Alexis Krayevsky

Research Plan

Title: Stimulating Innate Immunity via TLR9 agonist CpG ODN in a Non-Human Primate Model

Category: Cellular and Molecular biology

Rationale:

Alzheimer's disease (AD), the most common cause of dementia, is a neurodegenerative disorder that can be characterized by, neurofibrillary tangles, and deposition of amyloid-beta (Aβ) in the brain causing massive neuronal cell death leading to cognitive decline (Serrano-Pozo, 2011; Bennett, 2017). While previous research has determined that inflammation in the brain is part of the normal aging process, extensive inflammation can promote a neurodegenerative cascade (Cribbs, 2012). Alzheimer's disease deals directly with the immune system attacking the nervous system and causing dysfunction in many proteins and signaling cells (Cao, 2018). Immunotherapy has become the favored treatment for AD (Bittar, 2018). Previous research has demonstrated that innate immunity stimulation helps to reduce AD pathology (Wang, 2018). CpG ODN is a synthetic oligodeoxynucleotide which has been used to stimulate innate immunity via a toll-like receptor. CpG ODNs have been used in clinical trials for various diseases such as cancer and HIV to test for their immune-stimulating response (Scheiermann, 2014; Jahrsdörfer, 2008). Class B CpG ODN has been used to stimulate innate immunity in transgenic mice models and has been shown to ameliorate AD pathology (Scholtzova, 2017; Scholtzova, 2014; Scholtzova, 2009). Squirrel monkeys are very similar to humans and allow for a more accurate comparison between pathology in humans and pathology in squirrel monkeys making squirrel monkeys very useful for Alzheimer's research (Toledano, 2014; Walker, 1990) (Heuer, 2017). In the current study, squirrel monkeys will be treated with CpG ODN to determine its safety and efficiency. If CpG ODN is successful and is able to reduce pathology without worsening monkeys health, this treatment can be translated to humans to treat Alzheimer's disease in the future. If properly executed, this treatment can lead to a significant decrease in Alzheimer's symptoms and possibly halt the progression of Alzheimer’s in a patient. This can create a better quality of life for patients and their families, giving patients greater functionality and reducing financial burdens.

Research Question:

Aim:

- To complete a neuropathological evaluation in young and old squirrel monkeys.
- To determine the safety of class C CpG ODN in squirrel monkeys
- To determine the efficiency of class C CpG ODN in stimulating immune response

Procedure:

Histological Studies

PERL Prussian Blue Staining: A PERL staining will be performed to stain for microhemorrhages. 5% Potassium ferrocyanide with 5% HCL will be used as the reactant. Slides will be heated in the microwave submerged with. Once heated, potassium ferrocyanide is toxic; therefore, for safety, a mask, gloves, and a lab coat will be worn. The slides will then be left to sit in the solution under the fume hood for ten minutes and nuclear fast red will then be applied followed by dehydrating, and cover slipping the slides.

T-Cell Staining: A t-cell staining will be performed in order to detect t-cells in the brain tissue. A TRIS-EDTA buffer will be used for antigen retrieval. The slides will then be washed with PBS followed a 30-minute H_2O_2 incubation and a one-hour goat blocking. A primary will then be applied following PBS washes. The next morning, the primary will be taken off, and the slides will be washed with PBS-T. An anti-rabbit secondary will then be applied. Following the secondary and 3 PBS washes, the slides will be incubated in A+B. The DAB reactant will be made using nickel sulfate, and DAB powder. The DAB reaction will then begin starting with a concentration of 100ul of H202 per 10ml of DAB. Sodium acetate will be used to stop the DAB reaction, and the slides will be washed with PBS, dehydrated and cover slipped.

