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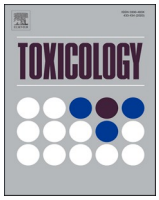

Ochratoxin A stimulates release of IL-1β, IL–18 and CXCL8 from cultured human microglia

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ABSTRACT

Exposure to mycotoxins has been associated with the development of neuropsychiatric symptoms and Ochratoxin A (OTA) has emerged as one of the main mycotoxins associated with neurotoxicity. However, the mechanism via OTA exerts its neurotoxic effects is not well understood, especially the importance of activated microglia and their contribution to neuroinflammation. Here we report the effect of OTA on cultured immortalized human microglia-SV40, as compared to the effect of neurotensin (NT) and lipopolysaccharide (LPS) used as "positive" triggers. OTA (1, 10 and 100 nM for 24 hrs) stimulated microglia to release in the supernatant fluids statistically significant amounts of IL-1β, IL-18 and CXCL8 assayed with ELISA. Preventing or inhibiting OTA-stimulated activation of microglia by luteolin could be an important way to limit mycotoxin-induced neuroinflammation and improve associated neuropsychiatric diseases.

To the Editor

Ochratoxin A (OTA) from exposure to either environmental or food sources has emerged as one of the main mycotoxins associated with neurotoxicity, as was reviewed recently in Toxicology (Obafemi et al., 2023). In fact, mycotoxins have been associated with the development of neuropsychiatric symptoms including brain fog, cognitive impairment, and headaches (Ratnaseelan et al., 2018). However, the mechanism via which mycotoxins exert neurotoxic effects is not well understood and the otherwise excellent review by Obafemi et al. (2023) did not include any discussion of the importance of activation of microglia and their contribution to neuroinflammation.

Microglia are known to be involved in neuroinflammation and neurodegeneration (Hickman et al., 2018) and could be the target of mycotoxins. We had previously reported that microglia can be activated by the peptide NT to release proinflammatory chemokines and cytokines (Patel et al., 2016). We, therefore, investigated the effect of OTA on the release of the proinflammatory IL-1β, IL-18 and CXCL (IL-8) from cultured human microglia.

The immortalized human microglia-SV40 cell line derived from primary human microglia was purchased from Applied Biological Materials Inc. (ABM Inc.; Richmond, BC, Canada) and cultured in Prigrow III medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in type I collagen-coated T25-flasks (ABM Inc.). Microglia-SV40 maintain their phenotype and proliferation rates for over 10 passages, during which all experiments were performed using multiple microglia thaws and sub-cultured cells. Experiments were carried out in type I collagen coated plates (BD PureCoat™ ECM Mimetic Cultureware Collagen I peptide plates, Becton Dickinson, Bedford, MA). Cell viability was determined by trypan blue (0.4%) exclusion and was greater than 95% at all concentrations tested. Human microglia (0.5×10^5 cells/ well) were stimulated at the concentrations shown with OTA, while NT and LPS were used as positive controls. Here we show that OTA can stimulate microglia to release statistically significant amounts of IL-1β (Fig. 1A), IL-18 (Fig. 1B) and CXCL8 (Fig. 1C) in a dose-dependent fashion (Fig. 1). At 100 nM of OTA, the effect was comparable to that of 10 nM NT, but less than 10 ng/ml LPS. These results are important because IL-1β and IL-18 have been implicated in brain diseases.

The only other reference of OTA and microglia was a report using immortalized mouse microglial cells (BV-2) where OTA (50–2000 nM for 24 hrs) induced microglial activation as determined by significant upregulation of IL-1β and IL-6 mRNA levels, but at concentrations higher than 100 nM with no cytotoxicity (Chansawhang et al., 2022). Another study using 3-D rat brain aggregate cultures (containing neurons, astrocytes, oligodendrocytes and microglia) reported that OTA (10 nM) resulted in significantly increased mRNA levels of IL-6 after 48 hrs of incubation (von Tobel et al., 2014). The ability of OTA and other mycotoxins to activate microglia is predicated on their crossing the blood-brain barrier (BBB) and entering the brain. The ability of at least T-2 toxin to disrupt BBB permeability has been reported (Ravindran et al., 2011).

These findings may be relevant to the pathogenesis of autism spectrum disorder (ASD) (Serkan et al., 2021) and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (Wu et al., 2022) whose patients had elevated serum levels of OTA and other mycotoxins. The mechanism of action of OTA activating human microglia is not presently known, but may involve toll-like receptors (TLRs) and the regulatory complex mammalian target of rapamycin (mTOR) (Patel et al., 2016).

