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NOD2 Investigating IBD with Autophagy and Interleukins

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Summary

Inflammatory bowel disease (IBD) is a chronic condition that causes inflammation and swelling in the digestive tract. Although two types of IBD exist, Crohn's disease (CD) and Ulcerative Colitis, this review will focus mainly on CD. The common pathology in IBD is the induction of interleukins and an abnormal Paneth cell phenotype. CD is caused by environmental and multigenic factors, including mutations in NOD2 and RUNX-3. NOD2 promotes the induction of specific antimicrobial peptides that strengthen the immune system's response against antigens. Mutations in NOD2 cause an overexpression of NF- κ B activity and IL-1- β processing, which increases susceptibility to CD. Deletion of RUNX-3 in mice induces colitis and causes an increase in certain interleukins. Moreover, IL-23R induces a pro-inflammatory response while IL-11R promotes an anti-inflammatory response. Defective autophagy has been linked to abnormal Paneth cells and an endotoxin-induced inflammatory response in mice. The most promising CD treatments involve procedures that reduce inflammation by decreasing interleukin levels. Specifically, injection of anti-interleukin 12 antibodies, increasing the production of glucocorticoids through LRH-1, and the addition of polysaccharide A (PSA) can all serve as possible treatments for CD by down-regulating the overactive immune system. Identifying more genes that increase susceptibility to CD can lead to novel treatments in the future.

Introduction

Inflammatory Bowel Disease (IBD) is a group of gastrointestinal disorders that causes inflammation in the intestines. The two main forms of IBD include Crohn's disease (CD) and ulcerative colitis (UC). While CD can affect any part of the gastrointestinal tract, UC is restricted to the colon and the rectum. CD was named after an American gastroenterologist, Burill Crohn, in 1932, who reported having patients with severely inflamed ileums (Vermeire et al., 2007). In his report, Crohn specifically described patients experiencing painful symptoms, including abdominal pain, diarrhea, weight loss, and rectal bleeding (Baumgart and Carding, 2007). In addition, bloating, nausea, fatigue, and fever often accompany bowel pain (Hanauer, 2006). CD also leads to mechanical problems within the intestine, such as obstructions and abscesses. However, patients often experience long periods of remission in which symptoms are not present.

Approximately 500,000 people in the United States suffer from CD. The onset of CD usually occurs during early adolescence or late middle-age (Bouma and Strober, 2003). However, the disease can occur at any age. Although CD equally affects men and women, people of

Eastern European descent and people from developed countries are more likely to be affected (Baumgart and Carding, 2007). Specifically, a group with a high incidence of CD is Ashkenazi Jews (Bouma and Strober, 2003).

In patients with CD, the immune system is chronically activated even in the absence of antigens. This abnormal inflammatory response causes chronic inflammation and ulceration. The cause of CD is unknown (Bouma and Strober, 2003). Environmental factors, such as smoking and genetic dispositions, can contribute to the pathology of the disease. Immediate relatives of patients with CD are more likely to develop the disease.

CD can be diagnosed by performing a colonoscopy, which allows for the direct visualization of the colon. Also, biopsies and blood tests are often used to help diagnose the disease (Hanauer, 2006). Currently, there is no known cure for CD. However, anti-inflammatory drugs, such as corticosteroids, are often prescribed for treatment. Other medications, including immunosuppressants, can reduce the inflammation. By managing their stress, nutritional intake, and sleep patterns, CD patients can help reduce their symptoms (Baumgart and Carding, 2007).

In CD, the inflammatory response mechanism is altered from the healthy pathway (Figure 1). There is an increase in pro-inflammatory cytokines observed in response to invading microbes. This immune response causes excessive inflammation. The immune system is unable to turn off, resulting in the symptoms seen in patients with CD (Fig 1). The specific factors that cause the increase in these proinflammatory chemicals are not well known. Also, the reason for the decreased ability of the immune system to destroy antigens remains unclear.

