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Gene-Microbiota Interactions Contribute to the Pathogenesis of Inflammatory Bowel Disease

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Abstract

Inflammatory bowel disease (IBD) is associated with risk variants in the human genome and dysbiosis of the gut microbiome, though unifying principles for these findings remain largely undescribed. The human commensal *Bacteroides fragilis* delivers immunomodulatory molecules to immune cells via secretion of outer membrane vesicles (OMVs). We reveal that OMVs require IBD-associated genes, *ATG16L1* and *NOD2*, to activate a non-canonical autophagy pathway during protection from colitis. *ATG16L1*-deficient dendritic cells do not induce regulatory T cells (T_{reg}) to suppress mucosal inflammation. Immune cells from human subjects with a major risk variant in *ATG16L1* are defective in T_{reg} responses to OMVs. We propose that polymorphisms in susceptibility genes promote disease through defects in 'sensing' protective signals from the microbiome, defining a potentially critical gene-environment etiology for IBD.

Intestinal microbiota modulate development and function of the immune system, and play a critical role in inflammatory bowel disease (IBD), a family of idiopathic intestinal disorders including Crohn's disease (CD) and ulcerative colitis (UC) (1-6). Concordance rates of 40-50% between monozygotic twins implicate gene-environment interactions contribute to CD (7-10), albeit in ways that are poorly understood. Advances in DNA sequencing

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technologies have empowered unprecedented insights into the human genome and the gut microbiome in IBD, enabling detailed genomic characterization of patients (11) and chronicling alterations in the composition and gene content of the gut microbiome (dysbiosis) (12).

Close to 200 risk loci have been proposed for CD, with several susceptibility genes linked to the regulation of autophagy (e.g., *ATG16L1*) (13-15) or to microbial sensors that activate autophagy (e.g., *NOD2*) (16-18). While previous studies have shown that disruption of ATG16L1 and NOD2 impacts CD susceptibility through defects in microbial clearance (19-23), recent reports reveal that immune cells impaired in autophagy are hyper-inflammatory (24-29). This suggests that deficiencies in ATG16L1 or NOD2 may contribute to CD risk through impaired anti-inflammatory responses, a hypothesis not mutually exclusive with microbial clearance functions.

The microbiome of CD patients is altered, with emerging evidence for cause and effects relationships to disease. Among other recent examples of host-microbe interactions (3, 5, 6), the human commensal *Bacteroides fragilis* has evolved beneficial immunomodulatory properties. During colonization of mice, *B. fragilis* capsular Polysaccharide A (PSA) is packaged in outer membrane vesicles (OMVs) and delivered to intestinal dendritic cells to induce interleukin-10 (IL-10) production from $CD4^+Foxp3^+$ regulatory T cells (T_{regs}), which protect from experimental colitis (30-32). To explore gene-environment interactions during host-microbiota symbiosis, we tested if genetic pathways linked to CD are involved in the immune response to *B. fragilis* OMVs.

Bone marrow-derived DCs (BMDCs) differentiated from wild-type (WT) and ATG16L1-deficient (*Atg16l1*$^{fl/fl}$ *Cd11c*Cre; $Atg16L1^{\Delta CD11c}$) mice were pulsed with OMVs harvested from wild-type *B. fragilis* (WT-OMV) or an isogenic mutant lacking PSA (ΔPSA-OMV), and co-cultured with $CD4^+$ T cells. As previously reported (33), WT-OMVs, but not vehicle or ΔPSA-OMVs, promote IL-10 production (Fig. 1, A to C, figs. S1 and S2). Conversely, ATG16L1-deficient DCs do not support IL-10 production in response to WT-OMVs (Fig. 1, A to C). We observe similar results using *Atg16l1*$^{fl/fl}$ *LysM*Cre mice (fig. S3). Purified PSA does not require ATG16L1 for its activity (Fig. 1, A and C, fig. S2). Next, we tested functional outcomes using *in vitro* T cell suppression assays. T_{regs} isolated from co-cultures with $Atg16L1^{\Delta CD11c}$ BMDCs treated with *B. fragilis* OMVs exhibit impaired suppressive activity (Fig. 1D and fig. S2A). Neither WT-OMVs nor pure PSA have any effect on IL-10 production among $CD4^+Foxp3^-$ type 1 regulatory T cells (fig. S4). ATG16L1, ATG5 and ATG7 are components of the autophagy elongation complex; BMDCs deleted in these genes likewise do not induce IL-10 production from T_{regs} (fig. S5). Further, recent reports reveal a role for autophagy components in T_{reg} homeostasis (34, 35). Our findings indicate that ATG16L1-deficient DCs fail to respond to *B. fragilis* OMVs, demonstrating that autophagy components in DCs are required for commensal-driven T_{reg} induction and function.

