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Review

Crohn's disease: A review of treatment options and current research

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ABSTRACT

Crohn's disease is an autoimmune disorder that affects nearly 1.4 million Americans. The etiology of Crohn's disease is not completely understood, however, research has suggested a genetic link. There is currently no known cure for Crohn's disease and, as a result, most government-funded research is being conducted to increase the quality of life of afflicted patients (i.e. reducing chronic inflammation and alleviating growth impairment in pediatric patients). A number of treatment options are available including an alpha-4 integrin inhibitor and several TNF-alpha inhibitors. Furthermore, research is being conducted on several alternative treatment options to help understand exactly which cellular mechanisms (i.e. inducing apoptosis in leukocytes) are required for clinical efficacy. This review seeks to chronicle the current available treatment options for patients affected by Crohn's disease to aid in understanding potential cellular mechanistic requirements for an efficacious drug, and shed light on potential options for future treatment.

1. Introduction

Crohn's disease, an inflammatory bowel disease (IBD), is an autoimmune disorder that may affect any part of the gastrointestinal (GI) tract from mouth to anus. The exact etiology of Crohn's disease is unknown, but both heredity and exposure to bacteria have been implicated. Crohn's disease affects 1.4 million Americans, out of which 140 000 are under the age of 18. Approximately 25% of all new cases in the population are under 20 years of age, and roughly 30 000 new patients are diagnosed annually [1]. Crohn's disease has a bimodal distribution for incidence as a function of age; the disease typically strikes individuals either in their early teens or after age 50. Crohn's disease occurs in all ethnic groups and races, but its incidence is highest in Caucasians and people of Jewish descent, particularly Ashkenazi Jews. There is an equal incidence of Crohn's disease in males and females, and no research has conclusively shown that Crohn's disease is genetically inherited [2].

Crohn's disease primarily involves the ileocolic region of the intestines. A small percentage of cases have reported Crohn's disease affecting only the small intestine or colon as well [3]. On a microscopic scale, histological specimens resected from portions of patients' intestines afflicted by Crohn's disease show masses of inflamed tissue referred to as granulomas and transmural inflammation, which can be present anywhere from the mucosa to serosa of the intestines. The granulomas are considered a hallmark of Crohn's disease. However, only 21–60% of Crohn's disease patients present with granulomas [4].

Crohn's disease can be subdivided into three categories including inflammatory, obstructive, and fistulating types. The inflammatory and obstructive types commonly present together due to inflammation-induced thickening of the intestinal mucosal wall causing obstructions in bowel [5]. The most common fistulas present in Crohn's disease patients are perianal fistulas and enteroenteric fistulas, in which portions of the bowel erode into neighboring bowel due to the transmural nature of the disease [6]. Furthermore, a small percentage of patients present with extraintestinal manifestations due to inflammation of fat cells under the skin or inflammation in large joints causing erythema nodosum and peripheral arthritis, respectively [7].

There is currently no known cure for Crohn's disease and current research focuses on controlling symptoms. The symptoms of Crohn's disease include chronic flare-ups due to inflammation, abdominal pain, weight loss, reduced appetite, perianal discomfort [2], growth impairment, and delayed sexual maturation [8]. The symptoms of Crohn's disease can be partially attributed to an abnormal activation of T-cells due to an exaggerated response to bacterial antigens in the gut lumen.

Typically, the number of mature T-cell lymphocytes in the body is stringently controlled by apoptosis, which can occur at anytime

Abbreviations: TNF, tumor necrosis factor; IL, interleukin; DC, dendritic cell.

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during a T-cell's lifespan. Apoptosis plays a vital role following antigen-specific lymphocyte activation; after antigen clearance, only a minority of generated T-cells survive to become memory-type T-cells while the rest undergo apoptosis. Failure of T-cells to undergo apoptosis via lymphocyte control can lead to the development of mucosal lesions as seen in Crohn's disease. In addition to the creation of these lesions, patients afflicted by Crohn's disease can create auto-antigens in the exocrine pancreas that are under attack by the immune system. Auto-antibodies against exocrine pancreatic tissue (PAbs) are highly specific to Crohn's disease. GP2, a major zymogen membrane glycoprotein, is the major auto-antigen of PAbs and is synthesized in Peyer's patches [9]. Consequently, there is an overactive immune response that leads to chronic inflammation. Furthermore, studies have shown that mucosal T-cells escape normal apoptotic regulatory mechanisms [10]. The lifespan of these T-cells is extended and the abnormal population remains in the mucosal compartment. One study has shown that these T-cells are resistant to apoptotic stimuli and have reduced levels of Bax, a pro-apoptotic protein, and increased levels of IL-6, a cytokine that can act as either pro-inflammatory or anti-inflammatory [11]. Another study examining the influence of enteric bacteria on leukocyte apoptosis showed that, with increased levels, IL-6 acts as a pro-inflammatory molecule and stimulates intestinal T-cells to express anti-apoptotic genes, including Bcl-2 [10].

