidly.[68]

Scanning studies of ALS patients demonstrate widespread microglial activation in the motor cortex, pons, dorsolateral prefrontal cortex, and thalamus.[161] The extent of the microglial activation was strongly correlated with the severity of the ALS. In addition, one sees high levels of ROS/RNS products released from the activated microglia. In ALS, oxidative and nitrogen stress begins very early in the course of the disease. The most toxic

of the radicals is peroxynitrite, which powerfully inhibits mitochondrial energy production, something commonly seen in ALS.

The reason for the prolonged microglial activation in the affected areas of the brain and spinal cord in ALS is not specifically known, but we know that the most commonly linked initiating factors, such as pesticide/herbicide exposure, aluminum and/or mercury accumulation, repeated minor injuries and certain persistent viruses all are known to cause prolonged microglial activation in a neurodestructive mode.

The relationship of superoxide dismutase-1 to ALS

In the past, it was thought that there were two distinct ALS disease forms, one sporadic with normal levels of the antioxidant enzyme SOD1 and a familial form with mutated SOD1. Newer studies have offered some important surprises. First, the loss of motor neurons was not related to a loss of functional SOD1, an antioxidant molecule that neutralizes superoxide.[26] Instead, these studies indicated that the mutated SOD was acting as a toxic molecule itself. Mutated SOD1-containing microglia were found to be more intensely activated when stimulated than were wild-type microglia. These activated microglia released higher concentrations of NO and superoxide.[6] The mutated SOD, when released from astrocytes was shown to react with CD14+ receptors on microglia and via TLR2 and TLR4 coreceptors, thus triggering CNS inflammation by releasing cytokines. In essence, the mutated SOD1 was acting as a powerful microglial activator, much like a foreign antigen. A previous study found that SOD1 was frequently oxidized in sporadic cases of ALS and that this altered enzyme, like the mutated SOD1 in familial cases, also activated CD14+ receptors, thus linking all forms of ALS, sporadic and familial, to toxic, immune activating forms of SOD1.[74]

One might conclude incorrectly that motor neuronal death in ALS was caused by the immune activation alone, yet mouse models of ALS in which IL-1ß and TNF-α have been deleted still demonstrated continued neurodegeneration, indicating that another pathological process was in operation and linked to the immune effect.[128] The strongest link to motor neuron loss has been excitotoxicity primarily caused by impaired glutamate transport by EAATs, which allows dramatic elevations in extracellular glutamate. These glutamate transporters play a critical role in keeping extracellular levels of glutamate very low by shunting the glutamate into microglia and astrocytes. Should they fail, or be impaired in their function, glutamate can rise to neurotoxic levels. Because these transporters (EAATs) are redox sensitive, the free radicals and lipid peroxidation products produced by the inflammation impair the glutamate transporter's function and this causes a rise in extraneuronal glutamate levels. Rothstein has shown that a large percentage of ALS patients have defective glutamate transport.[130] All ALS animal models show impaired glutamate transport. This transport mechanism is present on both microglia and astrocytes, but astrocytes are the major storage reservoir for glutamate. Abnormal, mutated, or oxidized SOD1-containing astrocytes are seen prior to the onset of symptoms in animal models.[25]

Excitotoxicity and its relation to neurological diseases and ALS

The high level of ROS/RNS and lipid peroxidation resulting from the widespread inflammation impairs the glutamate transporters, in particular GLT-1 (EAAT2), and impairs other essential metabolic enzymes and enzymes used to clear excess glutamate, such as glutamine synthetase. A number of studies have demonstrated elevated CSF glutamate levels in ALS patients as compared with controls.[129] A follow-up of this earlier study found elevated CSF glutamate in 40% of 400 patients with sporadic ALS and that elevation of CSF glutamate correlated with disease severity.[145] It is important to appreciate that excitotoxicity is not always dependent on elevation of glutamate levels, since a number of conditions, such as mitochondrial dysfunction, hypoglycemia, heavy metal poisoning, and inflammation, can dramatically enhance neuronal sensitivity to glutamate – so much so as to allow even normal levels of extracellular glutamate to trigger excitotoxicity.

Impairment of EAAT2 expression in the ventral horn of the spinal cord of experimental models occurs in the presymptomatic period. Interestingly, EAAT2 levels were almost completely abolished at the end-stage of the disease.[79] This loss of the glutamate removal transporter action means that excitotoxicity is a major mechanism of motor neuron destruction. This destructive process is enhanced by inflammation via a number of mechanisms. Impairment of EAATs causes a slow neurodegeneration because of the gradual accumulation of glutamate to excess. This initiates the excitotoxic cascade in which excess calcium enters the neuron, triggering a number of neurodestructive processes, including production of free radicals and lipid peroxidation products, impaired mitochondrial energy production, and activation of inflammatory prostaglandins.

