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Paneth Cell Alterations in the Development and Phenotype of Crohn's Disease

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Abstract

Pathogenesis of Crohn's disease (CD) involves immune and microbial dysregulation, induced by environmental factors in genetically susceptible individuals. There are believed to be multiple subtypes of CD, which contributes to its observed clinical heterogeneity. This concept has been reinforced by recognition of the complexity of the genetic, microbial, immune, and environmental factors that affect risk for CD. Paneth cells mediate immunity in and maintain the small intestinal epithelium; defects in activities of these cells have been observed in high proportions of patients with CD, and associated with more aggressive CD phenotype. Paneth cells integrate complex genetic, immune, and environmental signals, so alterations in their function could lead to different subtypes of CD, as observed studies in cohorts of primarily European descent. Subtypes of CD associated with Paneth cell function are observed even among patients from different genetic backgrounds. We discuss genetic susceptibility loci for CD and how these affect Paneth cell activity.

Graphical Abstract

Keywords

Crohn's disease; Paneth cell; autophagy; subtype

No conflicts of interests

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A combination of genetic and environmental factors contributes to the pathogenesis of complex diseases such as Crohn's disease (CD) ^{1, 2}. Although genetic factors determine susceptibility, most susceptibility loci increase risk by only a small amount—hereditary factors are only one piece of the puzzle. Most susceptibility loci have likely, over the ages, conferred some selective benefit ³. As society has evolved, such selection advantages may no longer be beneficial—subsequent changes in environmental pressures might now, instead, promote disease in genetically susceptible individuals. This paradigm can explain the increasing incidence of CD—new environmental factors induce inappropriate immune responses that cause inflammatory disorders in genetically susceptible individuals.

It is a challenge to determine the functional consequences of disease-associated variants. Typically, initial attempts to characterize functional changes utilize knockout and overexpression techniques in animal models and in relevant cell types. These studies often identify a cell type that is susceptible to altered function for a specific gene. For example, Paneth cell function is altered in mice with loss of function for ATG16L1 as well as other autophagy proteins ^{4, 5}. An important next step is to engineer mice with mutations in disease-associated genes and test for altered function of the gene products. As an example, mice with the *Atg16L1* mutation encoding T300A ⁶ confirmed which cell activities are disrupted by specific mutations.

A further challenge is to identify the environmental factors that affect disease susceptibility in people or animal models with risk-associated variants. In patients, this is done through studies of specific activities or lifestyle factors, such as diet. A more unbiased approach is to monitor the environment in home and workplace for exposures. However, it is a challenge to develop hypotheses for specific factors and these types of studies are difficult to perform, expensive, and prone to error and bias ⁷. A less biased approach would be to track markers of environmental exposures by screening urine, blood, or stool samples. However, there are not many validated biomarkers of exposures associated with CD. We propose that a cellular-based pathophysiologic target may leave a detectable imprint that is quantifiable in body fluid.

Inherited epigenetic changes could affect susceptibility to disease⁸, though this idea is has not been well studied in patients with CD. We propose that Paneth cells, located primarily in the small intestine (also in the proximal colon as a metaplastic response to inflammation), integrate genetic and environmental factors. Differences in Paneth cell activities and features might be used to classify different types of CD.

Paneth Cell Function and Abnormalities in CD Patients

Paneth cells are long lived (~60 days), ⁹ which makes them a good target to integrate information from other immune cells and components of the microbiome, compared with enterocytes, which live for only several days. Paneth cells are also a source of inflammatory signals ^{10–16}. Paneth produce, package, and export cytoplasmic granules that contain many different broad-spectrum anti-microbial proteins—some of these are constitutively produced whereas others are produced in response to microbial challenges.

Mice with engineered defects in autophagy proteins such as ATG16L1, and patients with CD who carry the mutation in *ATG16L1* that encodes T300A, have abnormal granule morphology and packaging of antimicrobial proteins. Localization of lysozyme and α -defensins (i.e. HD5 in humans) can be used to evaluate and quantify Paneth cell granule morphology. This method is superior to routine hematoxylin and eosin and Periodic acid-Schiff staining techniques, because the shape, size, amount, and distribution of these cytoplasmic granules can be determined. Each Paneth cell can be classified as normal or assigned to 1 of 5 different categories of abnormal: disordered (abnormal distribution and size of the granules); diminished (< 10 granules); diffuse (smear of cytoplasmic lysozyme or defensins, no recognizable granules); excluded (most granules do not contain stainable material); and enlarged (mega-granules) ^{4, 17–19}.