With an extended lifespan T-cells, along with dendritic cells (DCs) and T helper-1 (Th-1) cells, continue to secrete cytokines and initiate an inflammatory response. In this pro-inflammatory cascade, when T-cells secrete increased amounts of IL-6 and IL-17, DCs secrete IL-23. IL-23 acts to induce T-cells to secrete more IL-6 and IL-17, enhancing the inflammatory response. DCs also secrete another pro-inflammatory cytokine, IL-12. In the pathogenesis of Crohn's disease, IL-12 functions to convert T-cells into Th-1 cells, which secrete interferon-gamma (INF-gamma) and tumor necrosis factor-alpha (TNF-alpha). Increased levels of INF-gamma and TNF-alpha represent another major feature in the induction and perpetuation of chronic inflammation in Crohn's disease [12,13].

The most common treatments for Crohn's disease use a synthetic antibody against TNF-alpha or an alpha-4 integrin subunit rather than targeting specific interleukins in the pro-inflammatory cascade. In Crohn's disease, elevated TNF-alpha contributes to chronic flare-ups [14]. Infliximab (REMICADE®), adalimumab (HUMIRA®), and certolizumab pegol (Cimzia®) are examples of TNF-alpha inhibiting treatments. In 2008, the food and drug administration (FDA) approved a new anti-TNF-alpha drug, certolizumab pegol (Cimzia®), for patients with moderate-to-severe Crohn's disease who did not respond adequately to other TNF-alpha inhibitors [6]. During that same year, the FDA approved another drug for the treatment of Crohn's disease, natalizumab (Tysabri®), an alpha-4 integrin inhibitor [15]. Alpha-4 integrin, a molecule expressed on leukocytes, is responsible for cell migration to sites of inflammation. Therefore, drugs targeting alpha-4 integrin can limit the severity of inflammation in the intestines by hindering cell migration [16]. While these drugs are effective in controlling the symptoms of Crohn's disease, they have not been shown to alleviate growth impairment in pediatric patients as a complication of severe inflammation [17,18].

Other potential treatments for Crohn's disease currently under investigation include targeting T-cells and cytokines involved in the pro-inflammatory cascade and administering anti-inflammatory cytokines, conjugated linoleic acid (CLA), rifaximin, and human growth hormone (HGH). Various drugs undergoing clinical trials for the treatment of Crohn's disease focus on using antibodies to block specific cytokines in the pro-inflammatory cascade including IL-12, IL-17, and IL-23. These drugs include ustekinumab (Stelara®), AMG 139, and brodalumab (AMG 827®) [19–21]. Furthermore, studies have warranted exploring the effects of using antibodies against IL-6 and IL-12 in a larger population [22,23]. Blocking the activation and inducing apoptosis in T-cells has also been under investigation. Current research on manipulating T-cells for the treatment of Crohn's disease is exploring the effects of inhibiting the co-stimulatory signal required for T-cell activation (CD28 and CD80 or CD86) [24] and utilizing an antibody against CD3 in the T-cell receptor complex of activated T-cells [25].

Due to the imbalance of pro-inflammatory and anti-inflammatory cytokines in Crohn's disease, administering anti-inflammatory cytokines such as IL-10 and IL-11 has also been of interest to clinicians. Research has shown that there is insufficient production of IL-10, an anti-inflammatory cytokine, in patients with Crohn's disease. IL-10, secreted by monocytes and certain T-cells, functions to decrease the secretion of IFN-gamma by Th-1 cells and decrease the production of IL-6 and TNF-alpha in monocytes [26–29]. IL-11, a pleiotropic anti-inflammatory cytokine, has also been of interest to researchers due to its ability to stimulate crypt cell proliferation and down-regulate IFN-gamma and TNF-alpha [30,31].

CLA, a collective term used to describe the different isomers of linoleic acid, is a family of essential fatty acids that activate peroxisome proliferator-activated receptor-gamma (PPAR-gamma) in the nucleus of a cell. Upon stimulation from CLA, PPAR-gamma stimulates transcription of anti-inflammatory genes, which has been shown to help alleviate the symptoms of Crohn's disease [32]. Additionally, the role of enteric bacteria in the pathogenesis of Crohn's disease suggests that interfering with transcription of bacterial DNA via a nonsystemic drug like rifaximin could induce remission in patients [33,34]. Rifaximin acts by binding to the beta subunit of bacterial DNA-dependent RNA polymerase, an enzyme responsible for the synthesis of RNA using DNA genes as a template [35]. Research has also illustrated that human growth hormone (HGH) can block IL-6 signal transduction. IL-6, a cytokine overly expressed in Crohn's disease, has been shown to exacerbate inflammation. Thus, by blocking IL-6, HGH can reduce the severity of inflammation in Crohn's patients [36].

Current research focusing on Crohn's disease investigates whether neutralizing TNF-alpha, inducing apoptosis in leukocytes, or mediating antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) are requirements for helping patients achieve remission. One study reports that binding to monomeric TNF-alpha and obstructing the synthesis of pro-inflammatory cytokines is a potential requirement for efficacy in treating Crohn's disease. It also asserts that the induction of apoptosis and mediation of CDC and ADCC may not be a requirement [37]. Another report claims that inducing apoptosis may be necessary in treating Crohn's disease [11]. This review aims to illustrate the cellular mechanisms by which selected drugs alleviate the symptoms of Crohn's disease to aid in understanding the potential requirements for an effective drug, and to shed light on treatment options under current investigation.