Overexpression of EAATs, thus increasing the efficiency of glutamate removal, in experimental animal models of ALS delays the onset of motor loss and prolongs survival. An analysis of motor cortex and spinal cord extracts from ALS patients demonstrated nearly complete loss of EAAT2 in 25% of patients and some impairment in up to 80%.[130] The resulting elevations in extraneuronal glutamate leads to the generation of high levels of ROS/RNS and lipid peroxidation products, which

further impair glutamate transporters and suppresses mitochondrial energy production. Impaired energy generation markedly enhances excitotoxic sensitivity. One can easily envision a progressive neurodegenerative process from these interacting mechanisms that ends in widespread motor neuron loss.

Cannabinoid receptors

The cannabinoid system plays a critical role in modulating excitatory neurotransmission. Rodent microglial cells contain CB_1 and CB_2 receptors on activated microglia but are very low in ramified (resting) microglia.[10,11,150] The appearance of these receptors is quite rapid upon microglial activation. Activation of cannabinoid receptors on microglia reduce NO production and inhibit cytokine release resulting in a suppression of the inflammatory response.[28]

Experimental allergic encephalomyelitis

EAE is an animal model of multiple sclerosis and related inflammatory demyelinating disorders. A number of antigens have been used to produce the immune demyelination, including spinal cord homogenates, various myelin proteins (MBP, PLP, and MOG) or peptides of these proteins. The pathology produced varies to some degree based on the antigen used.

In the case of EAE, a model for human multiple sclerosis in animals, one sees upregulation of CB_2 receptors induced by the synergistic action of IFN-γ and GM-CSF.[104] CB_2 is also upregulated in microglia isolated from patients with AD, ALS, and AIDS-associated dementia.[180] In most cases, activation of cannabinoid receptors reduces microglial neurotoxicity while increasing microglial proliferation. This activation would switch the microglia from a proinflammatory cytokine-releasing phenotype into a phagocytic, neuroprotective phenotype.[55] This cannabinoid receptor activation is also demonstrated in the case of animal models of Huntington's disease in which stimulation of CB_2 receptors reduced brain edema, neuroinflammation and death of striatal neurons.[114]

From this abbreviated review of microglial receptors, it becomes obvious that brain immune reactions are under a great number of regulatory controls and it is the interaction of these various receptors that determines the dynamic changes in microglial phenotypes and thus their function.

Microglial migration and motility

Microglia are not fixed immune cells, but are characterized by a high degree of motility in the ramified (resting) state and are capable of extensive migration upon activation. As demonstrated previously, motility of resting microglial foot processes allows the microglia to conduct ongoing surveillance of the synaptic microenvironment and make adjustments of the concentrations of excitatory neurotransmitters such as glutamate and aspartate. This surveillance is critical not only for protection against excitotoxicity, but also for signal sharpness, as previously mentioned.

At the interface of cerebral blood vessels, microglial motility allows rapid identification of invasion by microorganisms. This rapid identification process is especially important near zones where the BBB is deficient, as with the CVOs, which includes the area postrema, subfornical organ, organum vasculorum of the laminia terminalis, pineal region, subcommissural organ, median eminence, and neurohypophysis. These areas contain abundant microglia, which when exposed to invading organisms or systemic proinflammatory cytokines, are rapidly activated and migrate over great distances in an effort to protect the rest of the brain.[91] They also represent areas of easy entry for activated macrophages from the periphery.

Motility appears to be mostly controlled through purinergic receptors on the microglial surface membrane. Purinergic receptors respond to free ATP and adenosine and on neurons can initiate an excitatory response. A number of subtypes of purinergic receptors have been identified. ATP and adenosine, both stimulate motility in ramified (resting microglia) microglia and act through P2Y12 subtype purinergic receptors.[49] This receptor stimulation attracts the foot process toward the site of damage. Upon microglial activation, P2Y12 expression is reduced.

In the face of pathological injury, activated microglia migrate to the site of damage mostly by following a chemical gradient. Like motility, microglial migration is controlled by a set of receptors. Studies suggest that a combination of P2X4 and P2Y12 receptors, subtypes of purinergic receptors, are involved in microglial chemotaxis.[112] CD39 and CD73, cellular ectonucleotidase enzymes, are essential for degrading ATP for stimulation of chemotaxis. That is, these enzymes break down the ATP, releasing adenosine, which then activates particular purinergic-subtype receptors on the microglial surface. It appears that costimulation of purinergic and adenosine receptors are required for microglial migration. This costimulation becomes important when considering microglia migration to areas of significant neural injury and neuron death, as occurs with strokes, brain trauma, and neurodegenerative disorders. Under such conditions, high concentrations of ATP are released from the damaged neurons and glial cells and can act as activators of microglial migration and motility.