Past studies have demonstrated that CD has a strong genetic link. Previous genome searches for CD-susceptibility genes have identified several loci, particularly on chromosome 16. (Satsangi, et. al., 1996) Recently, nine genes involved in the disease have been found, including *NOD2*, *DLG5*, *IL23* receptor, *ATG16L1*, *IRGM*, and *IL12B*, *NKX2-3*, *PTPN2*, and *NELL* (Kaser, et. al. 2008). Even though researchers have identified genetic mutations that increase susceptibility to CD, the mechanism by which these genes operate and their specific link to inflammation requires further investigation. The exact role of different interleukins should also be examined.

These gaps in knowledge are linked to the following hypothesis: mutations in certain genes, as well as defects in autophagy, can cause an increase in pro-inflammatory stimulants and a decrease in bactericidal killing activity, resulting in excessive inflammation seen in people with CD. Looking at the genetic basis of CD can potentially lead to improved treatments, and eventually a cure.

The Role of NOD2 in CD

NOD2 Activates NF- κ B and IL-1 β secretion

Since CD patients have exhibit increased levels of the transcription factor NF- κ B and a mutation in the NOD2 gene is increased, susceptibility to CD up to 40 times, the relationship between the gene and the nuclear factor needed to be investigated (Maeda et al., 2005). The NOD2 protein is active in a variety of immune system cells, including macrophages and dendritic cells, which protect the body against antigens. NOD2 is also active in epithelial cells, including Paneth cells found in the lining of the intestine. NOD2 is a pathogen-recognition molecule that detects muramyl dipeptide (MDP) in the intestine and triggers the

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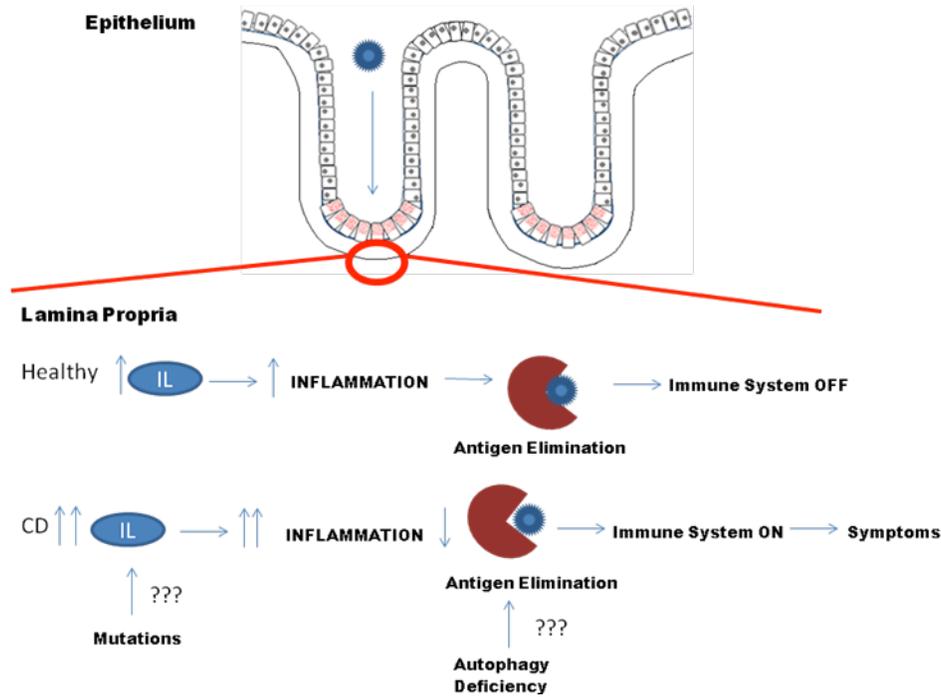


Figure 1: Model displaying a comparison between healthy and IBD immune system response. This figure depicts a comparison between a healthy and diseased inflammatory response to an antigen in the lamina propria. In the CD pathway, the levels of interleukins are significantly increased, and the bactericidal killing activity is decreased. The cause of these changes is unknown; however, this heightened pro-inflammatory response can result from specific gene mutations or deficiency in autophagy.