ATG16L1, ATG5 and ATG7 participate in both canonical and non-canonical autophagy pathways (36). Interestingly, the classical autophagy-specific genes *Ulk1*, *Fip200* or *Atg14* are not required for $CD4^+Foxp3^+IL\text{-}10^+$ T_{reg} induction upon WT-OMV treatment (fig. S6). We hypothesized that OMVs utilize the non-canonical autophagy pathway, LC3-associated

phagocytosis (LAP), which is specifically activated by microbial ligands delivered as particles rather than soluble molecules. LAP activation requires RUBICON, which represses canonical autophagy (36). *Rubicon*$^{+/-}$ but not *Rubicon*$^{-/-}$ BMDCs display increased accumulation of lipidated, membrane-bound LC3-GFP (LC3-II) upon *B. fragilis* WT-OMV treatment (Fig. 1E). As expected, neither ΔPSA-OMVs nor purified PSA are able to activate LAP (fig. S7). Moreover, treatment of *Rubicon*$^{-/-}$ DCs fails to induce T_{reg} responses (Fig. 1F). As RUBICON is upstream of ATG16L1 signaling, OMVs preferentially utilize the non-canonical autophagy pathway LAP to mediate tolerogenic responses to *B. fragilis*. Further, these data suggest a reconsideration of previous literature assigning the role of ATG16L1 in IBD to defects exclusively in autophagy.

As a CD-risk gene, we investigated the *in vivo* requirement for ATG16L1 in CD11c^{+} DCs during OMV-mediated protection from experimental colitis. Indeed, WT mice treated by oral gavage with WT-OMVs are protected from 2,4-dinitrobenzenesulfonic acid (DNBS) colitis (33), whereas Atg16L1$^{\Delta CD11c}$ mice exhibit acute weight loss and increased mortality similar to untreated mice (Fig. 2A and fig. S8A). WT, but not Atg16L1$^{\Delta CD11c}$ mice, orally administered OMVs are protected from shortening of the colon, a hallmark of colitis models (Fig 2B), with colitis scoring and cytokine profiles verifying protection from disease (Fig. 2C and fig. S8B). Prevention of colitis is not due to an overall defect in T_{reg} development in Atg16L1$^{\Delta CD11c}$ mice (fig. S9). Further, while proportions of CD4^{+}Foxp3^{+} cells are comparable in all groups of mice during colitis (fig. S10), Atg16L1$^{\Delta CD11c}$ mice produce significantly less IL-10 from gut Foxp3^{+} T_{regs} compared to WT mice following WT-OMV treatment (Fig. 2D and fig. S8C). Thus, WT-OMVs require ATG16L1 within DCs to induce IL-10 expression from Foxp3^{+} T_{regs} and to suppress intestinal inflammation in a colitis model.

In addition to impaired IL-10 production in response to OMV treatment, Atg16L1$^{\Delta CD11c}$ mice display an increase in IL-17A expression (Fig. 2E), but not IFN-γ (fig. S11), among mucosal CD4^{+}Foxp3^{+} T cells during colitis. Further, *in vitro* co-cultures of OMV-pulsed Atg16L1$^{\Delta CD11c}$ BMDCs result in impaired IL-10 expression among T_{regs} (Fig. 1C), and increased IL-17A production in CD4^{+}Foxp3^{+} T cells (fig. S12). Interestingly, while OMVs from other enteric bacteria each elicited a unique ATG16L1-dependent immune profile, only *B. fragilis* OMVs exclusively induce an anti-inflammatory response (fig. S13). Together, these data suggest ATG16L1-deficiency in DCs alters the quality of the T cell response to OMVs.

