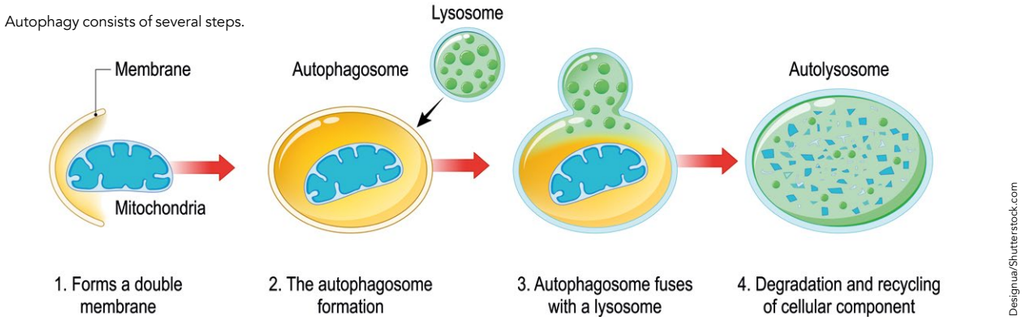
The importance of non-canonical autophagy pathways in Alzheimer's disease pathology and potential therapies

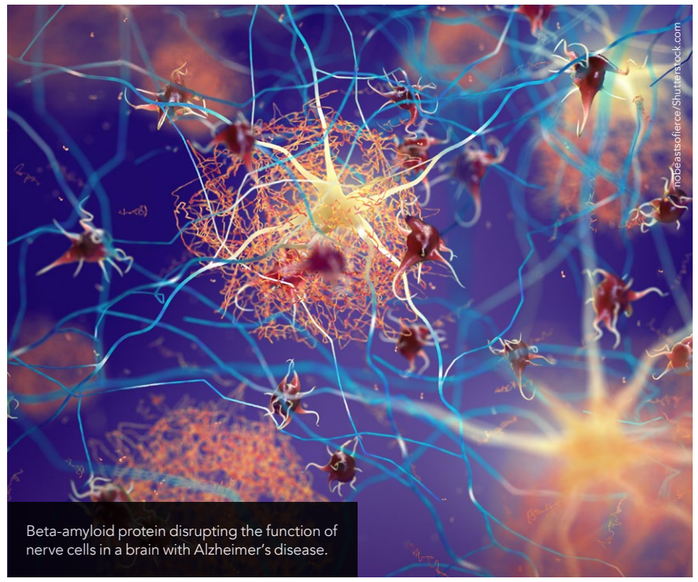
Autophagy is a vital process in the body: cells degrade unwanted or dysfunctional components to recycle nutrients, maintain energetic substrates, and support cellular homeostasis. However, defects in autophagy can lead to neuroinflammation and progressive neurodegenerative diseases such as Alzheimer's disease. Conventional autophagy pathways have been well established by past research. Now, Dr Bradlee L Heckmann, USF Health Neuroscience Institute, along with Dr Douglas R Green, discovered a non-conventional autophagic mechanism: the microtubule-associated protein light-chain 3 (LC3)-associated endocytosis (LANDO). LANDO plays a major role in regulating cellular energetics, cell death, and neuronal function.

The key phenomena involved in the inception and progression of neurodegenerative diseases such as Alzheimer's disease (AD) are neuroinflammation and neurodegeneration. Conventional pathways of macro-autophagy (hereby referred to as autophagy) involve the removal of damaged organelles, protein aggregates, and other cellular components through internalisation and endocytosis (a process by which a cell engulfs a foreign protein and lyses it internally) to maintain cellular and metabolic homeostasis. Proteins like Beclin1, ATG5, and ATG7 are instrumental in regulating such autophagic pathways. They also function in the brain's innate immune microglial cells to induce receptor-mediated endocytosis and prevent excess build-up of ß-amyloid (Aβ)-peptide oligomers, a classic hallmark for AD progression. Additionally, autophagy also regulates various immune pathways: it controls the secretion of pro-inflammatory cytokines such as type-I interferon (INF) and interleukin (IL)-1ß by targeting the IL-1ß precursor and pro-IL-1ß for degradation.