2. Current treatment options for Crohn's disease

Studies have shown that patients with Crohn's disease have increased levels of TNF-alpha, a cell signaling molecule responsible for chronic flare-ups [14]. By using TNF-alpha blockers such as infliximab (REMICADE®), certolizumab pegol (Cimzia®), and adalimumab (HUMIRA®), the amount of TNF-alpha able to bind to its receptors is reduced. Blocking formation of this ligand–receptor complex inhibits intracellular signaling cascades that produce pro-inflammatory proteins. Research has also shown that cell adhesion molecules (CAMs) such as integrins can be responsible for chronic flare-ups in patients with Crohn's disease. By blocking the ability of these CAMs to communicate with other cells, the

amount of leukocyte migration decreases, which in turn can decrease the frequency and severity of inflammation [16].

2.1. The role of TNF-alpha and TNF-alpha blockers

In the human body TNF-alpha, an inflammatory cytokine secreted by leukocytes, has pleiotropic properties. Many organs are affected by TNF-alpha; however, all of the functions of TNF-alpha are not well understood. It has been shown that TNF-alpha's function is dependent on its environment. For example, at sites of inflammation TNF-alpha stimulates neutrophil proliferation, but when TNF-alpha binds to the TNF-R55 receptor it induces neutrophil apoptosis. In Crohn's disease, TNF-alpha is present in high quantities, and is believed to cause excess inflammation [38].

Infliximab (REMICADE®), one commonly used drug to treat Crohn's disease, is a genetically constructed IgG1 murine-human monoclonal antibody with a murine variable region and human immunoglobin constant region [39]. It binds to the precursor of TNF-alpha and inhibits many biological activities of TNF-alpha [40]. Infliximab also promotes an anti-inflammatory atmosphere by manipulating the TNF-alpha cell signaling system. When TNF-alpha binds to TNF-alpha receptor type 1 (TNFR1), the ligand–receptor complex is internalized and induces an apoptotic signaling cascade. When TNF-alpha binds to TNF-alpha receptor type 2 (TNFR2), the extracellular domain of the receptor is cleaved and released into circulation as a soluble product. Naturally, soluble TNFR2 inhibits TNF-alpha [41].

One *in vitro* study on infliximab's mechanism of action showed that it increases TNFR2 release by monocytes and reduces surface TNFR2 expression on monocytes. By increasing the amount of soluble TNFR2, the cells contribute to the neutralization of TNF-alpha. Furthermore, the decline of surface TNFR2 expression limits the TNF-alpha responsiveness of these cells. Infliximab also selectively increased the synthesis of IL-10, an anti-inflammatory cytokine. In this study, infliximab did not compensate for the decline in TNF-alpha by increasing TNF-alpha production, which would reverse the anti-TNF-alpha effects [39].

Adalimumab (HUMIRA®), another TNF-blocking drug, is a fully human, monoclonal IgG1 antibody that binds to TNF-alpha, neutralizes it, and induces apoptosis in TNF-expressing mononuclear cells. This drug neutralizes the activity of TNF-alpha by inhibiting its interaction with p55 and p75 cell surface TNF-alpha receptors [42]. By neutralizing TNF-alpha, adalimumab inhibits a number of TNF-alpha events including: the release of IL-6, acute phase reactants of inflammation, and molecules enabling leukocyte migration. Furthermore, via the activation of intracellular caspases, TNF-expressing mononuclear cells undergo apoptosis [43].

The most recent U.S. food and drug administration (FDA) approved drug for the treatment of Crohn's disease is certolizumab pegol (Cimzia®). In 2008 the FDA approved certolizumab pegol for Crohn's disease patients who did not respond sufficiently to other TNF-alpha therapies [44]. Certolizumab pegol is a monoclonal humanized antibody without a constant region (Fc) that contains a PEGylated Fab'fragment that binds to TNF-alpha. The PEGylated Fab'fragment does not interfere with the antibodies' ability to neutralize TNF-alpha due to a free-cysteine residue in the hinge region. Since the certolizumab is PEGylated, its half-life is similar to that of a complete antibody [37].

In contrast to other TNF-alpha blockers, one study has reported that certolizumab pegol does not induce apoptosis *in vitro.* Unlike other TNF-alpha blockers, the proportion of activated T-cells and monocytes binding to annexin V did not increase following incubation with certolizumab pegol [37]. This suggests that on the extracellular side of their cell membrane, these cells did not express increased levels of phosphatidylserine, a phospholipid that flips to the extracellular domain when a cell undergoes apoptosis. However, there is evidence that lamina propria cells are more suitable to infliximab-induced apoptosis [45]. Furthermore, it is important to recognize that certolizumab pegol's ability to induce apoptosis *in vitro* is still an on-going research topic, and that the cell-killing mechanism of certolizumab pegol *in vivo* is still unclear.

Comparatively, certolizumab pegol's structure differs from both a whole antibody and other TNF-alpha blockers such as infliximab. Infliximab and adalimumab are based on the human IgG1 fragment crystalline (Fc) region, which has the potential to interact with cell surface receptors (Fc receptors) and proteins of the complement system. Since certolizumab pegol does not contain an Fc region, it does not mediate complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity like infliximab and adalimumab. On the other hand, certolizumab pegol can effectively bind TNF-alpha, similar to infliximab and adalimumab, since it contains analogous Fab regions. Furthermore, certolizumab pegol can be an attractive drug for pregnant women with Crohn's disease because polyethylene glycol does not traverse the placenta, unlike other un-PEGylated TNF-alpha blockers [46].

