PI: Anumantha Kanthasamy

Grant Number: R01ES026892

Grant Title: Protein Aggregation and Inflammasome Signaling in Manganese Neurotoxicity

Background/Context:
This is a new R01–aimed at neuroinflammatory pathology underlying neurodegenerative disease, but specifically will examine how Mn exposure triggers alpha-synuclein (αSyn) aggregation (a hallmark of PD) and activates glial mediated neuroinflammatory signaling. Importantly, Anumantha Kanthasamy is collaborating with Brad Racette who is studying neuropathological changes in postmortem brain tissue from Mn-exposed miners.

Key Translational Milestones

- Investigation of the neuroinflammatory pathology underlying neurodegenerative disease, specifically examining how Mn exposure triggers αSyn aggregation (a hallmark of PD) in brain cells.
- Computer modeling showing that the PKCδ isoform has the highest probability of phosphorylating Ser 595 on NLRP3 proinflammatory signaling.
- Characterization of the role of PKCδ in Mn-exosomal alpha-synuclein-induced inflammasome signaling bytesting whether Mn-exosomal αSyn aggregates activate inflammasome signaling in primary microglia and astrocytes.
- Investigation of this pathway in postmortem brain tissue from Mn-exposed miners.

Or more detailed:

- Characterize the cellular mechanism of Mn-induced impairment of endosomal trafficking, retromer dysfunction and exosome release in cell culture and animal models of Mn neurotoxicity. Expected finding is that *Manganese-induced exosomes containing αSyn aggregates (Mn-exosomal αSyn)* activate the NLRP proinflammatory *inflammasome pathways through* PKCδ*-dependent signaling.*
  Models *– Human mesencephalic dopaminergic neuronal cells (LUHMES) as well as wild-type and αSyn Tg mice*

- Determine the NLRP2/3 inflammasome neuroinflammatory signaling in microglia and astrocytes triggered by Mn-induced exosomal αSyn aggregates, and characterize the proinflammatory regulatory function of PKCδ in NLRP2/3 inflammasome activation in Mn neurotoxicity. i) Characterize the activation profile of NLRP2/3 inflammasome induction and PKCδ activation by Mn-exosomal αSyn in mouse primary microglia and astrocytes, ii) determine whether Mn-exosomal αSyn-induced PKCδ activation regulates NLRP2/3 inflammasomes and IL-1β proinflammatory signaling in microglial and astroglial cells, and iii) evaluate the role of PKCδ-dependent inflammatory responses in promoting nigrostriatal neuronal cell death using genetic approaches including siRNAs and PKCδ KO microglia. Expected finding is that *PKCδ regulates proinflammatory signaling cascade in Mn induced αSyn aggregation.*
  Models: *Primary microglia and astrocytes from wild type and* PKCδ *KO mice as well as primary striatal and nigral neurons.*

- Examine the role of PKCδ in mediating the exosomal αSyn aggregate-induced proinflammatory response in nigrostriatal neurons in animal models of Mn neurotoxicity, and confirm the presence of αSyn protein aggregation in Mn-exposed human brain tissues. i) Determine the role of PKCδ in Mn-exosomal αSyn-induced microglial activation and inflammasome signaling *in vivo* using PKCδ wild-type and PKCδ KO mice, ii) establish the pathological role of αSyn aggregation and PKCδ-mediated inflammasome signaling in Mn-induced progressive neuronal degeneration using αSyn A53T and PKCδ KO double-transgenic mice, and iii) characterize αSyn protein aggregation, microglial inflammasome activation, PKCδ upregulation and neuronal degeneration in Mn- exposed human brain tissues. Expected finding is that *exosomal αSyn and PKCδ proinflammatory signaling contributes to neurodegenerative processes in Mn neurotoxicity.*
  Models: Wild-type and PKCδ KO mice, human A53T αSyn mice, A53T αSyn/ PKCδ +/- double Tg mice, control human brain samples and Mn exposed human brain samples.

  Given the predominant roles of neuroinflammation and protein aggregation in neurodegenerative disorders, our proposed study will offer novel insights into the neuron-glia interactions relevant to neuroinflammatory mechanisms in Mn neurotoxicity and PD. This study will greatly advance our current understanding of how misfolded proteins released from neurons trigger proinflammatory responses in glial cells, promoting the spread of protein aggregates and neurodegeneration among neurons. Furthermore, this work will provide comprehensive information about how environmental exposure to Mn alters cellular mechanisms of endosomal trafficking, leading to cell-to-cell transmission of misfolded protein aggregates. Most importantly, the outcome of this study will offer therapeutic targets to modulate the propagation of misfolded αSyn and inflammatory responses for the treatment of Mn neurotoxicity, PD and other related disorders.