Other neurotransmitters are also involved in microglial migration, such as glutamate, dopamine and epinephrine via AMPA receptors, mGLuRs, dopamine receptors, and ARs, respectively.[58] Along with ATP, these neurotransmitters are also released with neural tissue damage. The high concentration of the ATP and

neurotransmitters released from the dying cells diffuse into the surrounding tissue and establish a chemical gradient that attracts the microglia.

Chemokines are also potent chemoattractants and are released from neurons and microglia. Damaged neurons express the chemokine CCL21, which attracts microglia. Monocyte chemoattractant protein-1 (MCP-1) regulates migration of microglia, monocytes, and lymphocytes to the sites of inflammation in the CNS.[144] Cannabinoids, acting through CB_2 receptors, can also act as stimulants for microglial migration.[166] They play a special role in increased microglial motility following ischemia.

A number of other factors also regulate microglial migration, but of special interest is nerve growth factor (NGF) and transforming growth factor-ß (TGF-ß) as both are involved in brain repair and reduction of neuroinflammation as well.[52] As the microglial transform to a reparative phenotype, release of these reparative NGF and TGF-B compounds can play a vital role in moving microglia to the sites of damage so as to enhance repair of the damage done.

Phagocytosis

Microglial cells are the main phagocytic cell type of the brain and can not only phagocytize dead cells, but also parts of neurons, for example, dendrites, and debris such as myelin and amyloid deposits. Microbes are recognized by TLRs and other PRRs, whereas apoptotic neurons utilize other receptors such as vitronectin and phosphotidylserine-mediated receptors.[176] Chloride channels play an important role in microglial phagocytosis and ciliary neurotropic factor increases phagocytosis via a $Ca2^+$ pathway.

A major control mechanism for microglial phagocytosis is by way of the $P2Y_6$ receptor, which is upregulated with neuronal damage.[81] This enhancement of a particular purinergic receptor indicates that purinergic receptors are important in several functions of microglia, which would be activated upon the release of ATP, which is associated with cellular damage.

Synaptic stripping and synaptic building

States of inflammation are often associated with removal of synapses and the process is driven by activated microglia. Neuronal electrical activity normally suppresses MHC class II activation on both astrocytes and microglia.[110] Yet, with immunoexcitotoxicity, where excitatory neurotransmission sensitivity is greatly increased, microglia can initiate synaptic stripping to reduce excitatory synaptic activity as a neuroprotective response.[160] This process principally removes excitatory glutamatergic synapses and spares inhibitory input.

When in a reparative phenotype, microglia can also promote synaptogenesis by secreting thrombospondins (TSPs), an extracellular matrix protein critical for synaptic formation.[34] As a result, microglia, when in a neuroprotective phenotype, are critical for restoration of neuronal connectivity following injury and after inflammatory reactions have terminated. The adaptive immune system, utilizing regulatory T-lymphocytes (CD4+, CD25+ Tregs), interacts with microglia to promote brain repair, again demonstrating the interaction between the innate immune system and the adaptive immune system. Tregs play a critical role in controlling brain inflammation and studies have shown that in diseases such as multiple sclerosis one sees a switch from infiltrating immune inhibitory Tregs to cytotoxic CD8+ T-lymphocytes during disease progression.[163] This switching process has also been shown in several neurodegenerative diseases, such as ALS and Alzheimer's dementia.[77] Microglial cells have a trophic function by upregulating the secretion of neurotrophins, such as BDNF. This functional switching allows microglia, while in this reparative phenotype, to repair the damage done during an immune attack. As shown in part I of this immune primer, CD200, fractalkines, IL-10, Il-4, IL-13, and TGF-ß also play critical roles in microglia switching to a reparative phenotype and in reducing brain inflammation.

Systemic immunity interactions with innate CNS immunity (Sickness behavior)

In the past it was assumed that the neurological and behavioral effects of viremia and sepsis were secondary to the infectious agent acting within the brain itself. It is now accepted that peripheral inflammation and immune activation secondarily effect brain function during the infectious process.[48] This secondary immune process has been named sickness behavior and is characterized by anorexia, hypersomnia, lethargy, reduced social interaction, reduced cognitive function, and weakness.

