Th17 response and autophagy - main pathways implicated in the development of inflammatory bowel disease by genome-wide association studies: New factors involved in inflammatory bowel disease susceptibility

Roberto Díaz-Peña1, Eliana Valdés2, Cecilia Cofré2 and Patricia Castro-Santos1

1Facultad de Ciencias de la Salud. Universidad Autónoma de Chile. Talca, Chile. 2Department of Gastroenterology. Hospital de Talca. Talca, Chile

ABSTRACT

Inflammatory bowel disease (IBD) is an entity that mainly includes ulcerative colitis (UC) and Crohn’s disease (CD). Improved health care, diet changes, and higher industrialization are associated with an increase in IBD prevalence. This supports the central role of environmental factors in the pathology of this disease. However, IBD also shows a relevant genetic component as shown by high heritability. Classic genetic studies showed relevant associations between IBD susceptibility and genes involved in the immune response. This is consistent with prior theories about IBD development. According to these, contact of the immune system with a high number of harmless antigens from the diet and the bacterial flora should originate tolerance while preserving response against pathogens. Failure to achieve this balance may originate the typical inflammatory response associated with IBD. Recently, genome-wide association studies (GWASs) have confirmed the implication of the immune system, particularly the Th17 immune response, previously associated to other autoimmune diseases, and of autophagy. In this paper, the mechanisms involved in these two relevant pathways and their potential role in the pathogenesis of IBD are reviewed.


INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition that mainly involves the gastrointestinal tract and includes ulcerative colitis (UC) and Crohn’s disease (CD), as well as in-between entities that are known as indeterminate colitis and share features of the former two. UC is characterized by inflammation and ulceration in the large bowel mucosa and submucosa, with distorted crypts and goblet cell depletion, whereas CD causes transmural inflammation with lymphocytic aggregates and usually non-caseating granulomas that may involve any intestinal segment (1).

The prevalence of this disease in western countries is 30-200 patients per 100,000 population. It was once considered a disease typical of western countries. However, its incidence in other countries is rapidly growing paralleling development (2). Thus, this increased prevalence is seemingly related to environmental rather than genetic factors (3).

However, the role of genetics in this condition is clear (Fig. 1). IBD is considered a polygenic disease, being familial in 5-10% of individuals and sporadic among the rest (4). Concordance in monozygotic twins is 50-75% for CD, and the risk of developing this condition is 800 times greater among patient relatives versus the general population (4). However, the phenotypic concordance of UC in monozygotic twins is lower (10-20%), which suggests heritability may be less significant (4). Genetic testing for candidate genes, linkage imbalance mapping, and most particularly genome-wide association studies (GWASs) have significantly increased our understanding of the relevance genetic susceptibility has in IBD (5). Specifically, we shall discuss the great importance attached by recently reported genetic studies to the role of the immune response (most particularly the Th17 response) and autophagy processes in the etiopathogenesis of this disease.

GENETIC FACTORS

IBD includes at least two distinct conditions, CD and UC, and a range of intermediate forms, known as indeterminate colitis, which share pathologic characteristics of the former two types. Such distinctions are also reflected...
by differences in heritability and in the increased risk for their development among patient relatives, as previously mentioned. This also applies to the genes historically implicated in the susceptibility to either disease. Overall, the fact that patients with IBD have altered cytokine levels as compared to the normal population is well known, hence associations between polymorphisms in cytokine-encoding genes and a greater susceptibility to disease have been researched (6). However, UC and CD show highly differentiated cytokine profiles, and the genetic associations found in one are not always applicable to the other. Best known among important associations are those between TNFα or IL-10 promoter polymorphisms and IBD susceptibility (7,8). Other significant associations include IL-1 and IL-1 receptor polymorphisms (9), and more recently IL-6 receptor or IL-23 receptor polymorphisms (10). Bacterial molecule receptors have also been studied as candidate genes, and associations have been found with several TLRs (10); specifically, the strongest such association found in CD so far is with the CARD15 (intracellular receptor for peptidoglycans) gene (11). Under normal conditions, the interaction of CARD15 with bacterial antigens should activate TNFα secretion, leading to the destruction and clearance of harmful microorganisms. When CARD15 has a mutation, such clearance fails to occur and the inflammatory response to the organism persists (12). These polymorphisms have also been associated with certain clinical characteristics, including ileal involvement, stenosis, early onset, and need for surgery (11,13).