2.2. The role of alpha-4 integrin and an alpha-4 integrin blocker

Alpha-4 integrin, a member of the cell-surface adhesion family, mediates adhesion to the extra-cellular matrix (ECM) and other cells. Alpha-4 integrins are primarily expressed on eosinophils, basophils, monocytes, and lymphocytes. Once activated by a ligand, alpha-4 integrin heterodimerizes with beta-1 and beta-7 subunits. One of the ligands for the alpha-4 integrin family is vascular cell adhesion molecule-1 (VCAM-1). When endothelial cells express VCAM-1 upon cytokine activation, alpha-4 integrin expressing cells such as leukocytes can firmly adhere to endothelial cells. Studies have shown that when alpha-4 integrin binds to VCAM-1, various cellular processes are activated to promote activation, proliferation, and migration of alpha-4 integrin expressing cells. Furthermore, alpha-4 integrin can bind to ECM proteins such as fibronectin [47]. Studies have shown that activation of the fibronectin binding site of alpha-4 beta-1 integrin is important for lymphocyte migration through the ECM towards sites of inflammation [48,49].

When a patient with Crohn's disease has a flare-up, cytokines expressed at sites of inflammation activate endothelial cells lining postcapillary venules and numerous cell adhesion molecules (CAMs) [33]. Natalizumab (Tysabri®) is a humanized monoclonal antibody that binds to the alpha-4 integrin subunit [47]. By binding to the alpha-4 integrin subunit, natalizumab inhibits the migration of leukocytes to areas of inflammation and reduces the frequency of flare-ups in Crohn's disease [16].

2.3. Alpha-4 integrin blocker vs TNF-alpha blockers

The mechanism of action of natalizumab differs from other drugs used to treat Crohn's disease in that it does not rely on inhibiting the production of pro-inflammatory proteins via binding to TNF-alpha upstream. Instead, natalizumab inhibits the ability of leukocytes to migrate to inflamed areas. Neutrophils, one class of leukocytes, do not express the alpha-4 integrin subunit [16]. As a result, neutrophils can continue to combat infections via phagocytosis, release of soluble anti-microbials, and producing neutrophil extracellular traps (NETs) [50]. Because the alpha-4 integrin subunit is not expressed on neutrophils, blocking the alpha-4 integrin is unlikely to suppress one's immune system, making natalizumab an attractive drug.

3. Contraindications

Infliximab is contraindicated for patients with congestive heart failure, those with a known sensitivity to murine proteins [51], and

those with untreated recurrent infections such as tuberculosis and hepatitis B or C [52]. Since infliximab is a chimeric antibody, derived from both mouse and human proteins, individuals with a known sensitivity to mouse-derived proteins should avoid using infliximab. Furthermore, infliximab lowers one's ability to fight infections and should not be taken if an individual has any untreated chronic infections.

According to the prescribing information for certolizumab pegol, there are no notable contraindications [53]. Adalimumab is contraindicated for patients with a known hypersensitivity to the drug. In the European Union, the combination of adalimumab and anakinra, an IL-1 receptor blocker, is prohibited. Furthermore, in the European Union, taking adalimumab while pregnant is contraindicated. In the USA, the food and drug administration (FDA) has assigned adalimumab to pregnancy category B. Pregnancy category B drugs have failed to demonstrate a risk to the fetus in animal studies but have not been tested in a well-controlled environment in pregnant women [54].

Natalizumab is contraindicated for patients with hypersensitivity to the drug and a history of progressive multifocal leukoencephalopathy (PML) [55]. Between July 2006 and November 2009, 28 cases of natalizumab-associated PML were documented [56]. As a result, patients with a history of PML are advised against taking natalizumab because their risk for PML increases.

4. Potential treatment options for Crohn's disease

Current research regarding the treatment of Crohn's disease investigates the efficacy of antibodies targeting interleukins involved in the pro-inflammatory cascade [19–23], anti-T-cell therapy [24,25,57–63], anti-inflammatory therapy [26–31], human growth hormone [64], conjugated linoleic acid [65], and rifaximin [66]. Administering anti-inflammatory cytokines, antibodies targeting the pro-inflammatory cascade, T-cells, human growth hormone, conjugated linoleic acid, and rifaximin differ from current treatment options because they focus on different cellular mechanisms, as seen in Table 1. Unlike current treatment options, human growth hormone inhibits Interleukin-6 from activating the synthesis of pro-inflammatory proteins [67]. In an open label pilot study, conjugated linoleic acid significantly suppressed $CD4^+$ and $CD8^+$ T-cells to produce TNF-alpha [65], illustrating its anti-inflammatory role in the body. In another study, 65% of patients refusing to take immunosuppressants or biologic therapies who were administered rifaximin achieved clinical remission [34].