There are four major links between peripheral immunity and CNS immunity, one a rapid system and the other three of slower onset. The slower-onset systems involve a passive diffusion of proinflammatory cytokines into the brain via the CVOs – organum vasculosum of the lamina terminalis, subfornical organ, neurohypophysis, pineal gland, median eminence, and dorsal vagal complex.[2] Diffusion of proinflammatory cytokines into the CVO areas brings them into contact with ramified (resting) microglia, which are then activated and become mobile.

The BBB has an energy-dependent, saturable, carrier-mediated transport system for cytokines, primarily IL-1, IL-6, and TNF-α.[8,73] Finally, when endothelial cells making up the BBB come into contact with these peripheral cytokines, they secrete various immune molecules into the brain parenchyma, including NO, prostaglandin E2, IL-1, and IL-6, all proinflammatory cytokines known to affect neurological function.[57] There is some question as to the sufficiency of these secreted cytokines to actually affect behavior directly.

The rapid system operates through the vagus. Several lines of evidence implicate the vagus nerve in this fast process. For example, subdiaphragmatic sectioning of the vagus mitigates sickness behavior induced by peripheral immune activation.[22] IL-1 receptors are found on vagal paraganglion and with LPS injection i.p., expression of both IL-1 and IL-1 receptors is enhanced on the vagal paraganglion with an increase in vagal afferent activity. Hansen *et al*. found that intraperitoneal injection of IL-1ß increased IL-1ß mRNA levels in the hypothalamus, hippocampus, and brainstem and sectioning of the vagus blocked induction of IL-1ß mRNA in the hippocampus and brainstem, but only partially blocked responses in the hypothalamus, suggesting the existence of other pathways in this area.[76]

Activation of brain microglia following peripheral immune stimulation is most intense in the face of preexisting brain pathology and some feel that without this preexisting pathology peripheral immune stimulation will not lead to neurodegeneration. For example, using a ME7 model of prion disease, researchers found that injecting this protein into the brain prior to peripheral immune activation, produced an exaggerated proinflammatory response with intense microglial activation, extreme sickness behavior, and acceleration of neurodegeneration.[44,47]

Clinical Correlation: This may be the case in such conditions a chronic traumatic encephalopathy (CTE) and would explain why the phenomenon does not occur in every person suffering repeated minor concussions.[18] Silent neurological conditions occur in a significant number of individuals and may act to prime microglia upon the first exposure to a systemic inflammatory event.[175] For example, cases of silent multiple sclerosis lesions are thought to be present in as many as 25% of examined brains.[56] Other chronic conditions, such as latent infections, glial scarring, neurodevelopmental defects, illegal drugs use, exposure to pesticides/herbicides, and toxic metal accumulations (aluminum, mercury, cadmium, and lead) could also act as priming agents. Aging itself also results in microglial priming. Subsequent injuries, ischemia/hypoxia or infectious processes affecting the systemic immune system would then trigger an enhanced microglial activation state in the CNS, leading to progressive neurodegeneration. Microglial activation is quite rapid following systemic immune activation, usually within minutes and results in immunoexcitotoxicity.

MICROGLIAL PRIMING

As we see in peripheral immune cells, in particular macrophages, microglia can switch from a resting phenotype to a primed state by an initial immune stimulus that is not excessively intense. For example, a mild head injury or episode of hypoxia can switch microglia from its resting state to a functional condition in which the enzymes and genetic activation is upregulated, but the active immune molecules, primarily proinflammatory cytokines and chemokines, are not released. We see priming of microglia also with aging of the brain.

With a second immune stimulus, these primed microglia began to release proinflammatory cytokines and chemokines in concentrations much higher than would microglia that have not been primed. Systemic immune stimulation can prime brain microglia, which means that either subsequent brain disturbances or systemic immune activation would trigger a magnified immune response within the brain. Is essence, it strongly indicates that systemic immune activation can worsen and speed up CNS degenerative disorders, such as ALS, AD, and PD.

We see similar enhancement of neurodegeneration by peripheral infections in rodent models. The destructive process develops secondary to local microglial priming by the preexisting pathological process, such as a stroke, closed head injury, multiple sclerosis, AD, PD, prior brain surgery, or penetrating injury. The peripheral immune stimulation by way of the interacting process discussed earlier, fully activate the primed microglia in an exaggerated manner.[119]

Because humans are exposed to a number of immune events throughout life, such as multiple infections, persistent viruses, exposure to neurotoxic metals, exposure to pesticides/herbicides and fungicides, head injury, microinfarctions, chronic stress and aging, one can see that each of these episodes is associated with microglia priming and activation, leading to a progressive loss of neurons in the most vulnerable parts of the CNS, such as the hypothalamus, temporal lobes (hippocampus, striatal area, amygdala, and entorhinal cortex) and prefrontal cortex.