immune system to respond to an infection. This response occurs by the activation of NF- κ B, which, in turn, regulates the activation of proinflammatory signaling pathways. The most common variation of NOD2, 3020insC, leads to the production of a slightly shorter NOD2 gene (Maeda et al., 2005). However, there is a great deal of conflicting evidence in regards to NOD2 function and the role it has on the proinflammatory response. Some past researchers believed that the NOD2 variants were defective in activation of NF- κ B, yet CD patients have exhibited increased levels of this nuclear factor (Maeda et al., 2005). In order to illuminate the mechanism by which NOD2 variants follow, cytosine was inserted into mice at position 2939 in the NOD2 locus, which corresponds to the 3020insC variant in humans (Maeda et al., 2005). The MDP stimulated Nod22939iC macrophages demonstrated increased NF- κ B DNA binding activity compared to wild type (WT) cells (Maeda et al., 2005). When analyzing the expression of NF- κ B target genes in terms of fold increase in mRNA expression, the results showed increased levels of a number of target genes, including IL-1 β , IL-6, and TNF- α (Maeda et al., 2005). However, only IL-1 β secretion was significantly increased in the Nod22939iC macrophages compared to WT controls (Figure 2A). When Nod22939iC mice were treated with dextran sodium sulfate (DSS) (a toxin involved in disrupting barrier wall function), there was an increase in apoptotic cells found in the lamina propria, which is linked to the activation of caspase-1 (Maeda et al., 2005). This is an enzyme required for the secretion of IL-1 β . In other words, a mutation in NOD2 overexpresses IL-1 β by stimulating the activation of caspase-1. These results support the hypothesis that NOD2 is necessary for the appropriate processing and release of IL-1 β . Thus, mutations in NOD2 increase susceptibility to CD by increasing NF- κ B activation and IL-1 β secretion, which causes a heightened proinflammatory response typically seen in CD patients.

NOD2 Activates hBD-2

In order to fight invading microbes, epithelial cells induce antimicrobial peptides, such as human beta-defensin-2 (hBD-2), as a defense response. Cytokines, such as IL-1, TNF- α , and IL-22, serve a role in activating hBD-2 (Voss et al., 2006). Since recent studies have shown an abundance of NOD2 in epithelial cells of the small intestine, among other regions, the relationship between NOD2 and hBD-2 needed to be clarified. Human embryonic kidney 293 cells (HEK293) that overexpress NOD2 were injected with both a hBD-2 promoter luciferase plasmid and with either a NOD2 or NOD2 3020insC expression vector. All samples were stimulated with MDP (Voss et al., 2006). Afterwards, the luciferase activity was measured, in order to analyze the hBD-2 promoter activity. The results showed significantly increased hBD-2 promoter activity for the NOD2 transfected cells upon MDP stimulation (Voss et al., 2006). To further verify that the activation of NOD2 by MDP mediates the secretion of hBD-2, a NOD2-specific siRNA was injected into primary keratinocytes to specifically inhibit NOD2 expression (Voss et al., 2006). Keratinocytes that were injected with NOD2 siRNA could not secrete hBD-2 when treated with MDP (Voss et al., 2006). These findings show that NOD2 plays a vital role in protecting the cell against invading microbes by stimulating antimicrobial peptides. A mutated NOD2, on the other hand, is unable to produce the needed antimicrobial peptides and thus cannot destroy the antigen. This can result in an excessive proinflammatory response and lead to the symptoms of CD.

The mechanism in which NOD2 activates hBD-2, which involves NF- κ B and AP-1 sites, still needed to be investigated. To demonstrate the importance of binding sites for transcription factors NF- κ B and AP-1 in the hBD-2 promoter region, hBD-2 promoter luciferase expression constructs containing mutations of the two NF- κ B sites as well as a mutated AP-1 site were used (Voss et al., 2006).