4.1. Drugs targeting pro-inflammatory cascade

Cytokines involved in the pro-inflammatory cascade include IL-6, IL-12, IL-17, IL-23, TNF-alpha, and IFN-gamma. The secretion of IL-17 and IL-6 from T-cells is indirectly influenced by IL-23 through induction. Therefore, blocking IL-23 will limit the secretion of not only IL-23, but also IL-17 and IL-6. Interestingly, IL-23 shares a common subunit, p40, with IL-12. Ustekinumab (Stelera®) takes advantage of this and inhibits IL-12 and IL-23 from binding to their receptors by blocking the p40 subunit on both interleukins. By blocking IL-12, ustekinumab inhibits the conversion of naïve T-cells to Th-1 cells stopping the production of TNF-alpha and INF-gamma. In addition, Ustekinumab indirectly blocks the production of IL-6 and IL-17 in T-cells by preventing IL-23 from reaching its receptor. Therefore, ustekinumab blocks the effects of IL-6, IL-12, IL-17, IL-23, TNF-alpha, and INF-gamma [67]. Ustekinumab is currently under phase 3 clinical trials for the treatment of Crohn's disease [19].

Developing antibodies solely against IL-17 or IL-23 has also been of interest to researchers. Brodalumab (AMG 827®), an antibody targeting the IL-17 receptor, has shown promising results during early clinical trials. However, due to poor safety and efficacy during phase 2 clinical trials, the study was terminated [68]. AMG 139, a drug targeting the p17 subunit of IL-23, showed promising results on a small population of individuals during phase 0 of clinical trials. As a result, AMG 139 is currently in phase 1 of clinical trials [21].

Studies have also looked into the therapeutic affect of blocking IL-6 receptor using a monoclonal antibody [22] and blocking the IL-6 binding site of the IL-6 receptor using tocilizumab (Actemera®) [69]. IL-6 plays a multitude of roles in the body including regulation of hematopoiesis, immune response, and inflammation. Excessive IL-6 levels have been shown to down-regulate the synthesis of erythropoietin, induce osteoclast differentiation to promote bone resorption, and increase the levels of serum amyloid A. As a result, clinicians have sought to develop an anti-IL-6 therapy to avoid excessive bone resorption, anemia, and unwarranted recruitment of immune cells to sites of inflammation [69].

BE-8, a murine IL-6 neutralizing antibody, was the first therapeutic approach towards the development IL-6 antibody. However, BE-8 showed limited efficacy. During phase 2 clinical trials, tocilizumab, a human monoclonal antibody against IL-6 receptor, demonstrated significant benefits in managing the symptoms of Crohn's disease. However, further studies are needed to measure the efficacy of tocilizumab in a larger population of patients afflicted by Crohn's disease [22,69].

Animal models have also illustrated promising results by targeting IL-12 with antibodies. Mice induced with colitis presented with Th-1 mediated gut inflammation characterized by increasing levels of TNF-alpha, IFN-gamma, and IL-12. Mannon et al. illustrated that the administration of an IL-12 antibody was associated with a decrease in Th-1 mediated inflammatory cytokines and may induce clinical remission in patients with Crohn's disease [23]. However, further research is needed to assess the efficacy of an anti-IL-12 drug.

4.2. Anti-inflammatory cytokine therapy

The use of anti-inflammatory cytokines such as IL-10 and IL-11 for the treatment of Crohn's disease is under investigation [26–31]. In the non-inflammatory state, IL-10, produced by monocytes and macrophages, is responsible for the maintenance of the intestinal epithelial barrier [26]. IL-10 deficient mice have been shown to develop severe transmural and granulomatous inflammation of the small and large intestines similar to Crohn's disease. Additionally, administration of IL-10 prevented inflammation in these mice. IL-10 functions to inhibit the antigen-presenting function of monocytes/macrophages and their production of pro-inflammatory cytokines (i.e., IL-6, and TNF-alpha), as well as to decrease the secretion of IFN-gamma by T-cells [28]. However, research has demonstrated that IL-10 administration for the treatment of Crohn's disease has a marginal, non-significant clinical benefit. Schreiber et al. and Buruiana et al. demonstrated that administration of IL-10 does not increase the number of remissions in Crohn's patients [26,29].

IL-11 is a pleiotropic cytokine of mesenchymal cell origin. It inhibits the secretion of pro-inflammatory cytokines by macrophages and T-cells [30]. In addition to its anti-inflammatory activity, IL-11 functions to preserve intestinal morphology. Rat models with induced colitis that were administered recombinant human IL-11 showed gross and microscopic resolution of colitis. In human trials, recombinant human IL-11 administration was well tolerated and successful in achieving short-term remission. However, IL-11 fails to maintain remission over a period of three months. IL-11 is currently undergoing clinical trials [31].

4.3. Anti-T-cell therapy

Inhibiting the activation of T-cells and inducing apoptosis in activated T-cell are other avenues by which clinicians are looking

Table 1
Cellular pathways and contraindications.

