



Microglial Activation and Neurodegeneration

by

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Microglia are the resident immune cells in the

central nervous system. Normally they remain in a quiescent state. When stimulated, they become metabolically active and assume an ameboid profile, with an ability to move about the CNS. Activation of microglia is quite easy and can be accomplished by a number of events, such as trauma, excitotoxic excitation, invasion of microorganisms, heavy metal toxicity, systemic immune activation and various pharmaceutical agents.

Under most circumstances this activation is short lived and terminates rather abruptly. Under such conditions, the microglia serves the function of any immune cell—that is, it releases immune cytokines, eicosanoids, free radicals, lipid peroxidation products and complement, which act to kill the invading organisms. In addition, microglia secrete several growth factors (brain derived growth factor and epidermal growth factor), which serves to repair any damage that might have been done. It also has ameboid, phagocytic activity, which allows these cells to remove cellular debris and dead or dying microorganisms. It can also remove soluble beta-amyloid, which can accumulate during such assaults. Because of their ability to move around and between neurons, they can specifically target the synaptic interface. In a great number of conditions, this synaptic unit is damaged and therefore is central to their pathophysiology.

On occasion, the activation of microglia is not terminated and a state of chronic activation occurs. When this happens, significant damage can occur to a number of brain microstructures, especially synaptic connections and dendrites. While short term microglial activation can be neuroprotective (because of the secreted growth factors) chronic activation appears to be neurodegenerative. Newer studies have shown that chronic microglial activation occurs in a number of pathological states, including CNS trauma, infections, heavy metal toxicity, pesticide exposures, neurodegenerative diseases, autism, Down' s syndrome and over-vaccination.

When microglia are activated, they also secrete two known excitotoxins—**glutamate and quinolinic acid**. In addition, they secrete a number of factors that are also known to enhance excitotoxicity, such as tumor necrosis factors—alpha (TNF—alpha), IL-1 β , IL-2, IL-4, IL-6 and IL-18, as well as interferons and other immune molecules. A number of studies have shown that several of these immune factors greatly enhance excitotoxicity, especially TNF—alpha and IL-1 β —two inflammatory cytokines that are elevated in all of the neurodegenerative diseases as well as autism.

Microglia also secrete a fatty molecule called **arachidonic acid**. This too enhances excitotoxicity. Arachidonic acid release increases the production of

inflammatory eicosanoids, primarily by the action of LOX and COX enzymes. Studies have shown that glutamatergic neurons also contain COX enzymes and that excitotoxic destruction of neurons follows the presence of COX-2 enzymes. Blocking COX and LOX enzymes significantly reduces excitotoxicity. This accounts for some of the selectivity of excitotoxicity, that is, it will severely damage and/or kill some neurons and result in no harm to others close by.

Another damaging effect of chronic microglial activation, is a concept called by-stander damage. When the inflammatory cytokines, excitotoxins, eicosanoids, free radicals and lipid peroxidation products are released, they diffuse toward surrounding neurons and their dendrites and synapses. The longer the microglia are active, the greater and more widespread the damage becomes. So, why the selective damage?—that is, How can some neurons survive in such an environment? Studies have shown that neurons possessing strong antioxidant defenses, or lacking free radical-generative enzymes (such as NADPH oxidase) can escape the damage much longer than poorly protected cells and synaptic connections.

Likewise, glutamatergic neurons have been shown to contain COX-2 enzymes, whereas protected neurons do not. Blocking COX-2 has been shown to be neuroprotective in cases of excitotoxic activation, as

mentioned. This explains the dramatic reduction in Alzheimer's disease seen in epidemiologic studies in those who regularly take NSAIDs. Experimentally, NSAIDs and other COX-2 and LOX blocking drugs and natural products can significantly attenuate excitotoxic damage.

In the excitotoxic cascade, PLA₂ (phospholipase-A₂) is activated by protein kinase-C, releasing arachidonic acid from the membrane of the neuron. LOX and COX enzymes convert arachidonic acid into leukotrienes and inflammatory prostaglandins, respectively. It is these eicosanoids that generate the reactive oxygen and reactive nitrogen species (peroxyl, superoxide and hydroxyl radicals) that ultimately damage the mitochondria, endoplasmic reticulum and nucleus during excitotoxic cascade activation.

During the opening of the calcium channel (in NMDA and AMPA glutamate receptors) the excess intracellular calcium also activates nitric oxide synthetase (NOS), which generates excessive amounts of nitric oxide (NO). The NO then reacts with superoxide to form peroxynitrite radical, which is a very powerful reactive nitrogen species (RNS). This RNS passes through the mitochondrial with great rapidity, and has been shown to be especially damaging to mitochondrial enzymes and mtDNA.

In essence, we see an interaction between

eicosanoid production and nitric oxide production that ultimately results in a significant loss of mitochondrial energy production. A number of studies have shown that neuronal energy loss, no matter the cause, greatly increases neuronal, dendritic and synaptic sensitivity to excitotoxin damage.

In addition, some of the NSAIDS are known to be rather potent inhibitors of microglial activation. This is especially beneficial in cases of chronic microglial activation. It is also known that inhibition of microglial activation, once triggered, is dependent of certain cytokines, such as IL-10 and TGF- β . Dysfunction in IL-10 activation, as seen with shifts from TH2 to Th1 cytokine activation, interfere with cessation of microglial activation. This switches microglia from a neuroprotective function to a neurotoxic one. In pathological states, such as Alzheimer' s dementia, Parkinson' s disease and autism, it may be that a loss or dysfunction in brain growth factors also plays a role. This would make microglia a source of inflammatory cytokines, complement, S100B, eicosanoids, arachidonic acid and excitotoxins, without the protective factors.

Reduced levels or dysfunction of antioxidant enzymes, such as catalase, SOD, glutathione reductase, glutathione peroxidase and glutathione itself would also greatly increase neuronal sensitivity to excitotoxicity and free radical and lipid peroxidation damage.

As we have seen in my discussion of mercury toxicity, mercury not only is a powerful activator of microglial activity at micromolar or even submicromolar concentrations, but also at these same concentrations powerfully inhibits the glutamate transport proteins. At 0.5 uM we see a 50% reduction in glutamate uptake. The majority of extracellular glutamate is taken up by the astrocyte, which is the site of the greatest mercury accumulation in the CNS. The microglia is the second most abundant site of mercury accumulation.

Mercury also is a powerful inhibitor of **glutamine synthase** and **glutamate dehydrogenase**, both of which also play major roles in controlling extracellular glutamate levels. At lower concentrations of glutamate, glutamine synthase is the most important mechanism for astrocyte glutamate clearing. But at higher levels, glutamate dehydrogenase becomes more important.

So, we can see that a single heavy metal can powerfully interfere with glutamate clearance by a number of mechanisms, resulting in excitotoxicity. In addition, mercury is a powerful inhibitor of mitochondrial enzymes and interferes with mitochondrial membranes function, both of which reduce neuronal energy production, as well as energy production by astrocytes and microglia. This energy loss, as we have seen, magnifies excitotoxicity.

Mercury is not the only thing that can precipitate

these chains of events. Pesticides, other heavy metals, elevated free radical presence, 4-hydroxynonenal, infectious organisms, glutamate itself, other excitotoxins and oxidized LDL-cholesterol in the brain can have the same effect. It is the synergistic effects of a number of environmental and metabolic toxins that, in my opinion, results in the neurodegenerative diseases, autism, Down' s syndrome and a number of other neurological conditions. Yet, central to the process in all these conditions is chronic microglial activation.