The NOD2 overexpressing HEK293 cells were injected with this construct, treated with MDP, and analyzed for hBD-2 promoter activity. The mutated sites caused a complete elimination of hBD-2 promoter activity. When only the two NF- κ B sites were mutated, NOD2 induced hBD-2 promoter activity decreased almost completely, while with only the AP-1 site mutation, hBD-2 promoter activity was decreased from 7- to 5-fold (Voss et al., 2006). The role of NF- κ B in the NOD2 mediated hBD-2 induction was confirmed by these findings, since they showed that mutating NF- κ B sites resulted in much more severe effects. These findings not only confirm the role that NOD2 plays in activating hBD-2, but also show the importance of the mediators involved in this pathway. Other studies have shown that CD patients have increased levels of NF- κ B, but the actual form (mutated or not) of the transcription factor needs to be researched.

Interleukin Function in IBD

IBD has been characterized as a multi-gene disease. One gene associated with IBD disease that is of particular interest is Runx3. Runx3 is a transcription factor known to be linked to human autoimmunity, specifically involving in the regulation of TGF- β (transforming growth factor) and the development of helper T cells (Brenner, et al., 2004). Successful deletion of Runx3 in mice induced colitis. A general increase in T lymphocytes and macrophages was also seen, indicating a heightened immune response (Brenner et al., 2004). Because Runx3 knockout mice developed colitis, a knockout of Runx3 is, therefore, linked to IBD.

Interestingly, Runx3 deletion was also associated with an increase in the expression of different interleukins. Interleukins are a group of cytokines synthesized by CD4+ T lymphocytes, monocytes, macrophages, and endothelial cells. Interleukins stimulate the immune response by promoting the differentiation and development of T cells, B cells, and hematopoietic cells (Brenner et al., 2004). Runx3 knockout mice exhibited an increase in several interleukins, including IL-4, IL-5, and IL-12. An increase in the transcription factors T-bet and GATA-3 was also seen (Brenner et al., 2004). T-bet and GATA-3 both indirectly stimulate the development and differentiation of T cells through interleukins. An increase in the levels of several different interleukins further confirms a relationship between interleukin levels and IBD.

IL23R As An IBD Gene

Previous findings have shown that Runx3 deletion induced colitis and increased the levels of interleukin, which led to the investigation of specific genes controlling interleukin expression (Cheung et al., 2006). IL23R is a gene located on chromosome 1p31 that encodes a subunit of the proinflammatory cytokine interleukin-23 (Duerr et al., 2006). IL-23R has a specific role in promoting activation of T cells during an inflammatory response. Past studies of IL23R have demonstrated that the gene plays a significant role in inducing colitis. Moreover, many different IL23R variants are associated with the inflammation observed in patients with IBD (Murphy et al., 2003).

A genome-wide association study of IL23R presented several SNPs, which caused both increased protection and increased susceptibility to Crohn's disease (Duerr et al., 2006). The coding variant Arg381Gln was uncommon, with an allele frequency of 1.9% in Jewish individuals and 7.0% in non-Jewish individuals (Duerr et al., 2006). The variant was shown to protect against the development of CD. In contrast, several different SNPs, including rs7517847, were found to be associated with increased susceptibility to IBD (Duerr et al., 2006). The

discovery of these twofold genetic variants, that both contribute to the pathogenesis of IBD and serve as protection against the disease, allowing for a continuum of susceptibility, neutrality, and defensive effects of different genes (Duerr et al., 2006).