Drug	Cellular pathway	Contraindications
Infliximab	Block TNF-alpha	Heart failure/sensitivity to murine proteins
Adalimumab	Block TNF-alpha	Hypersensitivity to drug
Certolizumab pegol	Block TNF-alpha	None
Natalizumab	Block alpha-4 integrin	Patients with PML
Human growth hormone	Block IL-6	Under research
Conjugated linoleic acid	Up-regulate PPAR-gamma	Under research
Rifaximin	Inhibit bacterial transcription	Under research
Ustekinmumab	Block p40 subunit of IL-23, IL-12	Under research
AMG 139	Block IL-23	Under research
Brodalumab	Block IL-17R	Under research
Tocilizumab	Block IL-6R	Under research
IL-10	Block IL-6, TNF-alpha, IFN-gamma	Under research
IL-11	Preserve intestinal morphology	Under research
Visilizumab	Induce apoptosis in T-cells	Under research
Abatacept	Block T-cell activation	Under research
Mesenchymal stem cells (MSCs)	Block CD80/86, IL-12, TNF-alpha, and increase IL-10	Under research

to treat Crohn's disease. Visilizumab (Nurion®) is a humanized monoclonal IgG2 antibody against activated CD3 cells. Visilizumab specifically induces apoptosis in activated T-cells, leaving quiescent ones unharmed. In a placebo-controlled study assessing the efficacy of visilizumab, no significant response rates were observed between the placebo and treatment groups. Additionally, the Data Safety Monitoring Board prematurely discontinued the trial due to its lack of efficacy and safety [33]. Research has also illustrated an association between visilizumab and cytokine release syndrome (CRS). CRS occurs when T-cells are activated and secrete cytokines initiating an inflammatory response before they are destroyed. A majority of patients administered visilizumab exhibited symptoms of CRS [57].

Abatacept (Orencia®) is a fusion protein consisting of a Fc region similar to that of the IgG1 immunoglobin and cytotoxic T-lymphocyte antigen 4 (CTLA-4) region. Normal T-cell activation requires a co-stimulatory signal from CD28 and CD80 or CD86, which are located on the antigen-presenting cell. When the T-cell becomes activated, it expresses CTLA-4, which has a high affinity for CD80/CD86. Abatacept's extracellular domain consists of CTLA-4 and therefore blocks the activation of T-cells by competing with CD28 for CD80/CD86 [32]. In human trials Abatacept was not shown to be efficacious for the treatment of Crohn's disease [58].

Research has also illustrated that mesenchymal stem cells (MSCs) exhibit immunomodulary effects potentially beneficial for the treatment of Crohn's disease. MSCs decrease the expression of CD80 and CD86, decrease the production of IL-12, decrease the production of TNF-alpha by myeloid dendritic cells, and increase the production of IL-10 by plasmacytoid dendritic cells [59]. *In vitro* experiments have shown that MSCs can suppress T-cell activation and proliferation, interfere with dendritic cell differentiation, and suppress IFN-gamma production [60]. Two phase 1 studies have been conducted on the use of MSCs for the treatment of Crohn's disease. One study assessed the effects of systemic administration of MSCs [61] and the other study assessed the effects of locally administering MSCs for the treatment of fistulas associated with Crohn's disease [62]. The systemic approach demonstrated no clear sign of efficacy and remission was not achieved in any patient. On the other hand, the local approach suggested a considerable therapeutic benefit. The use of MSCs for the treatment of Crohn's disease is still under investigation [63].

4.4. Hormone therapy

Human growth hormone (HGH) is the most abundant hormone secreted by the anterior pituitary gland of the brain and has a 191 amino acid sequence that comprises a single chain [70]. It is secreted by the pituitary gland via the hypophyseal portal system that surrounds the pituitary gland and simultaneously binds to two identical receptors, site 1 and site 2 [71]. After sequential dimerization, intracellular domains are brought into close proximity to activate cytosolic components and cell signaling pathways. HGH activates a number of signaling pathways including extracellular signal-regulated kinase (ERK), the signal transducer and activator of transcription and phosphatidylinositol-3 kinase (PI3) pathways. These pathways control cellular functions including target gene transcription, enzymatic activity, and metabolite transport [72].

When HGH attaches to its receptor and causes a conformational change, a number of cell signaling pathways are activated including the production of Insulin-like Growth Factor 1 (IGF-1). HGH also inhibits an important signaling cascade, the Interleukin-6 (IL-6) JAK2-STAT3 signaling pathway [73]. Interleukin-6, a protein in the body that acts as either pro-inflammatory or anti-inflammatory, is secreted by T-cells in response to trauma, including tissue damage resulting in inflammation. IL-6 activates a number of pathways, most importantly, the JAK2-STAT3 pathway in Crohn's disease. JAK2 is a kinase responsible for activating Signal Transducer and Activator of Transcription 3 (STAT3). Once STAT3 is activated, it travels into the cell's nucleus and transcribes its target gene, in this case the pro-inflammatory genes. As a result, high levels of IL-6 in Crohn's patients directly contribute to chronic inflammation and flare-ups [28].

Nicholson et al. *in vitro* study showed that HGH impedes the IL-6 signaling cascade by increasing the affinity of SHP-2 and SOCS-3 for gp130 on the intracellular side of the IL-6 receptor (SHP-2 to gp130 K_d = 550 nM; SOCS-3 to gp130 K_d = 42 nM). Once SHP-2, a tyrosine phosphatase, binds to gp130 on the IL-6 receptor, SOCS-3 binds to the SHP-2 binding site on the shared cytokine receptor unit. The IL-6 signal cascade is therefore blocked intracellularly, as seen in Fig. 1 [52]. By blocking the IL-6 signaling cascade, HGH essentially stops the creation of proteins responsible for exacerbating inflammation in patients with Crohn's disease.