Those with the least efficient protective systems, such as glutamate transport systems, antioxidant network, cellular glutathione, low CD200 and fractakline and tissue iron binding would be at the greatest risk for one of the neurodegenerative diseases. Under such conditions, the microglia would remain activated in the affected zones and immunoexcitotoxicity would be triggered over a long period leading to neurodegeneration.

Aging and immunoexcitotoxicity

Since aging itself is associated with priming of microglia and elevations in inflammatory cytokines, one would expect peripheral immune stimulation under such conditions to worsen any coexisting clinical picture significantly.[118] Aged mice exposed to i.p. LPS demonstrate more severe anorexia, depression-like behavior, learning deficits, and memory impairment than do younger cohorts.[69] In addition, it takes aged individuals longer to get over the symptoms of sickness behavior than it does younger persons.

Immunoexcitotoxicity and behavioral disorders

Immunoexcitotoxicity is thought to play a significant, if not central role in many behavioral disorders in humans as well[46] [Figure 10]. It is known that individuals developing two or more infections, of any type (mild or serious bacterial or viral infections), over a 4-year period increase their risk of developing AD by 2-fold.[54] It also explains why systemic infections can worsen multiple sclerosis and accelerate the progression of neurodegenerative diseases.[78,142] This magnification process has been confirmed in animal models of neurological diseases.[69] The link between systemic inflammation and brain neurodegeneration also explains the finding that those with general ill health, especially in the later years of life, have a higher risk of cognitive decline. This link between systemic inflammation and neurodegeneration occurs because frail individuals suffer not only from repeated infections, but also from prolonged infections. This interaction with systemic immunity serves to prime the microglia, which means that subsequent immune events will result in a greatly magnified release of proinflammatory cytokines and release of excitotoxins. The resulting brain inflammation significantly magnifies glutamate receptor sensitivity by a number of mechanisms, such as AMPA and NMDA receptor trafficking, mitochondrial energy suppression, suppression of glutamate removal mechanisms (EAATs), stimulation of glutaminase (converts glutamine to glutamate) and impairment of enzymes metabolizing glutamate (glutamate dehydrogenase and glutamine synthase).[18,146]

Immunoexcitotoxicity and other diseases

A growing number of neurodegenerative disorders, including strokes, schizophrenia, autism, CTE, and neuropsychiatric disorders are associated with primed or activated microglia.[14,17,18,33,60,67,119] Studies have also shown that inflammatory bowel disorders can prime/activate brain microglia and cause or worsen neurologic disorders.[131,164]

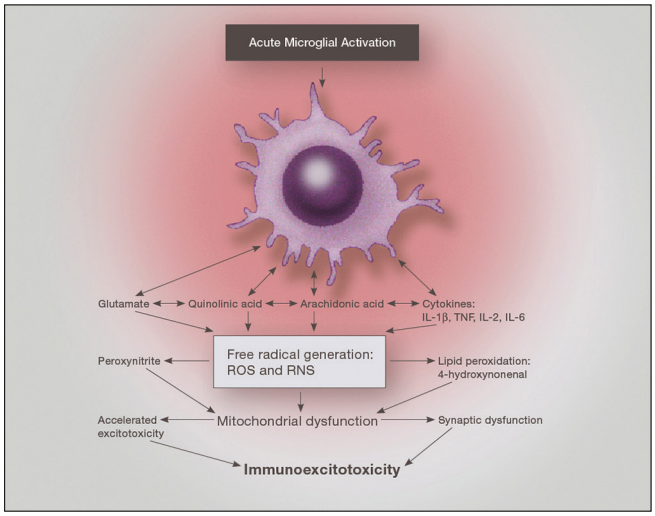

Figure 10: Illustration of the combined release of pro-inflammatory cytokines and chemokines along with excitatory amino acids during neurodestructive microglial activation. This demonstrates the interaction between immune factors, reactive oxygen and nitrogen species, lipid peroxidation species and excitotoxicity

Immunoexcitotoxicity is enhanced by vaccines

Also of concern is the present vaccine schedule for children, which entails a repetitive injection of concentrated powerful adjuvants during a period of intense brain pathway formation. The present US vaccine schedule recommended by the Center for Disease Control and Prevention (CDC), now mandated for most states, consist of giving small children up to age 6 years as many as 5-8 vaccines per office visit every 2 months for as total of 28 vaccines during the first year of life.[32] When given as multiple doses, for example, 6 doses to a small child during a single office visit, significant systemic immune activation occurs, with signs suggesting reactive brain inflammation–such as high pitched crying, fever, restlessness, and failure to eat.[14]