Further Investigation of Interleukin Genes: IL-11R

Interleukin 11 is a multifunctional cytokine that has been shown to exhibit protective effects on intestinal mucosa by significantly reducing the severity of colitis in animal models. IL-11 decreases the expression of many proinflammatory molecules, including IL-12 and IL-1 β . However, IL-11 expression is limited to colonic epithelial cells (Kiessling et al., 2003). IL-11 also triggers the activation of the Jak-STAT pathway, which regulates cell proliferation, differentiation, and apoptosis (Kiessling et al., 2003). Cells stimulated with IL-11 were found to contain higher levels of phosphorylated Jak (Kiessling et al., 2003). Stimulation of cells with IL-11 also resulted in decreased levels of caspase-9, increased Akt anti-inflammatory effects, and overall increased cell survival (Kiessling et al., 2003). Due to these properties, IL-11 therapy may be implemented as a possible treatment for IBD. Due to the cell proliferative effects of IL-11 in colonic epithelial cells, treatment using IL-11 should focus on the restorative effects of IL-11 phosphorylation, which results in diminished apoptosis (Kiessling et al., 2003).

Role of Autophagy in CD

Autophagy Background

Autophagy is an evolutionarily conserved pathway that comes in several forms. This review will particularly focus on macroautophagy, which will be referred to as autophagy (Kubella et al., 2008). Autophagy plays an important role in cell and tissue homeostasis and has been linked to an assortment of human diseases (Cadwell et al., 2008). The process of autophagy involves encapsulating cellular contents into a double membrane vesicle, or an autophagosome, and delivering these contents to the lysosome, where they are degraded and recycled (Kubella et al., 2008). Autophagy is a protective mechanism that is upregulated at times of cellular stress, starvation, or growth factor withdrawal (Kubella et al., 2008). In particular, a process in autophagy called xenophagy is thought to protect the cell by eliminating or limiting the growth of bacterial pathogens (Kubella et al., 2008). Thus, autophagy is suggested to be involved in the regulation of the inflammatory response.

Role of Atg16l1 in Mouse and Human Intestinal Paneth Cells

The genome-wide association study identified a coding polymorphism in an autophagy gene, ATG16L1, as a risk factor for the development of CD (Cadwell et al., 2008). Atg16L1 is required for formation of a high molecular protein complex with the Atg12-Atg5 conjugate, which is required for autophagosomal formation (Saitoh et al., 2008). Moreover, Atg 5/Atg 16L1 regulates the rate of autophagy (Kubella et al., 2008). In addition, Atg16L1 defines where LC3 is conjugated to phosphatidylethanolamine (PE), which is crucial for autophagy, because PE recruits the Atg3-LC3 intermediate to a source membrane of an autophagosome (Saitoh et al., 2008). Autophagosomes cannot function properly without these appropriate steps. A study performed by Cadwell et al. (2008) showed that Atg16L1 deficient mice displayed abnormal Paneth cells. Paneth cells secrete granules of antimicrobial peptides and lysozyme (Yano et al., 2009). These cells play a major role in the control of intestinal microbiota (Cadwell et al., 2008). The abnormalities in Paneth cells included disorganized granules and a decrease in granule number (Cadwell et al., 2008). In

addition, there was a significant decrease in lysozyme. These results show that Atg16L1 is required for retaining the integrity of Paneth cell granule exocytosis pathways in mice.

Similar abnormal phenotypes were seen in CD patients homozygous for the disease risk allele of ATG16L1 (Cadwell et al., 2008). Furthermore, these Atg16L1 deficient mice displayed an increase in the expression of genes involved in regulating injury responses, including certain genes in the PPAR pathway (Cadwell et al., 2008). Interestingly, in patients with CD, there is a significant increase in transcription of genes involved in injury response maintenance, specifically adipocytokines leptin and adiponectin (Cadwell et al., 2008).

These findings show an agreement between the abnormalities in Paneth cells from Atg16L1 deficient mice and the Paneth cells observed in CD patients with the risk allele ATG16L1.