Furthermore, HGH up-regulates the production of IGF-1, which is responsible for stimulation of cell growth, differentiation, and proliferation in bones and other tissues throughout the body. By up-regulating the production of IGF-1, HGH increases the amount of IGF-1 that is secreted by the liver and brought into systemic circulation. Once in circulation, IGF-1 binds to its receptor (IGF-1R) and stimulates systemic body growth [74].

Once in systemic circulation, human growth hormone has a half-life of 20–30 min [75]. HGH binds to HGH Binding Protein (HGH-BP), which can both prevent its metabolism during transport in the plasma and inhibit its binding to HGH receptors throughout the body. HGH-BP is generated through the proteolytic cleavage of HGH receptor proteins [76]. However, the exact function of

HGH-BP is not well established [77]. Additionally, about half of human growth hormone in circulation is cleared by the kidneys, while the remainder is internalized and degraded by lysozymes during receptor down-regulation [78,79].

4.4.1. Importance of hormone therapy for pediatric patients

Various phenotypic features, such as short stature, are observed in children and adults afflicted by Crohn's disease. Unique to pediatric patient populations, however, is the potential for growth impairment as a complication of severe inflammation [17,18,72]. This complication can compromise overall height at maturity. Studies have shown that human growth hormone (HGH) can be used to treat pediatric Crohn's disease patients via the inhibition of the IL-6-JAK2-STAT3 signaling pathway and stimulate growth via the IGF signaling pathway [48,72]. One of the most important cell signaling pathways in Crohn's disease is the JAK2-STAT3 pathway of IL-6. It has been proposed that HGH can block this cell signaling pathway (Fig. 1). Subsequently, the IL-6 signaling cascade is blocked and the activation of pro-inflammatory proteins via the JAK2-STAT3 pathway is impeded [72]. In addition to treating chronic inflammation and flare-ups in Crohn's disease, HGH can also stimulate growth in pediatric patients via the Insulin-like Growth Factor signaling cascade. Human growth hormone binds to its corresponding receptors (GHR) in bones and other tissues, and stimulates cell differentiation and proliferation. Studies have also shown that HGH can increase the quality of life, encourage mucosal healing, and promote linear growth in pediatric patients [48,80,81]. Another study illustrated that an intermittent elemental diet consisting of essential and non-essential amino acids can effectively reverse growth arrest [82]. Researchers are currently investigating the effects of a modified diet in conjunction with the administration of human growth hormone on inhibiting growth arrest in pediatric patients [83,84].

4.5. Conjugated linoleic acid

New research has revealed that patients with mild to moderately active Crohn's disease who were administered conjugated linoleic acid (CLA) expressed a decrease in disease activity, decline in production of pro-inflammatory cytokines, and an increase in quality of life [49]. CLA is a collective term used to describe the different isomers of linoleic acid. Linoleic acid, naturally present in dairy and meat products, is one of many essential fatty acids that are consumed for good health [85].

CLA has been shown to exert anti-inflammatory and anti-oxidant properties in various animal models including arthritis and intestinal inflammation [86]. In pigs with bacterial-induced colitis, it has been reported that CLA up-regulates peroxisome proliferator-activated receptor-gamma (PPAR-gamma) which binds to DNA and regulates transcription of anti-inflammatory genes. In a mouse model, dietary CLA ameliorated inflammation-induced colorectal cancer by activating PPAR-gamma in immune and epithelial cells [87]. Another mechanism by which CLA suppresses inflammation is down-regulating eicosanoid synthesis. Suppression of prostaglandin-E2 can be important in managing the symptoms of Crohn's because it plays a vital role in inflammation. Prostaglandin-E2 is synthesized in extensive amounts at inflamed sites in the body and acts as a potent vasodilator [88]. Suppressing