It is not the antigens that are of most concern, but rather the adjuvants, which initiate the most intense and prolonged immune stimulation. These adjuvants are designed to magnify the immune response, as the antigens (viruses and bacteria) themselves are too weak to provide protection. In addition, adjuvant additives, such as aluminum and mercury, are known powerful microglial activators and are neurotoxic, even in very small concentrations.[13,86,159] These neurotoxic metals have been shown to accumulate in the brain.[36,167] Because of the ability of immune adjuvants to prime microglia and trigger intense microglial activation with subsequent vaccinations, we see neurological side effects even in vaccines without aluminum or mercury.[13,24,36] Several studies have also shown that the aluminum adjuvant in human vaccines is distributed into the brain upon injection.[61,126]

Priming of the microglia

In the infant or small child, the priming event may come from a number of sources, such as vaccination of the mother during pregnancy or with intrauterine or early postbirth infections.[16,19] In other instances, the priming event may occur with the first vaccine inoculation, usually at birth (hepatitis B). Once primed, subsequent vaccinations, especially within months of the previous inoculation, will trigger full microglial activation and in the developing brain can result in abnormal pathways development.[1,14,15,133]

While natural infections can also produce this neurodestructive response, vaccinations produce higher levels of immune activation and the immune response can persist longer than natural infections – sometimes lasting years. In the case of the elderly, already having primed microglia, repeated, closely spaced, sequential

vaccination can also present a potential hazard, since an exaggerated microglial response could worsen any preexisting neurological disorder (even silent ones) or initiate such a disorder.[17,48,119,120]

Control of microglia activation and deactivation

A great number of factors can activate microglia, which makes sense when you consider that it is the main defensive arm of the innate immune system and must react rapidly to a number of stimuli. Microglial activation occurs rapidly upon disturbance of the brain's homeostasis, usually within minutes. The major activator of microglia is IL-1ß, which can be released locally with pathological damage within the CNS or can be transported from the systemic circulation.[23] Activation of a PRR signals the intracellular inflammasomes and this cleaves pro-IL-1ß, leading to microglial release of additional IL-1ß, thus further activating surrounding microglia. Other important cytokines in microglial activation include INF-γ and TNF-α. Thrombin can also activate microglia by way of a protease activated receptor 1 (PAR-1) receptor.[29] This would be important in cases of brain hemorrhaging and a leaky BBB. Activation in most cases takes place, as discussed previously, by the interaction of PAMPs and DAMPs with PRRs, which can include a number of the types of surface receptors mentioned above.

Recent studies have shown that microglial activation can be anatomically selective in terms of both the brain anatomy affected and the microglial phenotype being expressed.[38] This would explain the ability of systemic immune activation to produce differing patterns of behavioral and neurological effects, such as obsessive–compulsive disorder, depression or schizophrenia.[33,67,147,168] The final clinical presentation would depend on the activating principle and other associated factors, such as the patterns of neural net injury. Microglia are not only heterogeneously distributed, but also appear to have specialized subgroups, which would give them differing patterns of activation.

Newer studies have shown that many of the traditionally used antipsychotic and antidepressive medications can suppress microglial activation and reduce inflammation and excitotoxicity.[107] In fact, both lithium and St John's Wort reduce excitotoxicity and the latter has been shown to be antagonistic to the NMDA receptor, which has been shown to play a major role in depression.[90,181]

Of equal importance in brain inflammation are mechanisms utilized to downregulate or terminate microglial activation. Of principle importance are the cytokines, IL-10, IL-4, fractalkine (CX3CL1), and CD200 (cluster of differentiation 200) and its receptor CD200R. Suppression of the last two modulators is necessary for microglial activation. The immune suppressant cytokine IL-4 modulates CD200 so as to reduce microglial activation.[99] Mice lacking CD200 receptors demonstrate an exaggerated proinflammatory release following stimulation with LPS or other CNS or systemic immune activators and deficiencies in CD200 are linked to Alzheimer's dementia and Parkinson's syndrome.[165,170,182] Aged mice were found to have less CD200R on the surface of their microglia as compared with younger animals. This loss of CD200 would make aged individual more susceptible to intense, prolonged microglial activation following immune activation from any cause.

IL-10 reduces microglial activation and lowers secreted levels of TNF-α, NO, ROS, and superoxide following LPS exposure (immune activation).[122] Another antiinflammatory cytokine is TGF-ß a soluble factor produced by astrocytes in a constitutive and inducible manner. TGF-ß inhibits microglial activation.[123] TGF-ß has also been shown to selectively induce apoptosis in microglia in a mixed glial culture of microglia, astrocytes, and oligodendrocytes.[177] A reduction in microglia reduces brain inflammation.