Loss of Atg16L1 Enhances IL-1 β and IL-18 Production

Atg16L1 plays a major role in the control of the endotoxin-induced inflammatory response. Mice that were deficient for Atg16L1, when exposed to LPS, had a significant increase in IL-1 β and IL-18 (Saitoh et al., 2008). In previous studies, it has been shown that there is an irregular expression of inflammatory cytokines, such as IL-1 β and IL-18, which were shown to be involved in the development of colitis (Saitoh et al., 2008). Mice that were deficient for Atg16L1, when treated with DSS, showed a significant decrease in survival and loss of body weight compared to WT mice (Saitoh et al., 2008). Histological analyses performed on the colons of these mice revealed an increase in inflammation in Atg16L1 deficient mice (Saitoh et al., 2008). In addition, there was a large area of ulceration and increased infiltration of lymphocytes seen in these mice (Saitoh et al., 2008). Also, the levels of IL-1 β and IL-18 were elevated in the Atg16L1 deficient mice compared to WT mice (Saitoh et al., 2008).

The symptoms seen in DSS induced colitis are very similar to the symptoms of IBD patients. These results show that Atg16L1 plays an important role in regulating the immune system response by controlling the production of IL-1 β and IL-18. These increased levels of specific inflammatory cytokines are associated with IBD.

ATG16L1 Variant and Its Association With CD

In humans, a coding polymorphism in the autophagy gene ATG16L1 variant was recently identified as a potential risk factor for the development of CD (Kubella et al., 2008). ATG16L1 encoding for threonine at amino acid 300 (ATG16L1*T) provides protection to the cells; however, ATG16L1 carrying alanine instead of threonine (ATG16L1*300A) causes increased susceptibility to CD (Kubella et al., 2008). Human gut epithelial cells Caco2 that have ATG16L1*300A when exposed to pathogens like *Salmonella* fail to rescue cells knocked out for ATG16L1 (Kubella et al., 2008). However, the epithelial cells encoding for ATG16L1*300T resulted in vigorous anti-*Salmonella* autophagy (Kubella et al., 2008).

When representative confocal optical sections of infected ATG16L1-deficient and ATG16L1 allele-specific reconstituted Caco2 cells were observed, both of the variants were able to capture the pathogen within the autophagosome, yet the ATG16L1*300 captured the pathogen at a slower rate, showing that the variant has a reduced ability to regulate anti-bacterial autophagy (Kubella et al., 2008). These findings suggest that CD patients with the ATG16L1*300A allele, in comparison to patients with the ATG16L1*300T allele, will have an altered management of intracellular pathogens and internalized antigens (Kubella et al., 2008). Even though the results are significant for *Salmonellae*, *Salmonella* is not a causative agent of CD.

Kubella et al. (2008) believe that cellular autophagy mechanisms for microbe recognition and degradation are the same for different intracellular pathogens. Hence, they hypothesized that in patients with the ATG16L1*300A allele, the homeostasis function of autophagy would work properly (Kubella et al., 2008). Nevertheless, these patients may have altered responses to bacterial components or infection in the gut because the gut microflora has increased exposure to antigens (Kubella et al., 2008). Overall, these results do not suggest that the effect of the coding variation in ATG16L1 in humans would have a drastic effect.

However, it would be interesting to observe the response of mice with the ATG16L1*300A allele to flagellin and TNF- α , IBD proinflammatory stimulants, since these mice would have an anti-bacterial autophagy that is mediated slower compared to mice carrying the ATG16L1*300T allele.

Treatment

Polysaccharide A

Current research focuses on decreasing the proinflammatory response seen in both CD and UC patients by implementing treatments that lower levels of specific inflammatory agents. IL-17 is a cytokine involved in producing a pro-inflammatory response by overexpressing TNF- α and IL-6. *B. fragilis*, a commensal bacterium found in the gastrointestinal tract of animals, has been shown to suppress overproduction of IL-17 with the addition of polysaccharide A (PSA) (Mazmanian et al., 2008). To study the use of PSA as a potential treatment for IBD, PSA was administered orally to DSS treated mice. These mice experienced a decrease in intestinal inflammation through the reduction of TNF- α and IL-6 (Mazmanian et al., 2008). Also, the mice did not experience any significant weight loss, which is one of the common symptoms seen in IBD patients. Interestingly, it was found that IL-10 is necessary for PSA function. DSS treated mice without IL-10 experienced an inflammatory response even with PSA treatment (Mazmanian et al., 2008). In the future, this treatment might have similar effects in lowering inflammation in the human gastrointestinal tract. Since *B. fragilis* is already present in the human tract and PSA may be administered orally, this type of therapy can be easily implemented in IBD patients (Mazmanian et al., 2008). Furthermore, the role of IL-10 to specifically treat CD needs to be further investigated.