GWASs are considered a primary tool to determine genetic influence for a given disease. Multiple studies of this sort have been carried out, with over 900 primary big-scale studies in the last 5 years. In each study at least 100,000 single-nucleotide polymorphisms (SNPs) are genotyped in cohorts comprising over 1,000 subjects on most occasions. Therefore, GWASs provide an objective view of the whole genome, and an increased probability to detect associations with markers. For each of these associations identifying the genetic change likely responsible for an association is a labor-intensive endeavor. Hence, a fine mapping analysis is important before intensive functional studies are initiated.

In the last few years, GWASs have provided lots of information on the genetic factors associated with IBD. GWAS meta-analyses have thus far revealed approximately 163 genetic risk loci (14), including 23 specific for UC, 30 specific for CD, and 110 shared by CD and UC, which suggests a similar genetic contribution to disease predisposition in both instances, as well as common pathophysiological routes. Many of the associated SNPs correspond to non-encoding variants, as previously described for other complex diseases (15). Approximately 40% of SNPs are found in gene expression regulatory zones. Many (70%) of these IBD-associated SNPs have been also related with other complex diseases, and especially with the immune response. Of particular significance, because of their association with disease, are genes related with the regulation of cytokine production, activation of lymphocytes, and response to bacterial molecules, particularly mycobacterial ones. Such studies again focus on pathways previously identified in immunological studies, including IL-23 and T helper (Th) 17 cells (16,17).

The fact should be underscored that genetic contribution not only derives from our eukaryotic genome, as the genome of our microbiota, the microorganisms colonizing our body, must also be counted in. As in other conditions, microbiota changes (dysbiosis) have been associated with disease onset (18). Such changes result from multiple fac-
tors: Antibiotics, pollution, some infections, or disease management itself (18). The relevance of our microbiota is reflected by the fact that it comprises 10 bacterial cells for each eukaryotic cell in our body, and our microbiome, the genome of our microbiota, carries 150 bacterial genes for each of our own genes. The microbiota protects us from enteric pathogens, plays a role in the acquisition of energy and nutrients from our diet, and its interaction with the bowel mucosa is crucial in the regulation and activation of the immune system. As regards IBD, an exaggerated response to some microorganisms or excessive colonization by some others may underlie the origin of this disease (19). In fact, fecal flow bypassing induces remission whereas the infusion of fecal contents reactivates the condition. This is also the case with antibiotic therapy for UC, which demonstrates the important role of microorganisms in the pathogenesis of this disease. However, not all microorganisms exert equal effects – genera such as Lactobacillus, Bifidobacterium and Faecalibacterium have shown a protective effect against mucosal inflammation (19). Therefore, while some microorganisms induce an inflammatory response, others inhibit said response by, for instance, enhancing IL-10 production or regulatory T-cell activity (20). Thus, it is the predominance of proinflammatory over regulatory microorganisms that seems to bear a connection with the development of IBD. When comparing the microbiota of patients with UC or CD with that of healthy individuals no species could be directly linked with disease development (20), but decreased diversity and overexpression of species such as Ruminococcus spp. or Enterobacteriaceae spp.

Th17 RESPONSE

A meta-analysis of all IBD-related GWASs to date clearly points to the Th17 response, IL-23, and the IL-23 receptor (IL23R) as one of the mechanisms involved in IBD susceptibility (14). The gastrointestinal tract is characterized by the presence of a high number of harmless antigens from the diet and intestinal flora. It is therefore a place where the immune system must operate with a high degree of tolerance while keeping adequate responsiveness to pathogens. This is why an imbalance in this highly unstable system may give rise to a disproportionate inflammatory response to harmless antigens or, on the contrary, fail to respond to some pathogens, which may represent the etiopathogenic grounds of IBD.

Antigen presentation to naïve T-cell precursors in the periphery leads to the development of effector T-cells or regulatory T-cells. This process is strongly influenced by signals from the environment at the time of activation. Predominant signals condition cell differentiation to specific effector lineages while blocking other phenotypes, and dramatically remodel gene expression. From this cascade results a T-cell with a wholly specialized function. The helper T-cell populations initially described included Th1 (cell response, proinflammatory) and Th2 (humoral response, antibody production). However, since regulatory cells were first discovered, researchers have assumed that immune responses are much more complex (Fig. 2). These cells play a role in the maintenance of self-tolerance, and eliminate self-reactive lymphocytes using cell-to-cell contact mechanisms and the production of cytokines such as transforming growth factor (TGF) beta or IL-10 (21). Deficient numbers or functioning have been associated with several autoimmune diseases.