6E10 Staining: A 6E10 staining will be performed in order to detect amyloid burden. Using formic acid, antigen retrieval will be performed under the fume hood for 15 minutes. The slides

will then washed with PBS followed by a one-hour MOM blocking. Following three rounds of five- minute PBS washes, an anti-mouse primary will then be applied with a 1:1000 dilution and left overnight. The next morning, the primary will be taken off, and a n anti-mouse secondary will be applied for one and a half hours. Following the secondary, the slides will be washed in PBS-T and incubated in A+B for one and a half hours. The DAB reactant will be made using nickel sulfate, and DAB powder. The DAB reaction will then begin starting with a concentration of 100ul of H202 per 10ml of DAB. Sodium acetate will be used to stop the DAB reaction, and the slides will be washed with PBS, dehydrated and cover slipped.

GFAP Staining: A GFAP staining will be done to determine the presence of astrocyte pathology. Using a TRIS-EDTA buffer, antigen retrieval will be performed in a water bath for 25 minutes at 95°C. The slides will then be washed with PBS followed by a one-hour goat blocking.An anti-rabbit primary will then be applied with a 1:1000 dilution and left overnight. The next morning, the primary will be taken off, and a anti-rabbit secondary will be applied for 1 hour made in a 1:800 dilution. Following the secondary, there will be three five-minute PBS-T washes followed by an incubation in A+B for 1 hour. The DAB reactant will be made using nickel sulfate, and DAB powder. The reaction will then be done starting with a concentration of 50ul of H202 per 10ml of DAB and sodium acetate will be used to stop the DAB reaction.

Iba-1 Staining: An Iba-1 staining will be performed in order to determine microglial pathology. . Using a TRIS-EDTA buffer, antigen retrieval will be performed in a water bath for 25 minutes at 95°C. A goat blocking will then be applied, and the slides will be washed with PBS. An anti-rabbit primary will then be applied with a 1:1000 dilution and left overnight. The next morning, the primary will be taken off, and the slides will be washed with PBS-T to ensure for less precipitate on the slide. An anti-rabbit secondary will then be applied for 1 hour. Following the secondary, there will be a three five-minute PBS-T washes followed by an incubation in A+B for 1 hour. The DAB reactant will be made using nickel sulfate, and DAB powder. The reaction will then be done starting with a concentration of 50ul of H202 per 10ml of DAB and sodium

acetate will be used to stop the DAB reaction. In order to stop the DAB reaction, the slides will be rinsed in sodium acetate followed by PBS and then dehydrated, and cover slipped.

Cytokine Assays

Cytokines IL4, IL6, IL12p70, MCP1, IL1β, TNFα, IP10, IL10, and IFNγ will be quantified using a custom nine-plex detection kit. The assays will be performed as per the manufacturer's protocol. The Luminex 200 analyzer (New York University Langone Medical Center Immune Monitoring Core) will be used to measure the levels of each cytokine.

Risk and Safety :

- Use of gloves when working with chemicals, or tissue sample
- Use of lab coat, gloves, and mask when working with DAB, a carcinogen, and potassium ferrocyanide

Data Analysis:

Semi-Quantitative Analysis

Analysis of GFAP, Iba1, and T-cell immuno-stained sections was based on a semiquantitative analysis. Immuno-stained sections were evaluated on scales ranging from 0 to 5 based on pathological severity. Approximately nine cortical sections were analyzed per animal.

Potentially hazardous biological agents research:

- Squirrel monkey brain tissue was obtained from obtained from the Squirrel monkey brain tissue was obtained from the Squirrel monkey Breeding Research Resources (SMBRR) located at the University of Texas MD Anderson Cancer Center Michale E. Keeling Center for Comparative Medicine and Research. The BSL is 2 based on assessment. The lab uses agents associated with Alzheimers which is a human disease. Additionally it is required to wear protective equipment when in the lab setting.

- In order to stay safe in the lab, a lab coat, gloves, and a mask must be worn when working with any biological agents. Slides containing biological agents are not disposed unless the glass has been broken beyond repair, in which case they are disposed of in a broken glassware box in the lab and then disposed of by NYU's sanitation team.