Recently, TREM2, a DAP12-associated receptor and modulator of TLRs, has attracted a lot of attention as a regulator of microglial activation.[62] While TREM2 is found in a number of cell types, in the brain, the highest expression is in microglia. Increased expression of TREM2 switches microglia to a phagocytic phenotype, which is a phenotype known to secrete none of the proinflammatory cytokines. That is, it is mostly neuroprotective. Reductions in TREM2 are associated with a high risk of AD.[84]

Should we be taking microglial inhibitors in our diets?

Streit *et al*. put forth the hypothesis that with aging, microglia become dystrophic and therefore lose much of their ability to protect the brain utilizing both their immune and neurotrophic functions.[149] This dystrophic microglia concept would argue against the neurotoxic effect of primed microglia with brain aging. Sierra *et al*. examined this hypothesis utilizing a transgenic mouse line and found that with aging the microglia actually increased their production of proinflammatory cytokines (IL-1ß, IL-6, IL-12, and TNFα) as well as antiinflammatory cytokines as compared with younger mice, which would be consistent with destructive effect of priming of microglia with aging.[143] The proinflammatory cytokine levels were higher than the antiinflammatory cytokines, resulting in an inflammatory condition. This demonstrates that aged microglia have a fully functioning neurotoxic capability and because they are primed, exhibit an exaggerated proinflammatory response.

With the finding of abnormal functioning of CD200-CD200R regulatory control in neurodegenerative diseases, one can appreciate the effect of age-related priming on accelerating neurodegenerative pathology since the normal switching off of the microglia would

be impaired [Figure 11]. Abnormalities of T regulatory lymphocyte (Tregs) function, a major antiinflammatory mechanism discussed later, have been linked to several neurological conditions.[127,155] Abnormalities in these and other antiinflammatory molecules would mean that even low concentrations of proinflammatory molecules would have a greater impact on neuronal pathology because of the imbalance in the antiinflammatory and inflammatory systems.

While microglial activation would appear to be a useful target in treating a number of neurological diseases, recent studies indicate that general suppression of microglia may not be a good idea, as certain phenotypes of microglia are neuroprotective. The timing is important when considering suppressing microglial activation. For example, it is thought that in the early course of ALS, microglia and macrophages are in the M2 protective mode of activation and as the disease progresses, they switch to an M1 cytotoxic mode.[21] The same pattern may also appear in AD. Tregs are thought to control microglia/macrophage switching from M1 (neurodestructive) to an M2 (neuroprotective) phenotype.[185]

Safer targets would be downstream mechanisms, such as inhibiting glutaminase, enhancing neuronal glutathione, antioxidant protection of critical enzymes, such as glutamine synthase and glutamate dehydrogenase; suppression of NADPH oxidase or iNOS, stimulation of group III mGLuRs (inhibitory), enhancing EAATs and suppression of prostaglandin E2 production. A number of studies have been done using these various approaches with evidence of considerable success in animal models and some limited human studies. A growing number of natural products, most extracted from plants, have shown an ability to alter immunoexcitotoxicity.

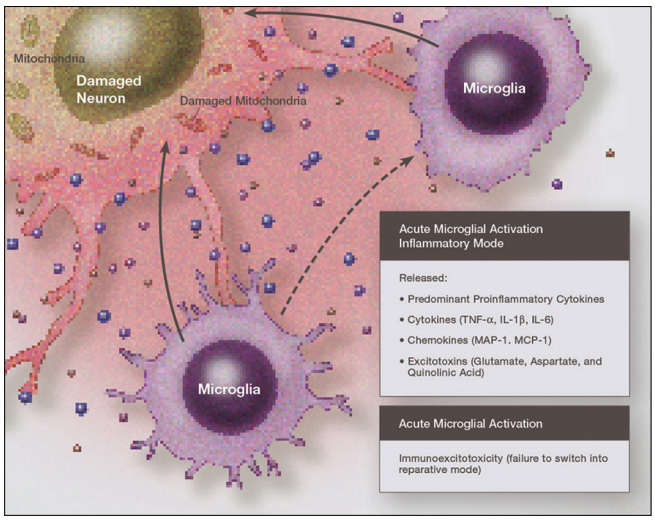

Figure 11: Illustration demonstrating chronic neurodegeneration resulting from a failure of activated microglia to switch to a resting state. Under such conditions immunoexcitotoxic cascades persist.