As DCs coordinate adaptive immunity, we sought to determine how Atg16L1$^{\Delta CD11c}$ DCs are impaired in promoting tolerogenic responses. Following OMV stimulation, we observe no differences by WT or Atg16L1$^{\Delta CD11c}$ DCs in internalizing OMVs, or in surface expression of MHC II, CD80 and CD86 (fig. S14) (27). However, stimulation with OMVs results in an increase transcription of multiple pro-inflammatory cytokines in Atg16L1$^{\Delta CD11c}$ DCs compared to WT cells (fig. S15). These data are consistent with previous reports of a hyper-inflammatory response in ATG16L1-deficient macrophages and DCs stimulated with other microbial ligands (24, 26). Abrogation of T_{reg} responses by ATG16L1-deficient DCs is likely due to increased pro-inflammatory cytokine production, which may impair DC-T cell interactions. Atg16L1$^{\Delta CD11c}$ mice do not display more severe

colitis than WT mice in the absence of OMV treatment (Fig. 2), suggesting that lack of protection is not due to more fulminant inflammation, but rather an inability to induce T_{regs} in mice deficient in ATG16L1 among $CD11c^+$ DCs.

NOD2 encodes for an intracellular sensor of bacterial peptidoglycan, and polymorphisms in this gene contribute to the largest fraction of genetic risk for CD (13). NOD2 has been shown to physically recruit ATG16L1 (20, 21), a process that is impaired in human cells homozygous for a *NOD2* frameshift mutation (20). Accordingly, $Nod2^{-/-}$ BMDCs pulsed with WT-OMVs are unable to support IL-10 production from $Foxp3^+$ T_{regs} during *in vitro* co-cultures (Fig. 3, A and B), revealing a crucial role for NOD2 signaling in microbiome-mediated immune tolerance. This notion is supported with *in vivo* studies showing that $Nod2^{-/-}$ mice are not protected from colitis by WT-OMV treatment (Fig. 3, C and D). Similar to $Atg16L1^{\Delta CD11c}$ animals, $Nod2^{-/-}$ mice produce significantly less IL-10 from $Foxp3^+$ T_{regs} of the MLN following WT-OMV treatment (fig. S16A), while proportions of T_{regs} remain unchanged during DNBS colitis (fig. S16B). Previous studies have shown that Toll-like receptor 2 (TLR2) is required for the PSA response (33, 37). While the role of NOD2 in inducing LAP is currently unknown, signaling through TLR2 potently activates LAP (36, 38). *B. fragilis* OMVs induce reactive oxygen species (ROS) from WT DCs, a known product of LAP activation (36), but at significantly reduced levels in $Nod2^{-/-}$ or $Tlr2^{-/-}$ DCs (fig. S17). Though further studies are needed to define the mechanism of LAP activation by OMVs, these data reveal that *NOD2* and *ATG16L1* may cooperate as part of a common pathway to promote anti-inflammatory immune responses to the microbiome.

To extend and validate gene deletion approaches, we tested responses to OMVs by immune cells carrying the CD-associated variant of *ATG16L1* (13, 14, 39). The *ATG16L1* T300A variant leads to protein instability and altered cellular responses (23). BMDCs from transgenic mice expressing the T300A allele are also unable to promote IL-10 expression from $Foxp3^+$ T_{regs} in response to WT-OMVs (fig. S18A). Further, *ATG16L1* T300A transgenic mice are not protected from DNBS colitis and do not mount a potent T_{reg} response when administered WT-OMV compared to WT mice (fig. S18, B to F). These findings prompted us to investigate if human immune cells from CD patients with the *ATG16L1* T300A risk variant (table S1) are also defective in promoting $Foxp3^+$ T_{reg} development by *B. fragilis* OMVs. Monocyte-derived dendritic cells (MoDC) from CD patients and healthy controls harboring either the protective allele (T300) or the risk allele (T300A) were pulsed with OMVs or PSA and co-cultured with syngeneic $CD4^+$ T cells. Consistent with our mouse data, human cells homozygous for the risk allele are unable to support induction of IL-10 from $Foxp3^+$ T_{regs} by WT-OMVs compared to MoDCs carrying the protective allele (Fig. 4). Remarkably, all samples tested display the predicted outcome based on genotype, and not disease status. However, cells from most subjects, regardless of genotype, respond to purified PSA (Fig. 4). Collectively, we conclude that mouse and human DCs require functional ATG16L1 for induction of $CD4^+Foxp3^+IL\text{-}10^+$ T_{regs} in response to *B. fragilis* OMVs.