More recently a new T-cell population has been described—Th17. These cells are of the effector type and promote inflammatory response, autoimmune disorders, and transplant rejection. It is clear that the proportions of Th17 cells and regulatory cells are inversely associated, and an increase in Th17 cells usually implies a decrease in regulatory cells. These cells are phenotypically characterized by the expression of nuclear receptor RORγt, IL-17, IL-22, and GM-CSF (granulocyte-macrophage colony-stimulating factor). Differentiation to Th17 initially requires IL-6 signaling together with TGF-β expression. TGFbeta concentration is crucial for the regulation of Treg versus Th17 cell development. A sub-differentiation of these cells ensues where IL-23 plays a key role. IL-6, IL-21, and IL-23 signaling pathways activate STAT3, and render it essential for Th17 differentiation (22). In fact, STAT3 mutation results in absence of these cells (23). STAT3 activation regulates RORγt, IL-17, and IL-23R expression. However, these IL23R+RORγt+ Th17 cells are not clearly proinflammatory – exposure to IL23 is necessary to trigger inflammatory functions, as was demonstrated in the experimental autoimmune encephalitis (EAE) model in the mouse (24). The effect of TGFβ is the opposite – while playing a role in Th17 differentiation, it also inhibits IL23R expression (25). Thus, a Th17(β) population has been described, which is not involved in autoimmunity, as well as a Th17(23) population, which is involved (26). While Th17(β) cells are characterized by IL-17A, IL-17F, and IL-10 (an immunomodulating cytokine) production, Th17(23) cells produce IL-17A, IL-17F, IL-22, and IFNγ.

Th17 cells exert their effects through IL-17 secretion. IL-17 exhibits pleiotropic actions, including the induction of proinflammatory cytokine and chemokine expression, which results in tissue infiltration and eventual destruction. In fact, IL-17 is a cytokine family that plays a relevant role in both the innate and adaptive immune responses, and includes six members (IL-17A to F) as well as five receptors (IL-17RA to RE). There is another Th17 cell population, the so-called natural Th17 cells, which emerge directly as such from the thymus instead of undergoing peripheral differentiation, as other effector cells. These cells, as well as other natural cells, express TCR with affinity for self-peptide-bound HLA, and therefore have escaped negative selection mechanisms. These are effector...
cells with an activated, memory phenotype pending contact with their corresponding antigen (24).

The physiologic role of Th17 cells is fighting extracellular bacteria and fungi via the secretion of IL-17A, IL-17F, and IL-22. However, dysregulated Th17 responses, characterized by IL-17A and IL-17F overproduction, are recognized as highly significant as a causal factor or add-on factor in autoimmune conditions such as multiple sclerosis and IBD (27). Their etiopathogenetic role seems clear, as they are proinflammatory cytokines inducing the secretion of other proinflammatory cytokines, of chemokines such as CCL20, and of metalloproteinases (MMPs). It comes as no surprise, therefore, that gene polymorphisms within this pathway are associated with IBD susceptibility. Specifically, IL-17A has been shown to have a protective role in mouse models of colitis, as Th1 response inhibition is among its functions, whereas IL-23 increases murine colitis severity via the generation of Th17 cells that release IFNγ-Th17β cells (28,29). This would again prove a dichotomy between Th17β and Th17(23) cells. Other responses potentially involved are Th9 and Th22, both of them recently described (30) and still lacking association data.

**AUTOPHAGY**

Another large group of genes unveiled by GWASs as relevant for IBD is involved in autophagy regulation (31). This process mainly consists of the degradation by lyso-
some autophagy is to engulf material to be destroyed (Fig. 3). This is a process related to inflammation that becomes activated in response to various types of stress (33). The process is regulated by mTORC1 levels. Its deactivation in response to starvation or other forms of stress activates autophagy. A phagophore, a membrane precursor to the autophagosome, develops first. PI(3)P recruitment into the membrane is key. Then, Atg proteins mature the autophagosome, which eventually fuses with endosomes and lysosomes that destroy its contents.