Microglial cells, the flag-bearers of immunity in the central nervous system (CNS), undergo autophagic mechanisms with the help of toll-like receptors (TLRs), Fc receptors, Immunoglobulin (Ig)-superfamily receptors, scavenger receptors (SRs), and complement receptors. The expressions of Beclin1, ATG5, and ATG7 reduce proportionally with age. Unsurprisingly, patients with neurodegenerative disorders like AD have shown drastically reduced levels of these proteins.

Dr Bradlee Heckmann and his collaborators have reviewed as well as conducted multiple studies investigating the role of the conventional autophagic mechanism and the non-canonical uses of the autophagy machinery in distinct pathways. Through these investigations, they identified the LC3-associated endocytosis (LANDO) pathway to be a key player in modulating neuroinflammation. It uses components of the autophagic machinery to target LC3 to endosomes, thereby preventing exacerbated Aβ accumulation and mitigating ß-amyloid





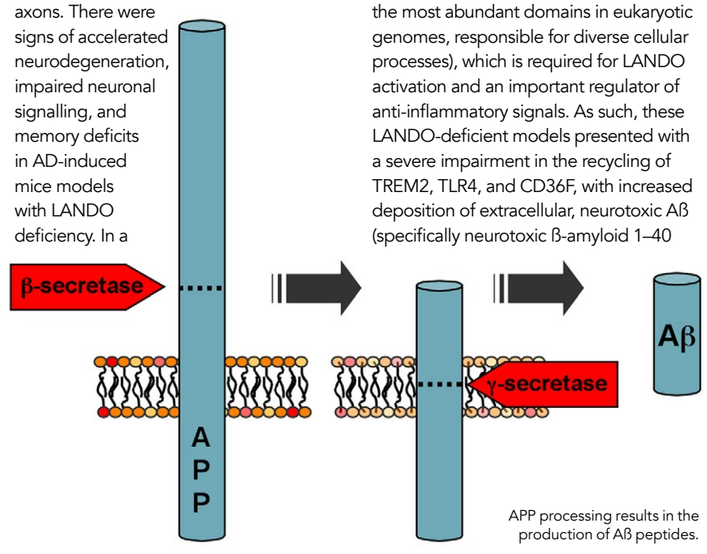
The LC3-associated endocytosis (LANDO) pathway is a key player in modulating neuroinflammation.

induced neuroinflammation in mouse models of AD.

IMPORTANCE OF NON-CANONICAL AUTOPHAGIC PATHWAYS

LC3 is pivotal in the selection of phagocytic markers and the biogenesis of autophagosomes. Previous pre-clinical studies by Drs Heckmann and Green have shown non-canonical autophagic pathways like LANDO to be crucial in the conjugation of LC3 to Rab5+, clathrin+ endosomes containing Aβ in murine CNS microglia. Considering the importance of accumulation of Aβ plaques and hyperphosphorylation of the tau protein in AD, Heckmann and his team of researchers have previously conducted various experiments to determine the importance of LANDO in mouse models with different combinations of conventional autophagic mechanisms.

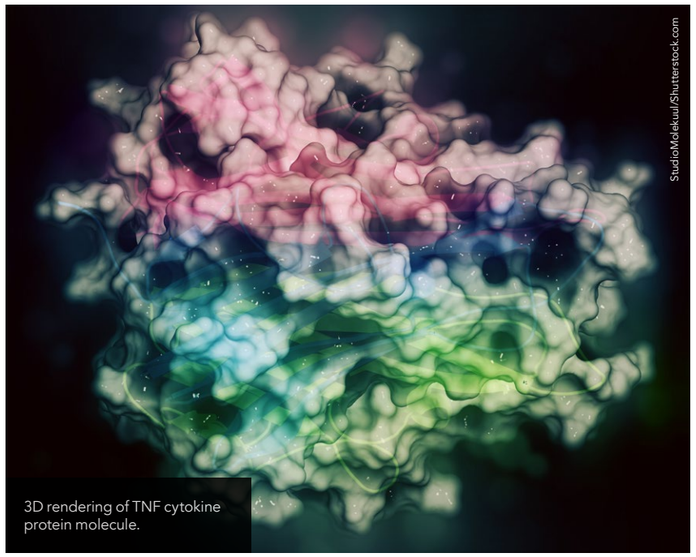
Experiments with murine models genetically engineered to lack LANDO by removing the Rubicon, a required regulator of LANDO but not canonical autophagy in microglia, showed a significant increase in pro-inflammatory cytokine production in the hippocampus. This was associated with increased levels of neurotoxic Aß protein, increased reactive microgliosis, and tau hyperphosphorylation – all of which are crucial markers of AD pathology. Untreated, these will eventually lead to the collapse of the microtubular architecture within the neurones and axons. There were signs of accelerated neurodegeneration, impaired neuronal signalling, and memory deficits in AD-induced mice models with LANDO deficiency. In a different experiment, another set of LANDO-deficient 5xFAD mice had a similar fate, with severe Aß accumulation that promoted reactive microgliosis (an inflammatory reaction by microglia, the primary innate immune effector cells in the CNS) and increased tau hyperphosphorylation (a process by which the otherwise integral, neuronal tau protein present in the microtubules, gets detached from the parent neurons, and forms neurofibrillary tangles, leading to neurodegenerative pathologies like AD).



APP processing results in the production of Aß peptides.

Previous research by Heckmann's team has also explored the possibility of deficiencies of canonical autophagic pathways by genetically engineering mice models and removing myeloid or microglia-specific FIP200 protein, which is required for conventional autophagic activation. Their data suggest that depletion of myeloid or microglia-specific FIP200 had no impact on tau phosphorylation, and thus concluded that depleting regulators of non-canonical autophagic mechanisms like Rubicon (global or microglia-specific) or myeloid ATG5 promoted tau phosphorylation throughout the hippocampus and the cerebral cortex. These data emphasise the importance of LANDO in neuroinflammation and progressive neurodegenerative disease.

Another experiment was conducted with animal models whose primary microglia lacked the WD-domain of ATG16L (one of the most abundant domains in eukaryotic genomes, responsible for diverse cellular processes), which is required for LANDO activation and an important regulator of anti-inflammatory signals. As such, these LANDO-deficient models presented with a severe impairment in the recycling of TREM2, TLR4, and CD36F, with increased deposition of extracellular, neurotoxic Aß (specifically neurotoxic ß-amyloid 1–40


3D rendering of TNF cytokine protein molecule.

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and 1–42) in the hippocampus and cortex, within two years.

THE DIFFERENCE BETWEEN LAP AND LANDO

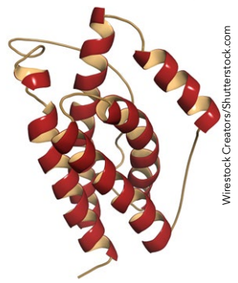
Of the different non-canonical autophagic pathways discovered thus far, LC3-associated phagocytosis (LAP) is another crucial pathway that uses the autophagic machinery with a similar mechanism as LANDO, albeit being different cellular entities. The role of LAP in mice concerning AD risks is not clearly understood.

In mouse models with FIP200-deficient microglia, there was no change as such in the development of AD-like pathology, similar to mice that are sufficient in autophagy. Researchers thus identified that a primary contributing factor for Aß accumulation in mice with LANDO-deficient microglia was not due to a defect in degradation, as is common for LAP-deficiency. Rather, it was caused by impaired recycling of receptors that recognise ß-amyloid, including TLR4 and TREM2. This indicated a difference in the importance of LAP versus LANDO in mitigating neuroinflammation, at least in the context of AD pathology.

The WD-domain of ATG16L, similar to Rubicon, interacts directly with the cytokine receptors responsible for anti-inflammatory cytokines and those responsible for adaptive immunity, including IL-10RB (for IL-22R, IL-26R) and IL-2R (for IL-2), that participate in downstream signalling events leading to LC3 lipidation. Removing the WD-domain of ATG16L, similar to the Rubicon-deficient animals, reduced anti-inflammatory signalling due to delayed endocytosis and insufficient recruitment of anti-inflammatory cytokine complexes leading to increased inflammation.

