Effect of Antibody to ß-amyloid on the Microglial Mediated Aß-induced Neurotoxicity

Introduction

Microglia are the phagocytes of the brain (McGeer and McGeer 2003), engulfing and ingesting cell wastes and any other foreign particles in an attempt to defend the brain against possible harm. Despite the beneficial capability of these phagocytic cells, there is much ongoing debate over whether "activated" microglia are beneficial or harmful in certain disease states.

Alzheimer's disease (AD), the most common form of dementia, is characterized by pathology including neuronal loss, β-amyloid (Aβ) plaques and neurofibrillary tangles (McGeer and McGeer, 2003). Clusters of activated microglia appear on the senile plaques of AD brain, while few, if any, are seen in similar regions of control brains (McGeer and McGeer, 2003), indicating a potential role for microglia in the disease, although it is unclear whether this role would be detrimental or protective or both under different conditions. There is considerable evidence that fibrillar Aβ-activated microglia produce neurotoxic material including proinflammatory cytokines, nitric oxide (NO), and reactive oxygen species (ROS), all inflammatory agents that consequently lead to neuronal damage. In addition to activated microglia, complement proteins are upregulated in AD. C1q, the recognition component of the classical complement pathway, has been found colocalized with the fibrillar plaques and C1q synthesis has been demonstrated to be induced in rat hippocampal slice cultures upon treatment with Aβ (Fan and Tenner, 2003).

The goal of this project is to determine if there is a difference in microglia-mediated neurotoxcity induced in the presence of MCSF and Aβ immune complex verses that induced in the presence of MCSF and Aβ alone. Although members of the Tenner Lab have shown convincing evidence of the neurotoxicity induced in the presence of Aβ and MCSF (M. Li, submitted for publishing), less is known about the interaction between Aβ-anti-Aβ immune complexes and MCSF. In addition, previous work done in the Tenner lab by Webster et al. involving anti-Aβ immune complexes demonstrated that the presence of C1q enhances microglial scavenger receptor-independent uptake of immune complexes composed of fAβ and suboptimal amounts of anti-Aβ Ab (2001). However, these analyses were performed after 30 minutes and no long-term effects on ingestion were studied. C1q's effect on neurotoxicty between MCSF and Aβ-anti-Aβ immune complexes at varying amounts of anti-Aβ has not been studied. Therefore, I plan to assess the levels of neurotoxicity induced by Aβ-anti-Aβ immune complexes in the presence and absence of MCSF, and compare that to the neurotoxicity induced by Aβ alone in the presence and absence of MCSF, and then finally assess the effect of C1q on the toxicity or modulation of toxicity by the presence of anti-Aβ resulting in the formation of immune complexes.

In general, antibodies are prevented from entering the brain by the blood-brain barrier (a tight cell layer that protects the brain from harmful substances in the bloodstream); however, others have shown that some antibody entered the brain of an APP transgenic mouse AD model immunized with Aβ and may have caused clearance of Aβ (Schenk et al., 1999). In the absence of antibody, Aβ uptake proceeds via the scavenger receptor (SR)-mediated pathway, while in the presence of anti-Aβ immune complexes, Aβ uptake proceeds via the Fc receptor (FcR)-mediated pathway (Paresce et al., 1996). Since the presence of antibody affects the method of uptake (Webster et al., 2001), I would like to know if this difference in uptake would also affect neurotoxicity.

In order to investigate these questions, I will first prepare Aβ-anti-Aβ immune complexes (IgG-fAβ) and verify that microglial ingestion is the same as demonstrated by Webster et al. (2001). Following this, we will verify the levels of neurotoxicity induced in the presence of both Aβ and MCSF and in the presence of Aβ itself. Next, we will determine if IgG-fAβ (with either high or low levels of antibody forming the complexes) affects neurotoxicity with and without the presence of MCSF. Finally, we will assess the effect of C1q bound complexes on neurotoxicity.

At the end of this research project, I hope we will have a better understanding of the effects of C1q and Aβ antibody on Aβ/MCSF microglial induced neurotoxicity. Since there are current studies involving the inoculation of anti-Aβ antibody as a possible treatment for Alzheimer's disease in this laboratory and others, this study will provide important information on the neurotoxicity involved in such a treatment and enable future researchers to be aware of the possible risks/advantages involved.

Materials and Methods

Formation of fAβ and Aβ-anti-Aβ Immune Complexes:
fAβ and Aβ-anti-Aβ immune complexes are formed as described by M. Li (submitted for publication).