LRH-1

As aforementioned, proinflammatory chemicals, such as IL-1- β , IL-6, and TNF- α , are involved in inducing inflammation in intestinal epithelial cells during an inflammatory response. LRH-1, a liver receptor homolog, is able to decrease the levels of these cytokines by inducing the synthesis of glucocorticoids (Coste et al., 2007). Glucocorticoids serve to lower intestinal inflammation by both upregulating the expression of anti-inflammatory proteins and repressing proinflammatory proteins (Coste et al., 2007). Since IBD patients have been shown to exhibit a reduced expression of LRH-1, a mouse model was used to illuminate the relationship between LRH-1 and IBD. LRH-1 deficient mice exhibited reduced expression of glucocorticoids in epithelial cells. These results show that LRH-1 deficiency in the intestines predisposes mice to inflammation due to insufficient glucocorticoid production. Colon biopsies of patients with IBD, consistent with these findings, show decreased expression of LRH-1, along with other genes involved in glucocorticoid production (Coste et al., 2007). Furthermore, healthy sections of IBD sufferers' colons showed increased levels of two enzymes: CYP11A1 and CYP11B1, which collectively produce glucocorticoids (Coste

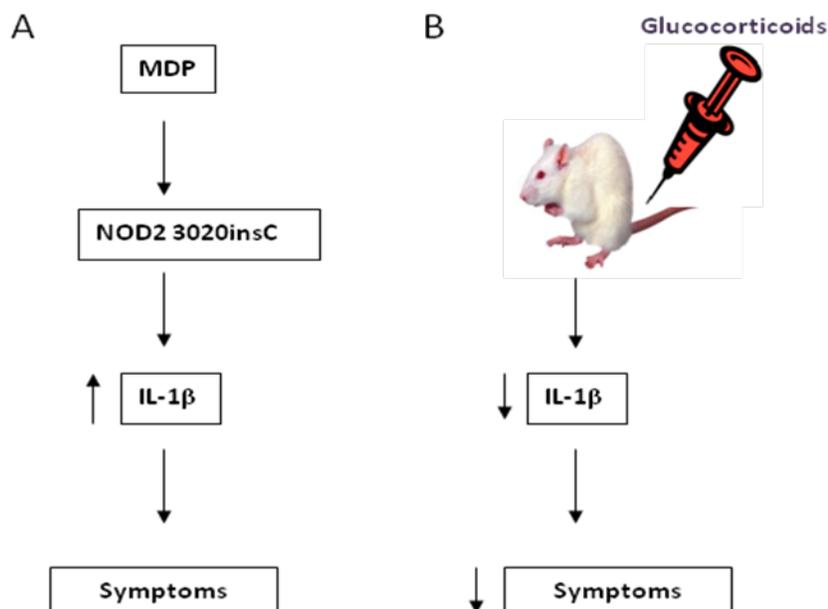


Figure 2: Utilizing possible causes of IBD as potential treatment options (A) Stimulation of the mutant NOD2 gene by MDP causes overproduction of IL-1 β , which is associated with CD. (B) Administration of glucocorticoids into mice causes a decrease in the production of IL-1 β and therefore serves as a viable treatment for CD.

et al., 2007). These findings suggest that the LRH-1 pathway is involved in decreasing inflammation. The oral administration of glucocorticoids could be tested as a potential treatment for IBD.