IBD impacts over 1.5 million people in the US, with rates of diagnosis increasing and treatment options remaining limited (40, 41). The etiology of IBD is complex and incompletely resolved (1). We describe herein that interactions between genetic (*ATG16L1*/

NOD2) and environmental (microbiome) factors cooperate to promote beneficial immune responses. *B. fragilis* OMVs utilize LAP, an ATG16L1-dependent cellular trafficking and signaling pathway, to induce mucosal tolerance. The hyper-inflammatory responses that occur with mutations in ATG16L1 likely alter antigen-processing pathways and impair signaling by DCs to T cells, and may explain why CD-associated polymorphisms abrogate T_{reg} induction by OMVs. Collectively, discovery of genetic circuits co-opted by the microbiome to engender health provides unprecedented functional insights into gene-environment interaction relevant to the pathogenesis of IBD. We propose an additional role for genes previously implicated in killing bacteria—namely, mutations in genetic pathways linked to IBD result in an inability to sense and/or respond to beneficial microbes. This hypothesis may represent a new perspective for the etiology of microbiome-related diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and Notes

1. Peterson DA, Frank DN, Pace NR, Gordon JI. Metagenomic approaches for defining the pathogenesis of inflammatory bowel diseases. Cell Host Microbe. 2008; 3:417–427. published online EpubJun 12. [PubMed: 18541218]
2. Hill DA, Artis D. Intestinal bacteria and the regulation of immune cell homeostasis. Annu Rev Immunol. 2010; 28:623–667. DOI: 10.1146/annurev-immunol-030409-101330 [PubMed: 20192812]
3. Honda K, Littman DR. The microbiome in infectious disease and inflammation. Annu Rev Immunol. 2012; 30:759–795. DOI: 10.1146/annurev-immunol-020711-074937 [PubMed: 22224764]
4. Brown EM, Sadarangani M, Finlay BB. The role of the immune system in governing host-microbe interactions in the intestine. Nat Immunol. 2013; 14:660–667. published online EpubJul. DOI: 10.1038/ni.2611 [PubMed: 23778793]
5. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014; 157:121–141. published online EpubMar 27. DOI: 10.1016/j.cell.2014.03.011 [PubMed: 24679531]

6. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012; 336:1268–1273. published online EpubJun 8. DOI: 10.1126/science.1223490 [PubMed: 22674334]
7. Brant SR. Update on the heritability of inflammatory bowel disease: the importance of twin studies. Inflamm Bowel Dis. 2011; 17:1–5. published online EpubJan. DOI: 10.1002/ibd.21385 [PubMed: 20629102]
8. Orholm M, Binder V, Sorensen TI, Rasmussen LP, Kyvik KO. Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. Scandinavian journal of gastroenterology. 2000; 35:1075–1081. published online EpubOct. [PubMed: 11099061]
9. Halfvarson J, Bodin L, Tysk C, Lindberg E, Jarnerot G. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. Gastroenterology. 2003; 124:1767–1773. published online EpubJun. [PubMed: 12806610]
10. Tysk C, Lindberg E, Jarnerot G, Floderus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. Gut. 1988; 29:990–996. published online EpubJul. [PubMed: 3396969]
11. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012; 491:119–124. [PubMed: 23128233]
12. Wlodarska M, Kostic AD, Xavier RJ. An integrative view of microbiome-host interactions in inflammatory bowel diseases. Cell Host Microbe. 2015; 17:577–591. published online EpubMay 13. DOI: 10.1016/j.chom.2015.04.008 [PubMed: 25974300]
13. Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet. 2007; 39:207–211. published online EpubFeb (ng1954 [pii]10.1038/ng1954). [PubMed: 17200669]
14. Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet. 2007; 39:596–604. published online EpubMay (ng2032 [pii]10.1038/ng2032). [PubMed: 17435756]
15. Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nat Genet. 2007; 39:830–832. published online EpubJul (ng2061 [pii]10.1038/ng2061). [PubMed: 17554261]
16. Gardet A, Xavier RJ. Common alleles that influence autophagy and the risk for inflammatory bowel disease. Curr Opin Immunol. 2012; 24:522–529. published online EpubOct (S0952-7915(12)00124-0 [pii]10.1016/j.coi.2012.08.001). [PubMed: 23041451]
17. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature. 2001; 411:599–603. published online EpubMay 31. [pii]. DOI: 10.1038/3507910735079107 [PubMed: 11385576]
18. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature. 2001; 411:603–606. published online EpubMay 31. [pii]. DOI: 10.1038/3507911435079114 [PubMed: 11385577]
19. Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science. 2005; 307:731–734. published online EpubFeb 4. DOI: 10.1126/science.1104911 [PubMed: 15692051]
20. Travassos LH, Carneiro LA, Ramjeet M, Hussey S, Kim YG, Magalhaes JG, et al. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. Nat Immunol. 2010; 11:55–62. published online EpubJan (ni.1823 [pii]10.1038/ni.1823). [PubMed: 19898471]
21. Cooney R, Baker J, Brain O, Danis B, Pichulik T, Allan P, et al. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. Nat Med. 2010; 16:90–97. published online EpubJan (nm.2069 [pii]10.1038/nm.2069). [PubMed: 19966812]

22. Murthy A, Li Y, Peng I, Reichelt M, Katakam AK, Noubade R, et al. A Crohn's disease variant in Atg16l1 enhances its degradation by caspase 3. Nature. 2014; 506:456–462. published online EpubFeb 27. DOI: 10.1038/nature13044 [PubMed: 24553140]

23. Lassen KG, Kuballa P, Conway KL, Patel KK, Becker CE, Peloquin JM, et al. Atg16L1 T300A variant decreases selective autophagy resulting in altered cytokine signaling and decreased antibacterial defense. Proc Natl Acad Sci U S A. 2014; 111:7741–7746. published online EpubMay 27. DOI: 10.1073/pnas.1407001111 [PubMed: 24821797]

24. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature. 2008; 456:264–268. published online EpubNov 13 (nature07383 [pii]10.1038/nature07383). [PubMed: 18849965]

25. Cadwell K, Patel KK, Maloney NS, Liu TC, Ng AC, Storer CE, et al. Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. Cell. 2010; 141:1135–1145. published online EpubJun 25 (S0092-8674(10)00545-3 [pii]10.1016/j.cell.2010.05.009). [PubMed: 20602997]

26. Hubbard-Lucey VM, Shono Y, Maurer K, West ML, Singer NV, Ziegler CG, et al. Autophagy gene Atg16L1 prevents lethal T cell alloreactivity mediated by dendritic cells. Immunity. 2014; 41:579–591. published online EpubOct 16. DOI: 10.1016/j.immuni.2014.09.011 [PubMed: 25308334]

27. Park S, Buck MD, Desai C, Zhang X, Loginicheva E, Martinez J, et al. Autophagy Genes Enhance Murine Gammaherpesvirus 68 Reactivation from Latency by Preventing Virus-Induced Systemic Inflammation. Cell Host Microbe. 2016; 19:91–101. published online EpubJan 13. DOI: 10.1016/j.chom.2015.12.010 [PubMed: 26764599]

28. Lu Q, Yokoyama CC, Williams JW, Baldridge MT, Jin X, DesRochers B, et al. Homeostatic Control of Innate Lung Inflammation by Vici Syndrome Gene Epg5 and Additional Autophagy Genes Promotes Influenza Pathogenesis. Cell Host Microbe. 2016; 19:102–113. published online EpubJan 13. DOI: 10.1016/j.chom.2015.12.011 [PubMed: 26764600]

29. Kimmey JM, Huynh JP, Weiss LA, Park S, Kambal A, Debnath J, et al. Unique role for ATG5 in neutrophil-mediated immunopathology during M. tuberculosis infection. Nature. 2015; 528:565–569. published online EpubDec 24. DOI: 10.1038/nature16451 [PubMed: 26649827]

30. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008; 453:620–625. published online EpubMay 29 (nature07008 [pii]10.1038/nature07008). [PubMed: 18509436]

31. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010; 107:12204–12209. published online EpubJul 6 (0909122107 [pii]10.1073/pnas.0909122107). [PubMed: 20566854]

32. Allan SE, Broady R, Gregori S, Himmel ME, Locke N, Roncarolo MG, et al. CD4+ T-regulatory cells: toward therapy for human diseases. Immunol Rev. 2008; 223:391–421. published online EpubJun (IMR634 [pii]10.1111/j.1600-065X.2008.00634.x). [PubMed: 18613849]

33. Shen Y, Torchia M. L. Giardino, Lawson GW, Karp CL, Ashwell JD, Mazmanian SK. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. Cell Host Microbe. 2012; 12:509–520. published online EpubOct 18 (S1931-3128(12)00275-2 [pii]10.1016/j.chom.2012.08.004). [PubMed: 22999859]

34. Wei J, Long L, Yang K, Guy C, Shrestha S, Chen Z, et al. Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. Nat Immunol. 2016; 17:277–285. published online EpubMar. DOI: 10.1038/ni.3365 [PubMed: 26808230]

35. Kabat AM, Harrison OJ, Riffelmacher T, Moghaddam AE, Pearson CF, Laing A, et al. The autophagy gene Atg16l1 differentially regulates Treg and TH2 cells to control intestinal inflammation. eLife. 2016; 5 published online EpubFeb 24. doi: 10.7554/eLife.12444

36. Martinez J, Malireddi RK, Lu Q, Cunha LD, Pelletier S, Gingras S, et al. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. Nature cell biology. 2015; 17:893–906. published online EpubJul. DOI: 10.1038/ncb3192 [PubMed: 26098576]

37. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, et al. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science. 2011; 332:974–977. published online EpubMay 20 (science.1206095 [pii]10.1126/science.1206095). [PubMed: 21512004]

38. Sanjuan MA, Dillon CP, Tait SW, Moshiach S, Dorsey F, Connell S, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. Nature. 2007; 450:1253–1257. published online EpubDec 20 (nature06421 [pii]10.1038/nature06421). [PubMed: 18097414]

39. C. Wellcome Trust Case Control. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447:661–678. published online EpubJun 7. DOI: 10.1038/nature05911 [PubMed: 17554300]

40. Packey CD, Sartor RB. Commensal bacteria, traditional and opportunistic pathogens, dysbiosis and bacterial killing in inflammatory bowel diseases. Curr Opin Infect Dis. 2009; 22:292–301. published online EpubJun. [PubMed: 19352175]

41. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology. 2012; 142:46–54. e42. quiz e30. published online EpubJan (S0016-5085(11)01378-3 [pii]10.1053/j.gastro.2011.10.001). [PubMed: 22001864]

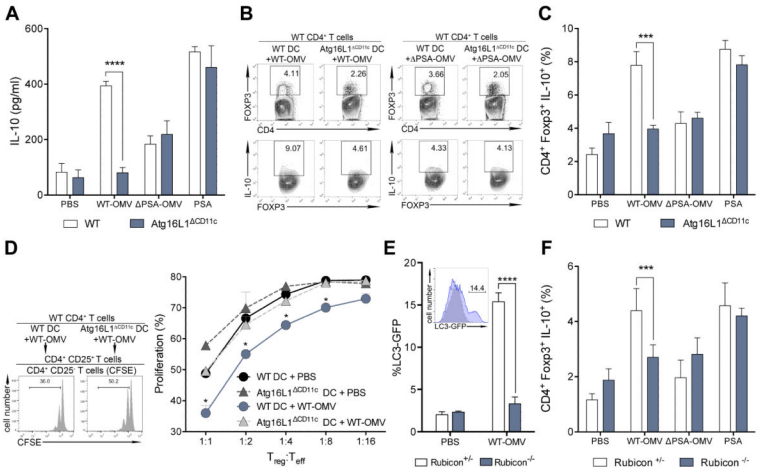

Fig. 1. ATG16L1 signals via a non-canonical autophagy pathway during OMV-mediated T_{reg} induction