- **Compare the extent of αSyn protein aggregation, inflammasome and PKCδ activation between Mn miners and non-Mn miners.** Dr. Brad Racette has established a unique international collaboration among Washington University in St. Louis, MO, the University of Washington in Seattle, WA, and the University of Witwatersrand in Johannesburg, to characterize neuropathological changes in Mn-exposed brain tissues using a well-established mining occupational health management program in South Africa. Dr. Racette will provide brain tissues for our studies and will serve as a consultant for the project.Mn exposures in these mines are similar to those found in developed countries (274). Brains from non-Mn miners were collected in the same mining region within the Northern Cape from miners with a history of mining other commodities other than Mn, such as asbestos or diamonds. Mn miner and non-miner brains to be used in this study, as described previously (275, 276), come from age-matched black male subjects totaling eight Mn miners and eight non-Mn miners. These Mn miners have a proinflammatory imbalance with a higher microglia:astrocyte ratio compared to non-Mn miners (276).

Translational Research Milestone 1:

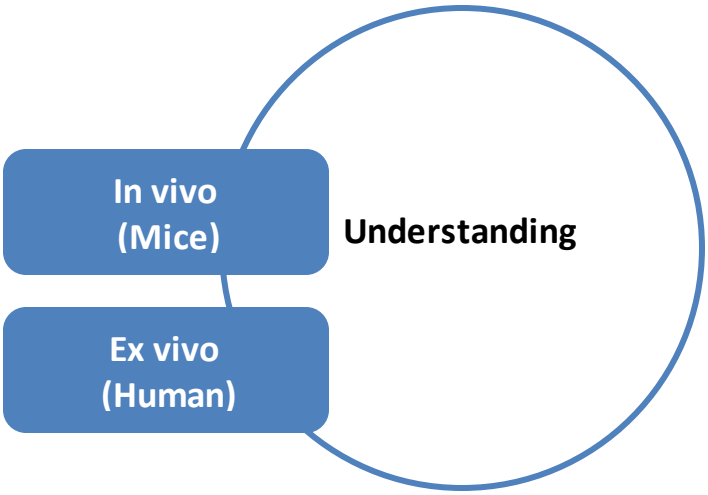
- Investigate the neuroinflammatory pathology underlying neurodegenerative disease, specifically examining how Mn exposure triggers alpha-synuclein aggregation (a hallmark of PD) and neuroinflammatory processes in neuronal and glial cells.

Fundamental Science Interactions Ring:

Driver: Mechanistic understanding

Experimental Setting: Ex vivo, In vivo

Organism: Human, Mice



Timeframe: Work is just starting in 2016

Collaborators:

- Anumantha Kanthasamy

Source:

Grant Proposal

Translational Narrative:

What led to the next step?

How did the idea evolve?

Who was involved?

What needed to happen (collaborations, tools, technologies, serendipity) to cross the translational bridge?

How did you know what to do next?

Translational Research Milestone 2:

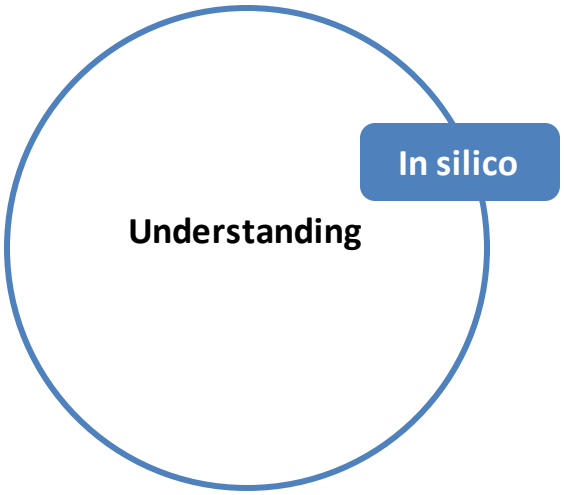
- Computer modeling showing that the PKCδ isoform has the highest probability of phosphorylating Ser 595 on NLRP3 proinflammatory signaling.