Hazardous chemicals, activities & devices:

- Hazardous chemicals will be disposed of into designated disposal bottles in the lab under the fume hood and taken by NYU for final disposal.

Bibliography

Bennett, Rachel E et al. "Enhanced Tau Aggregation in the Presence of Amyloid β." The American journal of pathology vol. 187,7 (2017): 1601-1612. doi:10.1016/j.ajpath.2017.03.011

Bittar, Alice, et al. "Prospects for Strain-Specific Immunotherapy in Alzheimer's Disease and Tauopathies." Npj Vaccines, vol. 3, no. 1, 2018, doi:10.1038/s41541-018-0046-8.

Cao, Wei, and Hui Zheng. "Peripheral immune system in aging and Alzheimer's disease." Molecular neurodegeneration vol. 13,1 51. 3 Oct. 2018, doi:10.1186/s13024-018-0284-2

Cribbs, David H et al. "Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: a microarray study." Journal of neuroinflammation vol. 9 179. 23 Jul. 2012, doi:10.1186/1742-2094-9-179

Heuer, Eric et al. "Amyloid-Related Imaging Abnormalities in an Aged Squirrel Monkey with Cerebral Amyloid Angiopathy." Journal of Alzheimer's disease : JAD vol. 57,2 (2017): 519-530. doi:10.3233/JAD-160981

Jahrsdörfer, Bernd, and George J Weiner. "CpG oligodeoxynucleotides as immunotherapy in cancer." Update on cancer therapeutics vol. 3,1 (2008): 27-32. doi:10.1016/j.uct.2007.11.003

Scheiermann, Julia, and Dennis M Klinman. "Clinical evaluation of CpG oligonucleotides as adjuvants for vaccines targeting infectious diseases and cancer." Vaccine vol. 32,48 (2014): 6377-89. doi:10.1016/j.vaccine.2014.06.065

Scholtzova, Henrieta et al. "Amyloid β and Tau Alzheimer's disease related pathology is reduced by Toll-like receptor 9 stimulation." Acta neuropathologica communications vol. 2 101. 2 Sep. 2014, doi:10.1186/s40478-014-0101-2

Scholtzova, Henrieta et al. "Induction of toll-like receptor 9 signaling as a method for ameliorating Alzheimer's disease-related pathology." The Journal of neuroscience : the official journal of the Society for Neuroscience vol. 29,6 (2009): 1846-54. doi:10.1523/JNEUROSCI.5715-08.2009

Scholtzova, Henrieta et al. "Innate Immunity Stimulation via Toll-Like Receptor 9 Ameliorates Vascular

Amyloid Pathology in Tg-SwDI Mice with Associated Cognitive Benefits." The Journal of neuroscience : the official journal of the Society for Neuroscience vol. 37,4 (2017): 936-959. doi:10.1523/JNEUROSCI.1967-16.2016

Serrano-Pozo, Alberto et al. "Neuropathological alterations in Alzheimer disease." Cold Spring Harbor perspectives in medicine vol. 1,1 (2011): a006189. doi:10.1101/cshperspect.a006189

Toledano, A., et al. "Does Alzheimer Disease Exist in All Primates? Alzheimer Pathology in Non-Human Primates and Its Pathophysiological Implications (II)." Neurología (English Edition), vol. 29, no. 1, 2014, pp. 42–55., doi:10.1016/j.nrleng.2011.05.006.

Walker, L. C., et al. "Cerebral Amyloid Angiopathy In Aged Squirrel Monkeys." Journal of Neuropathology and Experimental Neurology, vol. 49, no. 3, 1990, p. 299., doi:10.1097/00005072-199005000-00122.

Wang, Ming-Ming et al. "Innate immune activation in Alzheimer's disease." Annals of translational medicine vol. 6,10 (2018): 177. doi:10.21037/atm.2018.04.20

NO ADDENDUMS EXIST