Environmental Toxins Linked to Neurodegeneration and Autism Activate the Brain's Immune System

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Abstract
Microglia are the primary immune cells of the central nervous system and become activated in response to noxious stimuli, leading to a cycle of inflammation and cell death that has been implicated in the development of Parkinson’s disease and autism. This study examines the effects of environmental toxins, at levels commonly found in humans, on microglial cell survival and activation. The toxins used in this study include polybrominated diphenyl ether (PBDE) flame retardants, the food additive propionic acid (PPA), and the organochlorine pesticide dieldrin. These chemicals have been linked to neuronal damage, although their effects on microglial cells have not been fully studied. Our results indicate that microglial cell survival could be decreased by as much as 50% due to exposure to these toxins, without the production of certain cytokines produced by lipopolysaccharide (LPS)-induced activation. These effects are significant as further understanding of the role of microglia in neuronal damage could provide a pharmacologic target for future drug development as well as elucidate the pathology of neurodegenerative diseases.

Disciplines
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Comments
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Environmental Toxins Linked to Neurodegeneration and Autism Activate the Brain’s Immune System

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Abstract:
Microglia are the primary immune cells of the central nervous system and become activated in response to noxious stimuli, leading to a cycle of inflammation and cell death that has been implicated in the development of Parkinson’s disease and autism. This study examines the effects of environmental toxins, at levels commonly found in humans, on microglial cell survival and activation. The toxins used in this study include polychlorinated diphenyl ether (PBDE) flame retardants, the food additive propionic acid (PPA), and the organochlorine pesticide dieldrin. These chemicals have been linked to neuronal damage, although their effects on microglial cells have not been fully studied. Our results indicate that microglial cell survival could be decreased as much as 50% due to exposure to these toxins without the production of certain cytokines produced by lipopolysaccharide (LPS)-induced activation. These effects are significant as further understanding of the role of microglia in neuronal damage could provide a pharmacological target for future drug development as well as elucidate the pathology of neurodegenerative diseases.

Introduction:
In the central nervous system (CNS), microglia are the innate immune cells. Microglia are spread throughout the brain and when resting appear ramified, or branched (see Fig. 1). These branches extend into the surrounding tissue. Microglia wait in this resting state until contact is made with environmental triggers: damaged cells, foreign matter, plaques or other inflammatory triggers (e.g., pathogens or toxic chemicals).1 After contact, microglia change into an activated amoeboid form, where they phagocytize cellular debris and release pro- or anti-inflammatory factors and reactive oxygen species (ROS). Microglia can, however, become over-activated and damage neurons in a process known as reactive microgliaosis (see Fig. 2). Diseases such as Alzheimer’s disease and Parkinson’s disease have been associated with reactive microgliaosis and activated microglia. Environmental toxins, such as pesticides and flame retardants, are also known to activate microglia and may play a role in the development of neurodegenerative diseases.

Dieldrin is an organochlorine pesticide that persists heavily in the environment. Widely used in the U.S. until the 1980’s, exposure occurs via contaminated food.2 Dieldrin induces neurotoxicity in dopaminergic neurons through oxidative stress. Dieldrin has also been shown to induce microglia to produce ROS, which can activate apoptosis in dopaminergic neurons.2

Propionic acid (PPA), a natural component of butter and some cheeses, has been used as a pesticide due to its fungicidal and bactericidal properties. It is also an endogenous metabolite in humans, and as such is exempt from legal residue limits when applied to crops. Moreover, it does not have to undergo typical pesticide toxicity data and testing. PPA is readily able to cross the blood brain barrier and enter the CNS, where it has been linked to autism, and has been proven to activate microglial cells in vivo.3 The molecular effects of this activation are unknown.

PBDE flame retardants are commonly used in many household items, from computers and televisions to carpeting and furniture. They’ve also been found to be increasingly prevalent in the environment, with high levels recorded in soil, animals, and human serum and milk.4 Studies in rats and mice have shown low concentrations of PBDEs to cause reversible neuronal damage.5-7 PBDE-47, a common PBDE homolog, has been shown to induce ROS formation in human neutrophil granulocytes and primary rat hippocampal neurons in vitro.8

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Results:

- Dieldrin (FIGURE 3) significantly reduces microglial cell survival at doses of 10, 20, 30 and 40 µM, with dose-dependent death.
- PBDE-47 (FIGURE 4) caused a statistically significant reduction in survival of microglia at a dose of 60 µM.
- PPA (FIGURE 5) exposure also caused a significant decrease in microglial cell survival at concentrations of 1.5 and 3.0 µM.
- Neither dieldrin, PBDE-47, nor PPA activate microglia to release the cytokine TNF-α or nitrite, both typical markers of microglial activation.

Methods:
An immunolabeled rat microglial cell line (HAPI) was subjected to physiologic concentrations of PBDEs, dieldrin, or PPA, in vitro. Effects on microglia are measured via cell death, cytokine (TNFα), and nitrite production. Cell survival and nitrite production were measured for 96 h, while TNFα production was measured at 72 h. All results were measured 3 hours post-treatment. Statistically significant changes for each measure were determined using one way ANOVA with a post-hoc Bonferroni test; p-values < 0.05 were considered significant.

MTT Assay
The MTT assay was used to measure the metabolic viability of cultured microglia. Thiazole blue (MTT) is applied to the cell cultures and cell survival is based on a color change.

The production and release of TNFα was measured using an enzyme-linked immunosorbent assay (ELISA) according to manufacturer protocol (R & D Systems, Minneapolis, MN) and as published previously.9

Nitrile Assay
The amount of nitrite in the culture was determined using the Griess reagent method and measuring color change as previously described.8

Conclusions:
Microglia play a major role in immunity for the CNS, and chronic activation due to environmental toxins or neuronal damage has been shown to result in neuronal dysfunction and inflammation. This study shows that the environmental toxins dieldrin, PBDE-47 and PPA can cause microglial cell death at physiologically relevant levels, instead of the release of inflammatory cytokines, normally seen in response to threatening stimuli in the CNS (e.g., LPS and air pollution). This implies the presence of a separate mechanism of action of these toxins, resulting in cell death. This study implies that microglia may be compromised, and be unable to serve protectively when exposed to certain toxins, leaving neurons more susceptible.

Future directions of study involve exploring the mechanisms of toxin-induced microglial cell death. This includes the measurement of the activity of histone acetyl transferase (HAT) proteins. Histone acetylation allows for DNA to become transcriptional and gives rise to genes for transcription. Preliminary studies have indicated that HAT inhibitors may help protect against toxin-induced microglial cell death. Elucidating the pathways of neurodegeneration due to environmental toxins will offer new targets for investigative drug research.

References: