

Technical report:

This operating grant tests the hypothesis that **the ATG16L1 CD associated variant reduces the efficiency of autophagy in response to bacteria, thereby promoting persistent infection and intestinal inflammation.**

To date we have made excellent progress. We have presented this work at a National meeting, have one manuscript under revision for resubmission to Nature Immunology and another accepted scientific review in Seminars in Immunology. We have made progress in each of the three main aims of the plan.

Outlined below are the three main aims and progress that we have made:

1a) Determine if the CD-associated variant of ATG16L1 effects autophagosome formation

We have successfully knocked down ATG16L using shRNA and shown that it disrupts bacterial-mediated autophagy (*Shigella*, *H. pylori*).

We have obtained shRNA resistant constructs to reconstitute either wildtype and CD variant of ATG16L1.

In preliminary studies we have shown that Adherent Invasive *E. coli* (associated with Crohn disease) induces autophagy and plan to use this as our model to address the role of the ATG16L1 variants in bacterial-mediated autophagy as this is most relevant to Crohn disease. Our ongoing studies will utilize the ATG16L1 knockdown and reconstitution of the wildtype or CD-variant and assess the effect on AIEC induced autophagy.

1b) Impact of CD-associated variant of ATG16L1 on autophagy induction using lymphoblastoid cell lines.

We have determined that the CD-associated variant of ATG16L1 reduces Nod-like receptor stimulated autophagy in lymphoblastoid cell lines (see aim 1c and attached manuscript).

1c) Determine if Nod2 acts a sensor for induction of autophagy and determine if CD-associated Nod2 and ATG16L1 mutants alter the response.

See attached manuscript and positive response letter. Manuscript currently under revision for resubmission to Nature Immunology.

We demonstrated that Nod1 and Nod2 proteins were critical for the autophagic response to invasive bacteria by recruiting ATG16L1 to the bacterial entry site. We have also demonstrated that in cells homozygous for the Crohn's disease-associated Nod2 frameshift mutation, mutant Nod2 failed to recruit ATG16L1 to the plasma membrane, resulting in impaired engulfment of invading bacteria by autophagosomes suggesting that Nod2 may serve as a nucleating factor for autophagosome formation. We did not find a difference in the ability of Nod2 to recruit the wildtype or CD-ATG16L1. Thus ongoing studies will determine the exact mechanism by which the CD variant of ATG16L1 reduces the Nod-stimulated autophagic response. Together, our results implicate bacterial sensing by Nod proteins in autophagy induction and provide a functional link between Nod2 and ATG16L1, two of the most important genes associated with Crohn's disease.

2a) Determine if the CD-ATG16L1 variant alters autophagy in human peripheral blood monocytes (PBMC).

We have genotyped CD patients at Mount Sinai Hospital and have obtained ethical approval to call back individuals to obtain blood samples. We have also recruited and ATG16L1 genotyped normal individuals to serve as controls. We are currently optimizing the conditions to assess autophagy in PBMC using murine samples and normal controls.

2b) Examination of autophagy in gastrointestinal tissue from patients with the CD-ATG16L1 variant

We have received approval from ethics to utilize stored tissue specimens from genotyped patients and have begun to identify specimens to perform immunohistochemical analysis to assess for alterations in autophagy in those with and without the ATG16L1 and Nod2 CD-associated variants.

3a) Characterization of the role of ATG16L1 polymorphisms in autophagic responses in myeloid cells of mice

3b) Impact of CD variants in ATG16L1 *in vivo*: susceptibility to colitis

Once the knockdown reconstitution system is optimized (see aim 1) we can assess the effect of the ATG16L1 variants in myeloid cells of mice in the presence or absence of Nod2 mutants. In addition we can generate bone marrow chimeras with ATG16 wt or CD reconstituted cells and assess the effect of susceptibility to colitis.

In summary we have made excellent progress in the first year of our operating grant. We expect to continue with this current level of progress to help understand how the ATG16L1 variant predisposes an individual to develop Crohn disease and advance the mission of CCFC to find the cure.

Lay summary:

Crohn disease: failure of our cells to “go green”?

Recent large genetic studies have found that a variant in a gene involved in autophagy called ATG16, is associated with Crohn disease. Autophagy is our cells way of “going green.” It is important for recycling old or damaged material in the cell to keep it healthy, much like our recycling programs keep our environment healthy. Old or damaged material is put into a “recycling bin” or autophagosome where it is broken down or recycled. The cell can then reuse this recycled material. Interestingly, autophagy is also important for helping to eliminate infections in cells. Bugs within cells are put into these recycling bins or autophagosomes, where they are broken down and killed. Since we know that bugs in our gut are important for Crohn disease, we think that the ATG16 variant associated with Crohn disease might effect the cells ability to use the recycling bins to properly deal with bugs in the gut. Indeed, we have found that cells that have the Crohn disease variant of ATG16 don’t make recycling bins as well as normal cells in response to bugs.

Another gene called Nod2, which is also very important for Crohn disease, detects a particular substance present in bugs. Interestingly, we have found that when Nod2 detects this substance the recycling process (autophagy) is triggered. In addition, we have found that Nod2 recruits ATG16 to start the recycling process. Interestingly, in cells that have the Crohn disease variant of Nod2, ATG16L1 is not recruited and the recycling bins aren’t formed properly at the entry site where the bugs infect the cell. Therefore, we have shown that two very important genes in Crohn disease interact with each other to ensure that the cells recycling process (autophagy) occurs properly in response to bugs. We think that in Crohn disease, variants in these two genes might result in lack of proper recycling in response to bugs resulting in increased inflammation in the gut. We hope by understanding how this recycling process works and differs in Crohn disease we might be able to find better strategies to treat patients with inflammatory bowel disease.

Publications:

Hussey S, Travasso LH, Jones NL. Autophagy as an emerging dimension to adaptive and innate immunity. Seminars in Immunology 2009 epub ahead of publication. **Invited research review.** (manuscript appended)

Submitted Publications:

Travasso LH, Carneiro LAM, Hussey S, Yuan L, Magalhaes JG, Le Bourhis L, Ramjeet M, Boneca IG, Allaoui A, Jones NL, Girardin SE and Philpott DJ: Essential role of Nod1 and Nod2 in bacterial induced autophagy. Under revision for resubmission to Nature Immunology. **C, original research** (see attached response letter and manuscript appended)

Presentations:

Hussey S, Travassos L, Yuan L, Philpott D, **Jones NL**. The role of ATG16L1 in bacterial-induced autophagy. Oral presentation, Canadian Digestive Disease Week February 2009, Banff, Alberta.

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Research personal:

Esther Galindo-Mata: technician

Heidi Mascarenhas: summer student, 2nd year University

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