The microbiota in Parkinson’s disease and dementia: updates on the gut brain connection

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The story

• Why do people get sporadic Parkinson’s, Alzheimer’s or other related neurodegenerations?

• These disease all involve prion-like transmission of protein misfolding and neuroinflammation and new evidence suggests a primary role for inflammation in AD

• The microbiota (i.e., gut bacteria) have been shown to have important roles in human metabolism and immunity. There are several reasons to consider a gut origin of pathogenesis in neurodegeneration (anosmia, constipation, gut pathology)
Chief question: WHY?

1. Why is there protein misfolding – what initiates misfolding in the protein folding disorders (Alzheimer, Parkinson’s and others)?
   • Is it random?
   • Or induced by environmental factors?  

Prusiner, 2013

2. Why is there neuroinflammation - what initiates and sustains inflammation in the brain in these disorders?

Both protein misfolding as amyloid and inflammation may be needed for disease
What might be the trigger(s) of misfolding?

Gut exposure to amyloid proteins can cause noncatalytic seeding of endogenous protein misfolding resulting in neurodegeneration (bovine spongiform encephalopathy, BSE, and kuru)
What might be the trigger(s) of inflammation?

The microbiota are critical for the regulation of the systemic immune system, including the brain.
Bacteria make extracellular amyloid proteins

- *Bacillus subtilis*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Mycobacterium tuberculosis*
- *Salmonella enterica, typhimurium*
- *Staphlococcus aureus*
- *Streptococcus mutans, coelicolor*
- *Xanthomonas axonopodis*

[The list is incomplete]

- Genes for producing amyloids are also found in other phylum of bacteria: *Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes, Proteobacter, Thermodesulfobacteria*

Hufnagel et al, 2013; Schwartz et al, 2013; Bieler et al, 2005
Photo courtesy of Chapman lab
Bacterial amyloid and inflammation

- Toll Like Receptors (TLRs) are the principal sensors of the innate immune system.

- Bacterial amyloid is recognized by the innate immune system and activates TLR2/1, CD14 and other immune modulators.

- TLR2/1 and CD 14 is an important “trimolecular complex” and activates microglia, produces NFkB and iNOS, thus oxidative toxicity.

- TLR2 is a primary receptor for AD Aβ peptide and PD alpha synuclein to trigger neuroinflammatory activation.

Two hypotheses:

1. Bacterial amyloid proteins cause cross-seeding of amyloid misfolding of neuronal proteins in the gut (including the intestines, mouth and nose), which are subsequently transmitted via the autonomic nervous system to the brain in a prion-like manner.

Two hypotheses:

2. The innate immune system recognizes bacterial amyloids utilizing TLR2 and related pathways causing priming of immune cells. This enhances the response to the amyloid proteins deposited in the brain with aging, promoting neuroinflammation.

Friedland, Journal of Alzheimer's Disease, 2015
Exposure to the Functional Bacterial Amyloid Protein Curli Enhances Alpha-Synuclein Aggregation in Aged Fischer 344 Rats and Caenorhabditis elegans

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Curli exposure enhanced Toll like receptor 2 (TLR2) expression in hippocampus and striatum

Chen et al., Scientific Reports, 2016
Curli exposure enhanced AS aggregation

PK = Proteinase K

Chen et al., Scientific Reports, 2016
Interim summary 1

Exposure to the bacterial amyloid protein curli:
• enhanced Alpha synuclein (AS) expression in the gut and brain and AS aggregation in the brain (aged rats)
• enhanced neuroinflammation as assessed by microgliosis, astrogliosis and TLR2, IL6, TNF-α expression (aged rats)
• enhanced AS aggregation in muscle (transgenic C. elegans)

Chen et al., Scientific Reports, 2016
Interim summary 2

• Microbial amyloid enhances:
  
  • protein misfolding, though overexpression and perhaps cross-seeding
  
  • Neuroinflammation, through the innate immune system
The microbiota are our largest environmental exposure

Sneeze (up to 10 minutes)  Microbiota (lifelong)

Lok, 2016; Lifescience Global
Friedland, Chapman, the role of bacterial amyloid in neurodegeneration

*PLOS Pathogens* (December, 2017)
Routes of Exposure

Friedland, Chapman, PLOS Pathogens, 2017
Where is the mouth, nose, larynx and pharynx?
Olfactory receptor neurons are in direct contact with the environment

Urban and Tripathy, 2012; Hommet et al, 2012
What are the inflammatory mediators?

• Toll Like Receptors (TLRs), Inflammasomes
  • recognize pathogen and danger associated molecular patterns (PAMPs and DAMPs)

• ASC = Apoptosis speck like adaptor protein- containing a CARD (caspase 1)

• ASC assembles into large helical fibrils
  • ASC speck, in response to inflammasomes, such as NLRP3 [nodlike receptor pyrin containing 3] and AIM2 (absent in melanoma)

• TLR2 and NLRP3 recognize functional bacterial amyloid

• Diet influences these molecules

Rapinsky et al 2015; Hoss et al 2017; McManus, Heneka 2017; Christ et al 2018
ASC specks enhance Aβ aggregation

ASC specks can be visualized in brain sections of patients with AD and APP/PS1 transgenic mice, within microglia and extracellular space and bound to Aβ.