Fundamental Science Interactions Ring:

Driver: Mechanistic understanding

Experimental Setting: In silico

Organism: NA



Timeframe: prior to 2016

Collaborators:

- Anumantha Kanthasamy

Source:

Grant Proposal

Translational Narrative:

What led to the next step?

How did the idea evolve?

Who was involved?

What needed to happen (collaborations, tools, technologies, serendipity) to cross the translational bridge?

How did you know what to do next?

Translational Research Milestone 3:

- Investigate the role of PKCδ in Mn-exosomal alpha-synuclein-induced inflammasome signaling by testing whether Mn-exosomal αSyn aggregates activate inflammasome signaling in primary microglia and astrocytes.

Fundamental Science Interactions Ring:

Driver: Mechanistic understanding

Experimental Setting: In vivo

Organism: Mice

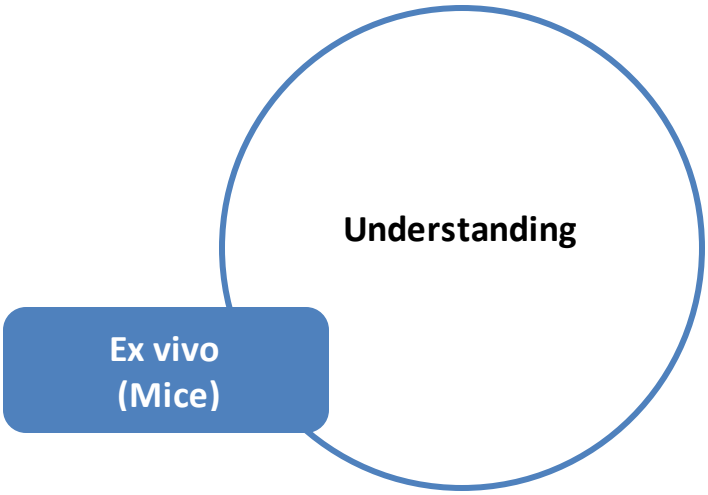
Timeframe: Work is just starting in 2016

Collaborators:

- Anumantha Kanthasamy

Source:

Grant Proposal



Translational Narrative:

What led to the next step?

How did the idea evolve?

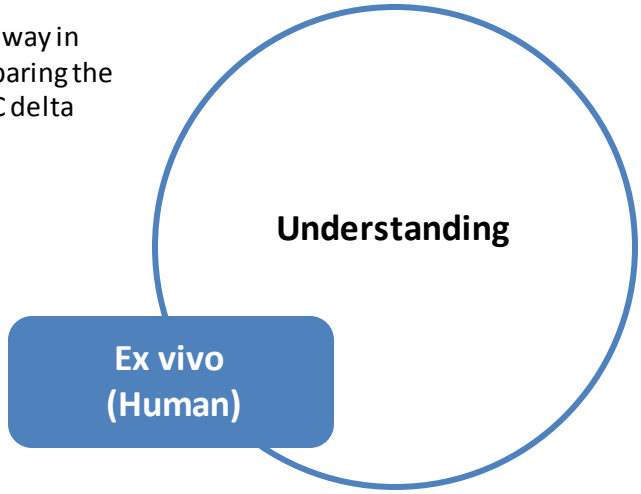
Who was involved?

What needed to happen (collaborations, tools, technologies, serendipity) to cross the translational bridge?

How did you know what to do next?

Translational Research Milestone 4:

- Investigation of this exosomal αSyn protein misfolding pathway in postmortem brain tissue from Mn-exposed miners by comparing the extent of αSyn protein aggregation, inflammasome and PKC delta activation between Mn miners and non-Mn miners.



Fundamental Science Interactions Ring:

Driver: Mechanistic understanding
Experimental Setting: Ex vivo
Organism: Human

Timeframe: Work is just starting in 2016

Collaborators:

- Anumantha Kanthasamy
- Dr. Brad Racette has established a unique international collaboration among Washington University in St. Louis, MO, the University of Washington in Seattle, WA, and the University of Witwatersrand in Johannesburg, to characterize neuropathological changes in Mn-exposed brain tissues using a well-established mining occupational health management program in South Africa.

Source:
Grant Proposal

Translational Narrative:
What led to the next step?
How did the idea evolve?
Who was involved?
What needed to happen (collaborations, tools, technologies, serendipity) to cross the translational bridge?
How did you know what to do next?