Paneth cell phenotypes of individual patients with CD or mouse models of IBD can be defined by the percentage of total abnormal Paneth cells in the sample. A 20% cut-off value has been used to differentiate type I Paneth cell phenotype ($\geq 20\%$ of abnormal Paneth cells) from a type II Paneth cell phenotype ($< 20\%$ of abnormal Paneth cells) ^{4, 17–19}. Using these criteria, Paneth cell abnormalities are found in 20%–50% of patients with CD.^{18, 19}

Paneth cell phenotypes can be analyzed using archived biopsy material obtained during endoscopy. Analysis of uninvolved ileal tissue from surgical resection specimens ¹⁸, allowed evaluation of Paneth cell morphology in areas with little or no inflammation. Systemic analyses of matched specimens and mathematical models showed that level of disease activity in specimens was not associated with Paneth cell phenotype, and that Paneth cell phenotypes remained stable over time (up to > 20 years). Biopsy specimens with 40–50 intestinal crypts were sufficient to generate results equivalent to those from resection specimens ¹⁹. It is therefore feasible to analyze Paneth cell phenotypes using materials obtained from routine endoscopic biopsies (our success rate is $\sim 70\%$), so this method of analysis might be used in studies of large patient populations.

Association of Paneth Cell Phenotypes With Patient Outcomes

Genome-wide association studies of North American and European cohorts identified >200 loci that increase susceptibility to inflammatory bowel disease (IBD), including >150 that increase risk for CD ^{3, 20}. Thus far, CD risk alleles associated with *ATG16L1* and *NOD2* lead to defects in packaging and secretion of antimicrobial peptides in Paneth cells ^{4, 17}.

In an analysis of the small intestinal Paneth cell phenotypes from large cohorts of genotyped adults with CD with well-annotated clinical information, the type I Paneth cell phenotype was associated with disease-associated genotype (*ATG16L1* T300A and single nucleotide polymorphisms in *NOD2*) ^{18, 19}. These variants appear to have additive effects as carriage of increasing numbers of *ATG16L1* T300A and CD-associated *NOD2* risk alleles in any individual correlate with increased proportions of abnormal Paneth cells in patients. Larger sample sizes are needed to study how these variants interact and what pathways they alter that lead to Paneth cell defects.

Laser-capture microdissection studies associated abnormal Paneth cells with an immune-activation gene expression signature. Furthermore, patients with CD and a type I Paneth cell phenotype were less likely to have granulomas, a characteristic of CD. Among adults with CD who underwent small intestinal resection and received post-surgical prophylactic therapy, those with a type I Paneth cell phenotype had a significantly shorter interval to disease recurrence than those with a type II Paneth cell phenotype¹⁸. Therefore, several different types of analyses have associated Paneth cell phenotype with mechanisms and development of CD.

These observations have been extended by numerous studies in mice. Disruption of *Atg16l1*, *Nod2*, *Lrrk2*, *Irgm1*, and *Xbp1* alter Paneth cell function, reducing expression and secretion of antimicrobial proteins and cell survival^{4, 13, 17, 21–24}. These studies have linked abnormal Paneth cells with defects in autophagy and the unfolded protein response, though other pathways are likely to be involved.

There is some evidence for functional synergy or epistatic interactions between *NOD2* and *ATG16L1*, *IRGM* and *ATG16L1*, as well as *NOD2* and *LRRK2*^{24–30}. Gene-gene interactions (where combinations of associated variants exponentially increase disease risk) have not been identified in patients with IBD. It is possible that identifying more homogenous sub-groups of disease, such as that characterized by Paneth cell changes, would facilitate these analyses³¹.