Pre-clinical studies highlight the potential of non-canonical autophagic mechanisms as a therapeutic target for treating AD and other CNS-based diseases.

Further research to differentiate between different stimuli, ranging from amyloids and extracellular aggregates to microbial pathogens, is needed to segregate specific triggers for specific pathways (LAP/LANDO) both globally and in the context of neuroprotection against AD and similar diseases. However, irrespective of these differences, it is now clear that the non-canonical autophagy machinery is crucial to suppress inflammation and any error in this machinery can lead to production of pro-inflammatory cytokines and subsequent neurodegeneration.


IL-1ß cytokine.

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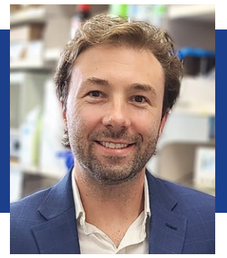
Moreover, neuroinflammation due to impaired autophagic pathways has also been identified as a risk factor for other CNS diseases including Huntington's and amyotrophic lateral sclerosis.

WHAT NEXT?

Every research experiment elaborated thus far highlights the potential of non-canonical autophagic mechanisms as a therapeutic target for treating AD and other CNS-based diseases. This would imply targeting inflammatory cytokine production and/or signalling for mediators including IL-1ß, TNF, and IL-6 – in short, the NLRP3 inflammasome. Heckmann and his team used this idea and have inhibited NLRP3 inflammasome using the NLRP3 inhibitor MCC950, which they tested on ATG16L WD-domain deficient mice with established AD-like disease for eight weeks. Results showed that MCC950-treated mice had comparable levels of Aß to those observed in vehicle-treated animals and restored approximately 80–90% behaviour and memory capacity. The vehicle-treated animals continued to have a decline in memory from the onset of therapy.

The root cause in treating AD-associated neurodegeneration is neuroinflammation, which is being addressed in future studies as well. Such studies also shed light on the importance of targeting specific inflammatory-cytokines like IL-1ß in an established disease model.

A further step in this regard would be to ensure the translational significance of determining such therapeutic targets, by implementing such studies in the human population and ensuring minimal adverse effects.



Behind the Research

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Research Objectives

The Heckmann laboratory examines LANDO-deficiency-induced neuroinflammation to develop new therapeutic avenues for treating diseases, including Alzheimer's.

Detail

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Bio

Dr Bradlee Heckmann is a member of the USF Health Neuroscience Institute and Director of the Spatial Biology, Neuroimmunology & Advanced Imaging Center. He is an Assistant Professor in Molecular Medicine and Affiliate Professor in Medical Engineering and is a scientific co-founder and chief scientific officer of Asha Therapeutics.

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- US National Institute of Neurological Disorders & Stroke
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- US National Cancer Institute
- Asha Therapeutics
- Ventus Therapeutics

Collaborators

- Douglas R Green, PhD, St Jude Children's Research Hospital
- Thomas Wileman, PhD, Quadram Institute

The Heckmann Lab

USF Health
Byrd Alzheimer's Center & Research Institute

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Personal Response

What inspired you to conduct these studies?

"I have always been fascinated with how diverse biology at the cellular level can truly be and how much interplay exists between seemingly unrelated mechanisms. The discovery of LANDO and its ability to regulate inflammatory signalling has been instrumental in opening new avenues for improving our understanding of a whole host of diseases, including Alzheimer's disease as highlighted herein.

Is there any other biomarker/biochemical test that can be used to screen for LANDO-deficient autophagic AD patients?

We are continuing along with other collaborators and colleagues in the field, including Drs Green and Wileman, Dr Oliver Florey, and many others, to improve our understanding of the mechanisms which regulate these important cellular pathways. In addition, our group is working to design new approaches to screening for deficiencies in non-canonical autophagy pathways including LANDO as well as immune markers. We are optimistic that these studies will lead to new clinical diagnostics for diseases including AD, and are simultaneously evaluating putative therapeutics strategies to restore LANDO function, modulate cellular energetics, or as suggested, reduce inflammation downstream of LANDO-deficiency. These efforts are aimed at restoring hope for patients suffering from devastating diseases such as AD."