Immune cell adaptive responses in the CNS

Over 90 years ago it was shown that an implanted tumor in the brain of a rat was resistant to systemic immunity, thus establishing the idea that the brain was an "immunologically privileged site" and therefore isolated from systemic immunity. Over the past several decades growing evidence has supported the idea that this immune isolation is not absolute. It is true that among lymphocytes only activated lymphocytes can enter the CNS, but do so with reduced efficiency.[5,172]

Under normal conditions, once within the CNS parenchyma, the activated CD8+ cytotoxic T-cells are treated as being hostile and most undergo apoptosis or conversion to regulatory T-cells (Tregs). Neurons protected themselves from activated lymphocytes by upregulating and secreting TGF-ß, an inflammation-suppressing cytokine that converts CD8+ cytotoxic T-cells to immune suppressant regulatory T-cells (Tregs). Newer studies have shown a preponderance of Tregs in the face of CNS inflammation.[96,97] This indicates that neurons themselves are critical in immune modulation.

Lymphocyte migration into the CNS is regulated by chemokines and their receptors. In the normal brain, few lymphocytes are found in the brain parenchyma. Dramatic increased infiltration of T-cells occurs with strokes, brain trauma, and CNS infections. Lymphocytic infiltration into the CNS is increased in neurodegenerative disorders but not to the scale of brain trauma or infection. Gemechu and Bentivoglio found increased CNS lymphocyte infiltration with brain aging.[64]

Aging is associated with structural and functional alteration in the BBB, including alteration in the carrier-mediated mechanisms.[139] A leaky BBB allows normally excluded immune cells, as well as antibodies, to enter the brain parenchyma. Entry of T-cells into the brain parenchyma is also facilitated by chemokines secreted by reactive astrocytes and microglia such as monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-1 (MIP-1).[4]

The fate of these entering T-cells depends on the state of brain inflammation and particular responses of lymphocyte interaction with glia cells. It appears that in most cases of prolonged brain inflammation, even with aging, T-cells are in a neuroprotective phenotype (Tregs).[30,135,137] This new concept has changed thinking regarding lymphocyte infiltration of the CNS in such diseases as multiple sclerosis, ALS, brain and spinal cord injury, and other neurodegenerative diseases.[136] It appears that in most cases of CNS inflammation, the preponderant T-cell phenotype consist of antiinflammatory Tregs, yet with disease progression, they may switch macrophages to a preponderant M1 cytotoxic phenotype.

SUMMARY

Over the past several decades we have learned a great deal about microglia and innate brain immunity. While microglia are the principle innate immune cells, other cell types also play a role, including invading macrophages, astrocytes, neurons, and endothelial cells. The fastest reacting cell is the microglia and despite its name, resting microglia are in fact quite active. Motion photomicrographs demonstrate a constant movement of ramified (resting) microglial foot processes, which appear to be testing the microenvironment for dangerous alteration in extracellular fluid content. These foot processes, in particular, interact with synapses and play a role in synaptic function. In event of excitatory overactivity, these foot processes can strip selected synapses, thus reducing activation states as a neuroprotective mechanism. They can also clear extracellular glutamate so as to reduce the risk of excitotoxicity.

Microglia also appear to have number of activation phenotypes, such as: principally phagocytic, principally neuroprotective and growth promoting or primarily neurodestructive. These innate immune cells can migrate a great distance under pathological conditions and appear to have anatomic specificity. There is some evidence that there are several types of microglia. Macrophage infiltration into the embryonic brain is the source of resident microglia and in adulthood macrophages can infiltrate the brain and are for the most part indistinguishable from resident microglia, but may react differently pathologically.

Activation itself does not imply a destructive phenotype and can be mostly neuroprotective via phagocytosis of debris, neuron parts and dying cells and by the release of neurotrophins such as NGF and brain BDNF. Evidence is accumulating that microglia undergo dynamic fluctuations in phenotype as the neuropathology evolves. For example, in the early stages of neurotrauma and stroke, microglia play a mostly neuroprotective role and only later switches to a neurodestructive mode.

A great number of biological systems alter microglia function, including neurohormones, cannabinoids, other neurotransmitters, ATP, adenosine, and corticosteroids. One can appreciate that with aging, many of these systems are altered by the aging process itself or diseases, thus changing the sensitivity of the innate immune system.

The realization that CNS microglia can be activated by stimulation of the systemic immune system is of great concern both in terms of natural disease and attempts to protect against disease by vaccination. It appears that not only the intensity of the immune stimulation is important, but also how closely spaced apart it occurs. Closely spaced, repetitive immune stimulation maximizes CNS innate immune activation and immunoexcitotoxicity.

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