(**A**) ELISA for IL-10 production during DC–T cell co-cultures with WT or Atg16L1$^{\Delta CD11c}$ BMDCs treated with PBS, *B. fragilis* WT-OMV, ΔPSA-OMV or purified PSA. (**B** and **C**) Representative flow cytometry plots (B) and frequency (C) of CD4^{+}Foxp3^{+}IL-10^{+} T_{regs} from DC–T cell co-cultures with WT or Atg16L1$^{\Delta CD11c}$ DCs treated with PBS, *B. fragilis* WT-OMV, ΔPSA-OMV or purified PSA. (**D**) T cell suppression assay analyzing *in vitro* generated T_{regs} from WT or Atg16L1$^{\Delta CD11c}$ DCs treated with WT-OMVs. (**E**) Quantification of LC3-GFP accumulation by *B. fragilis* WT-OMV treatment of *Rubicon*$^{+/-}$ or *Rubicon*$^{-/-}$ DCs. Representative flow cytometry histogram plot (inset). PBS, grey; WT-OMV, blue. (**F**) Frequency of CD4^{+}Foxp3^{+}IL-10^{+} T_{regs} from *Rubicon*$^{+/-}$ or *Rubicon*$^{-/-}$ DC–T cell co-cultures treated with PBS, *B. fragilis* WT-OMV, ΔPSA-OMV or purified PSA. Error bars represent S.E.M. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$. Two-way ANOVA, followed by Tukey's post-hoc analysis. Data are representative of at least 2 independent experiments.

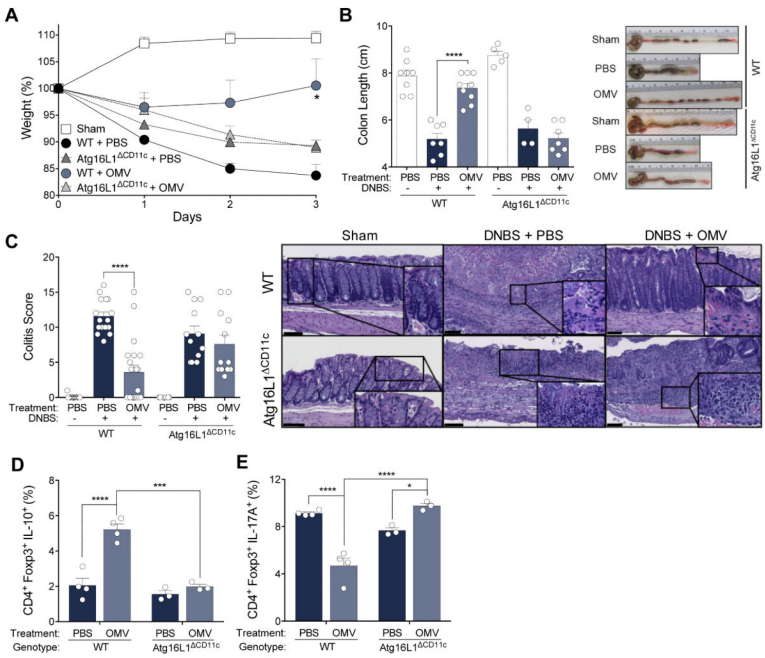

Fig. 2. *B. fragilis* OMVs require ATG16L1 in CD11c$^+$ DCs for protection from colitis

(**A** and **B**) Weight loss (A), colon length and gross pathology (B) of WT and Atg16L1$^{\Delta CD11c}$ mice orally treated with PBS or *B. fragilis* WT-OMV during DNBS colitis. Sham groups were treated with ethanol. **(C)** Colitis scores by a blinded pathologist using a standard scoring system, and representative H & E images. Scale bar represents 100 μm. (**D** and **E**) Mesenteric lymph node (MLN) lymphocytes isolated post-DNBS analyzed for IL-10 (D) and IL-17A (E) production among CD4$^+$Foxp3$^+$ T_{regs}, as assessed by flow cytometry. Error bars represent S.E.M. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$. Two-way ANOVA, followed by Tukey's post-hoc analysis. Data are representative of at least 3 independent experiments, with 3-9 mice/group.

