Effects on the apoptotic marker Cytochrome c following a chlamydial infection in neurons and astrocytes: Implications for Alzheimer's Disease

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Abstract

Neurodegeneration has been well documented in the CNS of Alzheimer’s individuals and evidence suggests that apoptosis may be a contributing factor in the pathogenesis of Alzheimer’s disease. Initiating events that occur in apoptosis have been identified in Alzheimer brains; however, complete apoptosis of the neuronal process is not well understood. In earlier studies Chlamydia pneumoniae which is an intracellular respiratory pathogen was identified and isolated from brains of patients who had been diagnosed with sporadic AD [1]. Our initial hypothesis suggested that Chlamydia pneumoniae could involve the apoptotic process. Chlamydia pneumoniae has been found to inhibit apoptosis in neuronal cells [2] and monocytes [3], although the precise apoptotic pathway inhibited is not defined. Inhibition of apoptosis may be one mechanism by which Chlamydia pneumoniae can sustain an infection in the host to maintain an optimal intracellular environment. This infection may influence the contrastive findings to the completion of the apoptotic process in the Alzheimer’s brain.

Given the previous data, our current hypothesis is that astrocytes and neuronal cells infected with Chlamydia pneumoniae can sustain an infection that resists the cells resistant to apoptosis. Since mitochondrial damage has been identified in the pathogenesis of Alzheimer’s disease, the focus of this study was to determine whether cytochrome c, an electron carrier protein that is essential to the mitochondrial respiratory process, was affected following Chlamydia pneumoniae infection of astrocytes and neuronal cells. Following damage to the mitochondria, cytochrome c is typically translocated from the mitochondria to the cytoplasm whereby cytochrome c activates the apoptotic process. In our studies, apoptosis was experimentally induced by staurosporine in astrocytes and SK-N-MC neuronal cells that were both uninfected and infected with Chlamydia pneumoniae for 72hrs. Cytochrome c production was analyzed by immunofluorescent microscopy utilizing an antibody specific to cytochrome c. Our results suggest that Chlamydia pneumoniae infected neuronal cells differentially activate cytochrome c as compared to infected astrocytes. In both infected neuronal and astrocytes, induction of apoptosis with staurosporine did not appear to induce the apoptotic event. Thus, our data appear to indicate that an infection of both cell types blocks apoptotic induction with staurosporine which may be independent of the cytochrome c pathway.

Introduction

Neurodegenerative disorders such as Alzheimer’s disease (AD) are characterized by the chronic degeneration of synaptic function and a progressive loss of cortical neurons. Cytokine-induced changes occur in both cortical and subcortical areas in the formation of paired helical filaments (PHFs) into neurofibrillary tangles (NFTs), signature pathologies of AD. As a result of NFTs, microglia, astrocytes, and neurons can generate AP-1-42 peptides in response to mitochondrial dysfunction and the final common pathway of cell death is mitochondrial damage. Mitochondrial damage has been identified in the pathogenesis of sporadic AD. APP or stimulus may be one mechanism by which Chlamydia pneumoniae may trigger a proinflammatory response in the Alzheimer’s brain.

Materials and Methods

Tissue Culture

The SK-N-MC (HTB-10 ATCC) human neuroblastoma cell line was cultured in Eagle’s Minimal Essential Medium (MEM) containing non-essential amino acids and 10% heat-inactivated FBS. The CD-AHA (ATCC CRL-3541) murine astrocyte cell line was cultured in Dulbecco’s Modification of Eagle’s Medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS). All cell lines were maintained at 37°C in 5% CO2.

Infection of Cells

Chamber slides (4 well/slide CultureSlides (BD Falcon) were seeded with approximately 1 x 10⁵ cells. Within 24 hours of seeding the chamber slides, cells were infected with Chlamydia pneumoniae AR-39 (ATCC) at an MOI of 1. Cells were then then incubated in 50% of growth medium for 72 hours.

Induction of Apoptosis

Cells were incubated in chamber slides in 1µl staurosporine solution (1µM stock staurosporine solution from Sigma Aldrich, St. Louis, MO) for 8 minutes. Cells were washed with PBS and placed in culture media for 15 minutes at room temperature (RT). The slides were then incubated in 1 µg/ml Annexin V-Alexa Fluor 594 conjugate (BD Bioscience) for 15 minutes at room temperature (RT). The slides were then rinsed with PBS and counterstained with 1 µg/ml 4,6-diamidino-2-phenylindole (DAPI) (BD Bioscience) for 15 minutes at room temperature (RT). The slides were then rinsed with PBS and mounted with ProLong Antifade Mounting medium (Invitrogen). Images were captured with a Zeiss Axioskop microscope equipped with a 38x oil objective and phase contrast 10x objective. Apoptotic induction with staurosporine which may be independent of the cytochrome c pathway.

Results

Figure 1: Neuronal cells (panel A) and astrocytes (panel B) infected with Chlamydia pneumoniae (panel B). NFTs (in red) can be identified within the cells cytoplasm (red).

Figure 2: Apoptotic induction with staurosporine which may be independent of the cytochrome c pathway.

Conclusions

Inhibition of apoptosis with staurosporine did not appear to induce the apoptotic event in both infected neurones and astrocytes.

Chlamydia pneumoniae infected neuronal cells differentially activate cytochrome c as compared to infected astrocytes.

Infection of both neurons and astrocytes appear to block apoptotic induction with staurosporine which may be independent of the cytochrome c pathway.

References


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