Microglial Cell Culture:
Cortical tissue is obtained from 2-4-day-old Sprague Dawley rat pups and exposed to 0.25% trypsin/calcium-magnesium-free buffer (CMF) solution at 1 ml per brain for 10 min at 37 °C. Following this incubation, the trypsin is replaced with DMEM supplemented with 10% fetal calf serum (FBS) and the cells are centrifuged at 1200 rpm for 7 min. The tissue is then dissociated by titration using flame constricted Pasteur pipettes. After addition of DMEM-10%FBS and a second centrifugation (at same settings as above), the supernatant is removed and the pellet is resuspended to a final volume of 1ml DMEM-10%FBS per brain. Finally, 1ml of this tissue/DMEM solution is added to each of the poly-L-lysine coated flasks previously filled and incubated with 10ml DMEM-10%FBS. After 24 hours, 9ml of the supernatant is replaced with fresh media. The microglia cells are collected after 7-10 days by shaking flasks using a rotary shaker at 140rpm and 37°C for 90 minutes, centrifugation at 1200rpm for 7 min, and washing in neurobasal media to remove serum, and resuspending in NB/N2 media.

Flow Cytometric Assessment of Phagocytosis:
Microglia are obtained from flasks as described above and resuspended in NB/N2 media (this media differs from that used by Webster, et al, but is critical to support neurons in the subsequent neurotoxicity assay). SR ligands are added to cultures 25 minutes before addition of peptide to saturate microglial SR. Cells untreated are controls assessed in parallel to the SR-ligand treated cells. Following exposure to Aβ or Aβ-immune complexes for 30 min at 37 °C, microglia are washed twice with HBSS to remove unassociated fAβ and treated with 250μg/ml trypsin/EDTA for 10 min at 37 °C to eliminate surface-bound fAβ and to detach the cells from the culture surface. The trypsin reaction is stopped with 1 ml DMEM supplemented with 10% FBS and the wells are washed 2 times with 1ml DMEM-10%FBS. Microglia are then fixed in solution by exposure to 4% paraformaldehyde for 10 min, washed twice, and permeabilized by addition of 0.1% Triton X-100 (in PBS) for 5 min at room temperature. Cells are washed and resuspended in 250μl residual FACS buffer (HBSS + 0.1% BSA + 0.01% NaN3), followed by addition of 3 μg mouse monoclonal anti-Aβ antibody 4G8 and incubation at 4°C for 1 hour. Cells are then washed twice at 4 °C and FITC-goat anti-mouse IgG F (ab') was added (3μg/250μl). After 30 min incubation at 4 °C, the cells are washed twice, resuspended in FACS buffer and cell-associated fluorescence is determined using FACSCalibur.

Neuronal Cell Culture:
Neuron cells are obtained from E18 Sprague-Dawley rat embroyos as described by M. Li (submitted for publication).

Co-Cultures of Microglia and Cortical Neurons:
Co-cultures are prepared by plating 1 x 10^5 microglial cells into each well of a 24-well plate previously plated with 50,000 neurons (used at 3 days in culture) in NB/N2. One hour later, co-cultures are treated with or without MCSF in the presence of fAβ and IgG, and in the presence or absence of C1q. After 72 hours, the media is collected for the nitrate assay and the cultures are fixed for immunocytochemistry.

Immunocytochemical Assay for an Analysis of Neurotoxicity:
Neurotoxicity is assessed as described by M. Li (submitted for publication).

Level of Preparation

While working in the Tenner lab for the last two quarters, I have been continuously performing microglial preps on rat pups. The primary microglia cells obtained through these procedures have been used successfully in cell culture models including various neurotoxicity and RPA experiments. I have observed and am able to perform the neuron cell prep, in addition to being trained to perform immunostaining of these neuron cells. I am currently in the process of learning Fluorescence Activated Cell Sorter (FACS) analysis and thus will be proficient at most, if not all, necessary techniques for this project by the start date.

Timeline

Experiment #1 (Week 1):
There will be six conditions involved here: (1) fAβ, (2) fAβ + IgG10 (prepared using 10μg/ml anti-Aβ Ab), (3) fAβ +IgG100 (prepared using 100μg/ml anti-Aβ Ab), (4) fAβ + Clq, (5) fAβ/Clq + IgG10, and (6) fAβ/Clq + IgG100. (Plus untreated control for background fluorescence.) Prepare Aβ-anti-Aβ immune complexes. Verify C1q's enhancement of IgG10-fAβ uptake by microglia at 2 μM fAβ at 30 min of exposure through FACS analysis as demonstrated by Webster et al. (2001).

Experiment #2 (Weeks 2&3):
Repeat experiment #1 at concentration of 10 μM fAβ (because neurotoxicity experiments are done at this concentration) and see if the results are the same as those of the lower concentration of amyloid.

Experiment #3 (Weeks 4&5):
Assess ingestion at times of 30 min, 2 hours, and 18 hours using the same conditions as experiment #2, while Karntipa Pisalyaput performs parallel co-cultures of microglia and cortical neurons to assess these treatments on neurotoxicity (at 48 and 72 hours). Neuron only cultures will be assayed in parallel to determine that any toxicity seen is microglia-mediated. I will also participate in the staining and be a second independent scorer of the neurotoxicity experiments.

Experiment #4 (Weeks 6&7) :
Repeat experiment #3 with the addition of MCSF.

Week 8:
Repeat any necessary experiments (using different concentrations of MCSF).

Weeks 9&10:
Complete data analysis and prepare my write-up and talk.

References

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