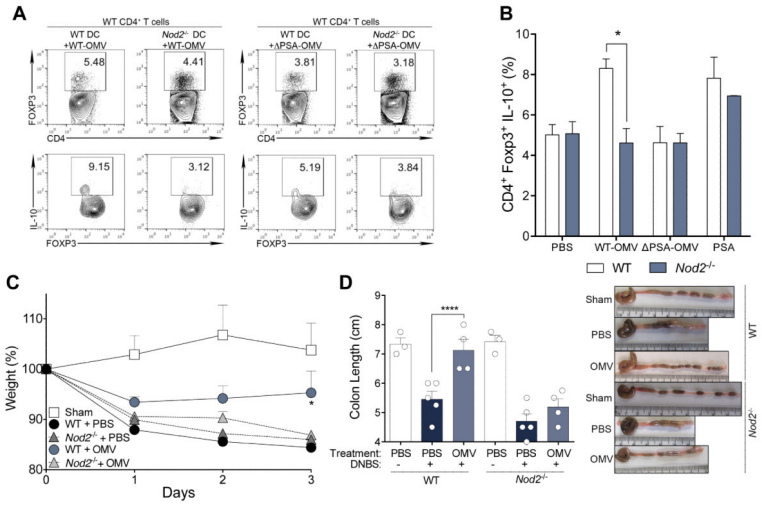

Fig. 3. NOD2 is required for OMV-mediated T_{regs} induction and protection from colitis

(**A** and **B**) Representative flow cytometry plots (A) from WT-OMV (left) and ΔPSA-OMV (right) treated BMDCs co-cultured with $CD4^+$ T cells, and frequency (B) of $CD4^+Foxp3^+IL\text{-}10^+$ T_{regs} from DC–T cell co-cultures. (**C** and **D**) Weight loss (C), colon length and gross pathology (D) of WT or *Nod2*$^{-/-}$ mice treated with PBS or *B. fragilis* WT-OMV during DNBS colitis. Error bars represent S.E.M. * $p < 0.05$, **** $p < 0.0001$. Two-way ANOVA, followed by Tukey's post-hoc analysis. Data are representative of at least 3 independent experiments, with 3-5 mice/group.

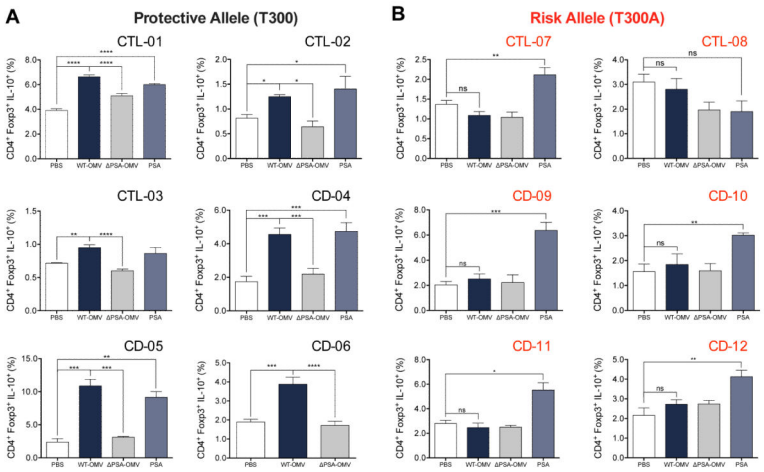

Fig. 4. The T300A risk variant of *ATG16L1* in human cells is unable to support OMV responses (A and **B)** MoDCs with either the protective (A) or risk (B) allele were treated with PBS, *B. fragilis* WT-OMV, ΔPSA-OMV or purified PSA, washed and co-cultured with syngeneic $CD4^+$ T cells. IL-10 expression was analyzed by flow cytometry among $CD4^+Foxp3^+$ T_{regs}. Human samples were processed and analyzed in a blinded fashion. CTL, control subjects; CD, Crohn's Disease subjects. Error bars represent S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns, not significant. One-way ANOVA, followed by Tukey's post-hoc analysis.