Effects of *Atg16l1* T300A on Paneth Cell Function in Mice and Patients

Researchers have generated knock-in mice that express *Atg16l1* encoding the T300A mutation^{6, 32, 33}. The *Atg16l1* T300A mice and patients who carry this mutation have similar percentages of abnormal Paneth cells, but a lower percentage than in *ATG16L1*-null mice. These studies indicate that *ATG16L1* is required for proper Paneth cell functions that are relevant to development of CD. *NOD2* and *ATG16L1* variants have been associated with abnormal Paneth cell phenotypes¹⁸ and with an inflammatory gene expression signature¹⁸.

Do these variants have direct or indirect effects on Paneth cells? Loss of function of autophagy-associated proteins affects activities of macrophages, dendritic cells, and T and B cells. Most of these studies have based on gene knockouts in cells of a specific lineage (all other lineages are competent for the pathway). It is not clear what effects the *Atg16l1* T300A variant has in other cell types. The effects of this mutation on Paneth cells may be indirect, and due to altered activities of other cell types. Studies are needed to determine what factors determine Paneth cell function. Viral infection affects Paneth cell activity in mice, but this has not yet been observed in humans. Abnormal Paneth cells have been associated with alterations to the microbiota; interactions between the intestinal microbiota and environmental have been reported^{17, 34}.

Paneth Cells as Biomarkers

There are few markers of CD disease development or progression, or markers that identify pathways of pathogenesis or predict patient outcome³⁵. Serum antibodies to *Saccharomyces cerevisiae* have been associated with CD, and have an additive effect with *NOD2* and

ATG16L1 variants in predicting susceptibility. This is similar to Paneth cell phenotype, raising the possibility that there are peripheral markers that can identify patients with different subtypes of CD. Further studies are needed ³⁶.

Paneth cell defects are more prevalent in children than adults with CD, and are associated with unique mucosal microbial composition and transcriptomic signatures. In an analysis of more than 500 adults with CD and more than 200 pediatric patients, the type I Paneth cell phenotype was found in a higher proportion of pediatric patients with CD (approximately 50%) than adults with CD (approximately 20%; $P<.001$). In pediatric patients, the type I Paneth cell phenotype is associated with reduced diversity and abundance of specific mucosa-associated microbiota. Changes in the microbial community include increased abundance of inflammatory microbes (such as *Corynebacterium* and *Erysipelotrichaceae*) and reduced abundance of barrier microbes (such as *Faecalibacterium* and *Blautia*). Importantly, the changes in microbial community associated with Paneth cell phenotypes are not observed in individuals without IBD (Liu et al, in press).

Paneth cell phenotypes also correlate with transcriptional profiles of ileal tissue. The most notable is decreased expression of genes that regulate oxidative phosphorylation in patients with CD with type I Paneth cell phenotype. Importantly, reduced expression of oxidative phosphorylation genes also correlates with changes in mucosal microbiota, when patients are stratified by Paneth cell phenotype. Importantly, Paneth cell phenotypes did not correlate with downregulation of oxidative phosphorylation gene cluster in individuals without IBD. There are therefore complex and dynamic interactions among Paneth cell functions, the mucosal microbial community, and epithelial energy metabolism and reactive oxygen species pathways (Liu et al JCI Insight in press).

Future Directions

Paneth cell changes are associated with genetic variation in *NOD2* and *ATG16L1* in humans and other genes that regulate autophagy or unfolded protein responses genes in mice. Paneth cell function is also affected by dysbiosis of the intestinal microbiome and associated with clinical outcomes in patients with CD. Studies are needed to identify additional genetic loci associated with this phenotype in humans. This is particularly true for populations of non-European ancestry, including East Asians, which have Paneth cell abnormalities but do not carry *NOD2* and *ATG16L1* variants associated with CD susceptibility.

More human and mechanistic studies are needed to evaluate the association between microbiome dysbiosis and Paneth cell changes. The association between Paneth cell changes and a more aggressive phenotype of CD, and the possible association of *ATG16L1* variants with a lack of therapeutic response to antagonists of tumor necrosis factor, indicates a need for additional, expanded, and ideally prospective cohort studies, to identify other phenotypes associated with Paneth cell changes ³⁷. A peripheral marker of CD development and related changes in Paneth cell activities could lead to new strategies to detect and treat CD, as well determine disease phenotype and predict disease progression.

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