ASC specks in human hippocampal microglia (10uM) 
Venegas et al, Nature 2017

M. Heneka, Bonn
Neuroinflammation and Microglia in Alzheimer’s disease

“These findings raise the possibility that innate immune activation may actively contribute to AD pathogenesis, rather than arising as a pure bystander reaction to amyloid deposition, as previously assumed”

Heneka et al 2015, Salrlus, Heneka 2017

The microbiota influence microglial morphology and function and the microglia are involved in development and learning, as well as protection form pathogens

Mazmanian and colleagues, J Exp Med, 2019
Challenges

- How to explain the diversity of the diseases?
  (early or late onset, asymmetry of onset, severity of progression)

**Strains !**

[as well known for prions]
Further implications - Strains

- Strains
  - Diversity of conformational states
  - Multiple forms of disease
  - Incubation periods
  - Host specificity
  - Deposition patterns in the brain
  - Proteolysis digestion patterns
  - Stability

Best demonstrated for prions, also reported for neurodegenerative disease proteins AS, TDP43

**Hypothesis:** Strains of bacterial amyloids induce cross-seeding in a strain-specific manner, thereby initiating CJD, AD, PD, ALS, PSP and their complex phenotypes.

Amyotrophic lateral sclerosis

- TDP-43 and SOD1 have potential for seeded aggregation in vitro
- In cultured cells TDP-43 fibrils induce aggregation of otherwise soluble protein
- SOD1 misfolding is self propagating
- ALS patients have elevated serum endotoxin levels and altered microbiota
- Tight junctions in the gut are affected by the G93A gene and also by curli
- C9ORF72 and immunity

Oppong et al 2013, 2015; Wu et al 2015; Zhang et al 2017 Thompson, in press
Potential therapies

- Prebiotics, probiotics, synbiotics, postbiotics (Metchnikioff, 1907)
- Curds
- Fecal microbiota transplants (FMT)
- Phages
- Diet
- Dental care
- Antibiotics
- Vaccines
- Medical foods
- Amyloid inhibitors (polyphenols)
- MYD88 inhibitors? (6,378 papers on Medline, 2/9/2018)
Gene therapy and the microbiota

- It is difficult to change the human genome, but the metagenome can be adjusted through diet.
- This can be done in as little as 2 weeks, with consequences for inflammation, oxidative toxicity and cancer in humans.

Greer and O’Keefe, 2011; Ou et al., 2012
"...CsgA fibrils cross-seeded fibrillation of Amyloid-β, providing further support for the proposed structural resemblance and potential for cross-species amyloid interactions”

Perov et al, 2018, http://dx.doi.org/10.1101/493668
CsgA fibrils share structural characteristics with cross-β amyloids and seed the fibrillation of human Aβ.

D-peptides inhibit fibrillation of CsgA but not of PSMα3.

Perov et al, 2018, http://dx.doi.org/10.1101/493668
“Unrelated fibrillation systems may share ... common fibril formation mechanisms, allowing inhibitors of one fibrillating protein to affect a completely different protein”

FapC, an amyloid protein of *Pseudomonas*  

Christensen et al 2019
Association between Apo E genotype and the gut microbiome composition in humans and mice

• The APOE genotype is associated with specific gut microbiome profiles in both humans and APOE model mice.

Tran et al. bioRxiv preprint first posted online Nov. 19, 2018
http://dx.doi.org/10.1101/473694
Conclusions 1

• Exposure to bacterial amyloid may:
  • 1. enhance aggregation of neuronal proteins in the gut, nose and mouth leading to prion-like propagation of misfolded proteins to the brain via the autonomic nervous system.
  • 2. Activate the innate immune system to cause priming of immune cells through TLR2/1, CD14, NFkB and iNOS, leading to increased immune responses to cerebral amyloid deposits

• These mechanisms may be exploited to develop preventive and therapeutic measures as it is relatively easy to alter the content of the human microbiota.
Conclusions 2

• The term mapranosis may assist in recognition of the opportunities for molecular mimicry involving tertiary structures to be involved in disease.

• The microbiota may be involved in neurodegeneration, as well as stroke, via other mechanisms, not involving microbial amyloid.

• Microbiota studies must consider interactions involving oral, nasal, pharyngeal and laryngeal structures, as well as the intestines.
Acknowledgements

- Axial Biotherapeutics
- Michael J. Fox Foundation
- Jewish Heritage Fund for Excellence
- University of Louisville
- Mary and Mason Rudd Family
- M. Chapman (Univ. of Michigan)
- Family of E.A. Ford II and Dr. W. Cowan
Imagination must not be limited by silos.
“Theories are not true or false, they are fertile or sterile”

Claude Bernard (1813-1878)