One may raise the possibility that mycotoxins could have synergistic action with that of SARS-CoV-2 spike protein in the pathogenesis of Neuro-COVID since we recently reported that SARS-CoV-2 spike protein can stimulate human microglia to release IL-1β and IL-18 (Tsilioni and Theoharides, 2023). In this context, activation of microglia by OTA may be inhibited with the use of the natural flavonoid luteolin (Theoharides, 2021), which has been reported to reduce OTA-associated oxidative stress (Liu et al., 2020). Pretreatment of BV-2 microglia with luteolin

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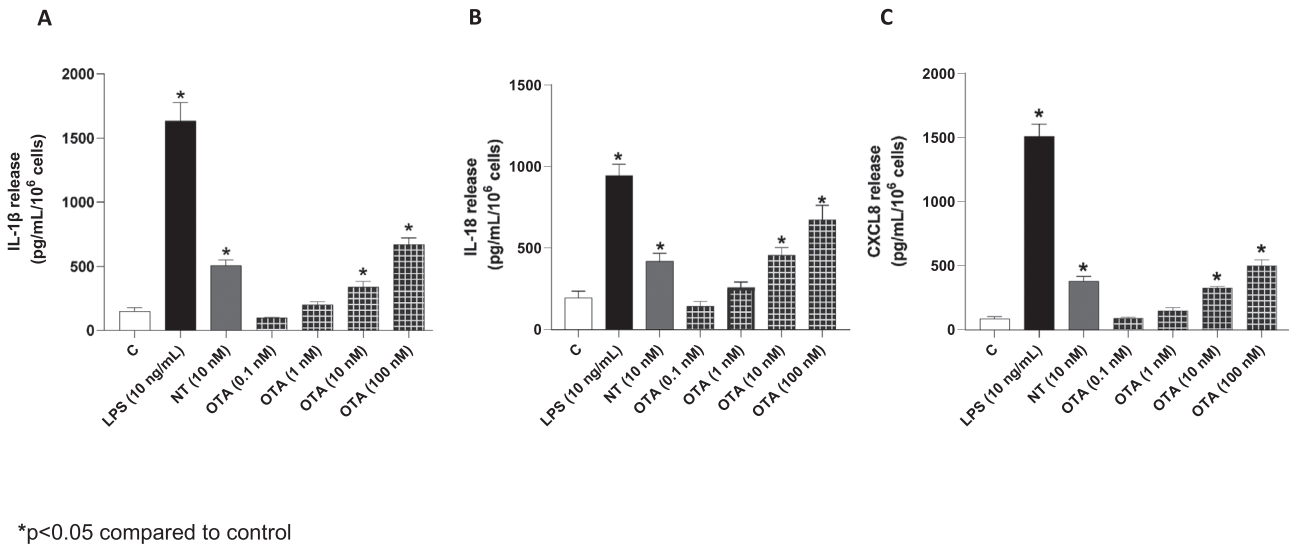

Fig. 1. Ochratoxin A activates human cultured microglia. Human microglia (0.5×10^5 cells/ well) were stimulated at the concentrations shown with Ochratoxin A (OTA), while neurotensin (NT) and lipopolysaccharide (LPS) were used as positive controls. After 24 h, supernatant fluids were collected and concentrations of IL-1β, IL-18 and CXCL8 were measured using commercially available ELISA DuoSet kits from R&D Systems (Minneapolis, MN) according to the manufacturer's instructions. Control cells were treated with equal volume of culture medium. All conditions were performed in triplicate, and all experiments were repeated at least three times (n = 3). Results are presented as mean ± standard error of the mean (SEM). Differences between each group and the control were assessed using the Student's t-test. Statistical significance is denoted as: *$p < 0.05$.

inhibited LPS-stimulated IL-6 production (Young et al., 2008) and methoxyluteolin inhibited NT-stimulated human microglia-SV40 (Patel et al., 2016). In fact, liposomal luteolin formulations have been successfully used (NeuroProtek®) for ASD (Tsilioni et al., 2015) and (BrainGain®) for Long-COVID (Theoharides et al., 2022).

The present findings provide a novel mechanism via which mold exposure may result in neuropsychiatric symptoms and provide a target for drug development.

Authors' contributions

IT and TCT conceived the project. IT performed all experiments. IT and TCT reviewed the results and their significance. TCT reviewed the literature and wrote the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. TCT is the Scientific Director and shareholder of Algonot LLC which develops and sells dietary supplements. TCT has been awarded the following patents: US 5,855.884; US 6,020,305; US 8,268,365.

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