Anti-Interleukin-12 Antibodies

CD is associated with overactive cytokine activity. IL-12 (anti-interleukin-12) is a key cytokine that drives the inflammatory response by initiating helper T-cells. It is involved in regulating both the normal and abnormal inflammatory responses that are accompanied by autoimmune diseases, including CD. Since CD is characterized by an increased production of IL-12, the efficacy of implementing a human monoclonal antibody against IL-12 was studied as a possible treatment. CD patients were given seven weekly injections of either one or three milligrams of anti-interleukin-12 per kilogram of weight or placebo (Mannon et al., 2004). The results showed a decrease in IL-12, TNF- α , and interferon- γ secretion (Mannon et al., 2004). Most of the patients receiving anti-interleukin-12 experienced significant clinical improvements. The treatment is, therefore, associated with decreased inflammatory cytokines at the site of the disease. Nevertheless, adverse effects, including diarrhea, dehydration, and abdominal pain, were seen in more than ten percent of the patients a short time after the study (Mannon et al., 2004). Thus, although all of the aforementioned treatments work to decrease the symptoms of IBD, a cure for the disease is yet to be discovered.

Future Advances in CD Research

CD has been shown to result from inappropriate regulation of homeostasis in intestinal mucosa in response to antigens. CD patients exhibit a malfunctioning gastrointestinal system, including excessive inflammation and painful symptoms. However, the cause of the malfunction remains poorly understood. Also, the exact role of interleukins and

autophagy in the inflammatory response pathway was vaguely outlined. Based on the aforementioned findings, mutations in specific genes, including NOD2 and Runx3, suggest a potential cause for CD, due to the upregulation of certain inflammatory cytokines (Ogura et al., 2001). Specific interleukins, such as IL-11, decrease symptoms of CD and increase cell survival. However, other interleukins, such as IL-23, increase the likelihood of developing CD (Abraham et al., 2008). Overall, it has been shown that interleukins play a complex role in the inflammatory response in CD.

Furthermore, ATG16L1 has been identified as a CD susceptibility gene and has been shown to play a role in the innate immune system response. The presence of this variant allele has been shown to cause defective autophagy, which results in decreased bactericidal activity and keeps the immune system from shutting down (Okazaki et al., 2008). Although numerous genes have been identified as susceptibility factors for CD, the interaction and collective effect of these genes is unknown. Specifically, in the future, the interaction between NOD2 and ATG16L1 needs to be further investigated (Okazaki et al., 2008). The synergistic relationship between these genes may prove to be a significant contributor to the pathology of CD.

Since a mutation in NOD2 has been shown to overexpress NF- κ B and stimulate the immune system response, decreasing levels of NF- κ B may reduce the inflammatory response. This could serve as a potential treatment for CD patients. Also, since mutations in NOD2 significantly increase IL-1 β secretion, injecting an anti-interleukin-1 β antibody into CD patients may reduce symptoms (Figure 2B). In addition, it has been shown that injections of antibodies of IL-1 β and IL-18 into DSS treated mice lower the severity of symptoms (Saitoh et al., 2008). Such injections can be tested on humans and incorporated into future treatments for CD. Furthermore, identifying genetic loci that contribute to CD susceptibility can lead to the discovery of new genes that play a significant role in the development of CD (Parkes et al., 2007). Therefore,

treatment of CD would need to take into account the many different factors associated with the onset of this complex disease.

Conclusion

In Crohn's disease, the immune response fails to be deactivated and contributes to the development of painful inflammation of the digestive tract. Many genetic factors, such as NOD2, Runx3, and ATG16L1, have been shown to increase susceptibility to CD. However, the specific interaction between these genes is unknown. The discovery of these genes and the role of inflammatory cytokines have led to the development of a prodigious number of treatments. Of these treatments, the targeting and suppression of inflammatory ILs leads to remarkable outcomes in patients suffering from CD. Patients who had previously been non-responsive to conventional treatments of steroids and glucocorticoids experienced profound results, where elimination of one IL resulted in a suppression of inflammatory cytokine cascades in the digestive tract, resulting in lowered inflammation of epithelial cells. These discoveries bring scientists increasingly closer to finding a cure for CD.

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