GWASs have associated CD susceptibility primarily with 3 genes involved in autophagy: ATG16L1, IRGM, and PTPN2 (34). ATG16L1 (autophagy-related protein 16-1) plays a role in the elongation of the autophagosomal membrane (35). ATG16L1-deficient fibroblasts have been shown to fail in forming phagosomes in response to starvation. Mutations in this gene have also been associated with Paneth cell abnormalities similar to those seen in patients with CD. Furthermore, patients with CD and mutated ATG16L1 exhibit large numbers of autophagosomes in the cytoplasm of Paneth cells likely due to defective fusion with lysosomes, which prevents their destruction (35). The presence of such mutations in ATG16L1 also activates inflammasomes, structures made up with various proteins that play a role in programmed cell death by pyroptosis (32). In turn, IRGM (immunity-related GTPase family M-protein) is in charge of autophagy for intracellular pathogens, both viruses and bacteria. It is also involved in the antigenic presentation of peptides resulting from microorganism degradation via HLA class I and class II molecules, thus taking part in the origin the adaptive immune response (36). Mutations in this gene may therefore entail a poorer clearance of pathogens from the intestinal mucosa in IBD. The role of adherent-invasive *E. coli* has been particularly highlighted because of its higher numbers in the intestine of patients with CD, and its clearance hinges upon IRGM-mediated autophagy (37). In addition, miR-196, the micro-RNA that binds the normal

**Fig. 3.** Main stages of macroautophagy. Different signals initiate macroautophagy. This involves molecules such as IRGM, ULK1 or PTPN2. Nucleation with phagophore formation, likely from the endoplasmic reticulum with participation of Atg2A and Atg2B. Elongation to engulf the material to be digested with the intervention of Atg9 (provided by vesicles from the Golgi apparatus), LC3, ATG16L1, Atg5, Atg12, Atg4, Atg7, and Atg3. Closure of autophagosome and fusion with lysosomes and endosomes. The prior dissociation of Atg present in the autophagosome is required. Maturation, where XBP1 plays a role. Autolysosome contents are digested by proteases including cathepsins B and D. Molecules associated with CD susceptibility by GWAS are shown in bold text.**
irgm allele, is increased in patients with CD. The role of this micro-RNA is unclear in this process but seems to relate to IRGM expression control and, therefore, autophagy (36). PTPN2 (protein tyrosine phosphate non-receptor type 2) is an important inhibitor of the response to proinflammatory cytokines such as IFN-γ and IL-6 because of its ability to dephosphorylate STAT1 and STAT3. Gene ptpn2-knockout mice develop systemic inflammation, and elevated messenger levels for this protein have been found in the inflamed mucosa of patients with CD (38). It has been seen to also play a significant role in autophagy control since the knockdown of this gene results in impaired autophagosome formation and dysfunctional autophagy (39), and this gene’s inactivation with siRNA in the T84 cell line increases mTOR phosphorylation and beclin 1 expression, while inhibiting Atg5 and Atg7 activation by IFN-γ and TNF-α (31). Specifically in the CD setting, in cultures of lamina propria fibroblasts from patients, their stimulation with IFN-γ and TNF-α was shown to increase autophagosome formation in wild-type subjects but not in individuals with PTPN2 mutation, which has been associated with higher CD susceptibility (39). This is therefore a protein involved in the connection between inflammatory response and autophagy, and possibly in the maintenance of epithelial integrity as well (31).

There is also a clear association between receptor NOD2 and autophagy, as this receptor—under normal conditions—can activate autophagy vacuole formation in dendritic and epithelial cells. Mutated NOD2 variants associated with CD cannot trigger such a response (40).

Closely related to autophagy is the response to protein misfolding as induced by endoplasmic reticulum stress. The XBP1 gene is involved in this process, also associated with increased susceptibility to IB (41). The inactivation of this protein in intestinal epithelial cells results in endoplasmic reticulum stress, Paneth cell dysfunction, and enteritis (41), and defects in this gene, together with defects in Atg16, give rise to severe, CD-like transmural ileitis similar to CD in animal models (42).

CONCLUSION

GWASs in the IBD setting have revealed genes that are particularly relevant for the immune response, specifically the Th17 response, as well as for autophagy processes. This confirms the results of previous studies showing an association of these two pathways with the etiopathogenesis of this disease, and opens the way to deeper understanding, improved categorization, and future treatment prospects for IBD.

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