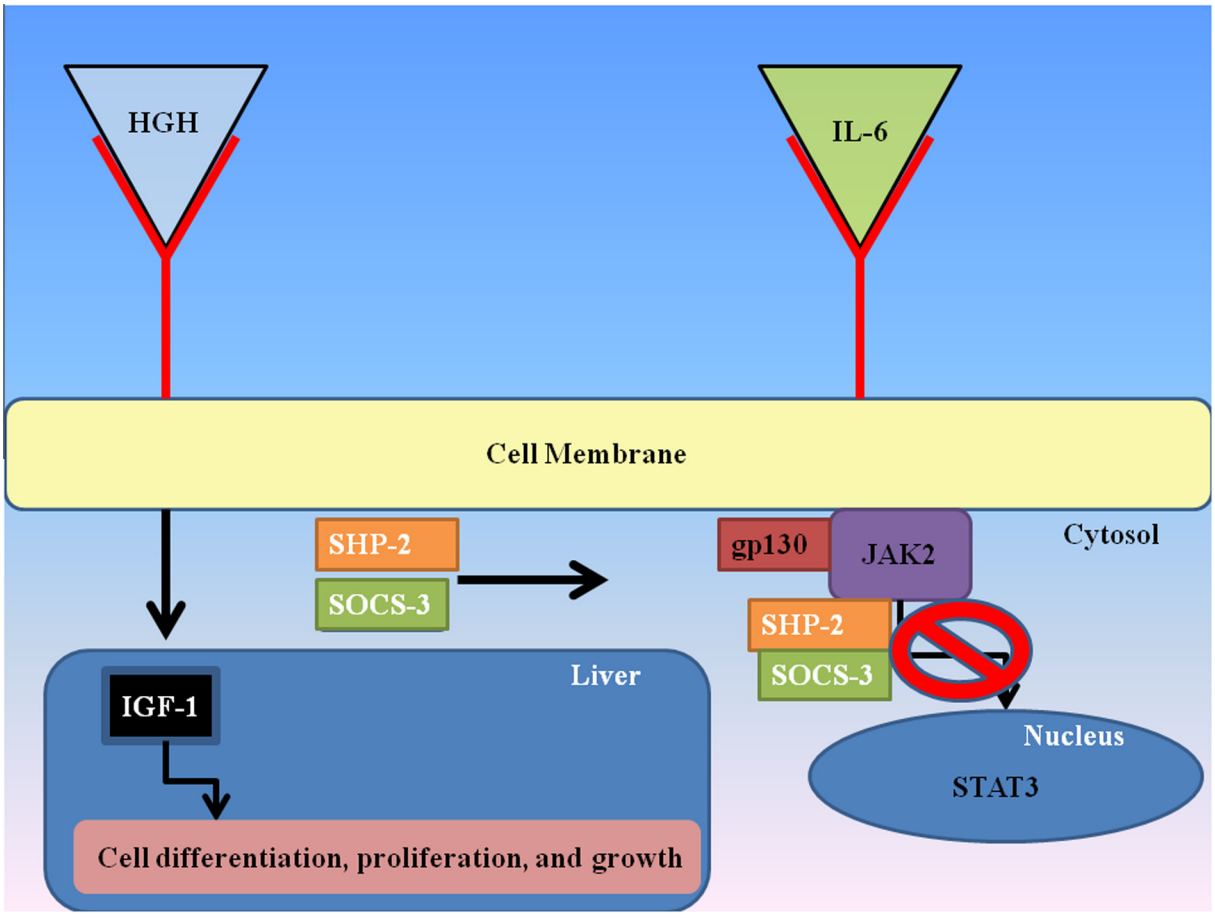

Fig. 1. Human growth hormone (HGH) signal transduction pathway. When HGH binds to its receptor (GHR) it causes a conformational change, increasing SHP2 and SOCS-3's affinity for intracellular IL-6 receptors. SHP2 and SOCS-3 inhibit JAK2, a protein implicated in signaling cytokines. When JAK2 is inhibited it cannot activate STAT3 (Signal Transducer and Activator of Transcription 3), a transcription factor responsible for the production of pro-inflammatory proteins.

the amount of free-floating prostaglandin-E2 can help alleviate inflammation in patients with Crohn's disease. In pigs, CLA was reported to significantly suppress prostaglandin-E2 release from the trachea [89]. Further research is necessary to determine how well CLA suppresses prostaglandin-E2 in patients afflicted by Crohn's disease.

4.6. Rifaximin

The suggested role of enteric bacteria in the pathogenesis of Crohn's disease encourages the investigation of an antibiotic treatment therapy. Current research asserts that rifaximin, an antibiotic generally used to treat travelers' diarrhea, is a good candidate because of its lack of interaction with other drugs [33]. Rifaximin is a nonsystemic drug and is therefore not completely absorbed in the blood stream, minimizing systemic exposure (more than 95% is excreted in feces). Furthermore, rifaximin has no known drug-drug interactions and lacks resistance to any identifiable antibiotics. As a result, this drug offers the advantage of minimizing off-target systemic effects and reducing the risk of side effects [32,33].

A recent research study conducted tested the efficacy of rifaximin in inducing remission in 68 patients with Crohn's disease in either the small intestine, large intestine, or in multiple locations. The study evaluated adults with Crohn's disease and attempted to induce remission in patients with Crohn's disease and improve their Crohn's disease activity index (CDAI), a tool used to quantify the symptoms of Crohn's disease. The results of the study reported that at least 55% of every patient group (i.e. patients with Crohn's disease in multiple locations, patients with Crohn's disease in the small intestine, etc.) achieved remission. Furthermore, the CDAI score of 90% of every patient group decreased by at least 70 points. Despite the fact the results of this study seem promising, selection bias in this study could be of concern. Selection of patients with Crohn's disease for this study depended on patient opposition to other therapies, lack of responsiveness to other treatments, and CDAI score [33]. Currently, the potential benefits and safety of rifaximin are being further evaluated in a phase 2 clinical trial [65].

5. Conclusion

Crohn's disease affects over one million Americans of out which 140 000 are under the age of eighteen. Crohn's disease is not presently curable, but there are many existing avenues by which one can achieve remission including the commonly used TNF-alpha blockers and natalizumab, an alpha-4 integrin blocker. Despite the fact that there are various treatment options available, specific requirements for an effective drug still remain unclear. Some researchers have claimed that neutralizing TNF-alpha is a potential requirement, but are unsure if inducing apoptosis in leukocytes or mediating antigen-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) are requirements for clinical efficacy. Others believe that inducing apoptosis in leukocytes is a key requirement [10,36]. Furthermore, current treatment options are lacking in their ability to alleviate growth suppression and promote normal sexual maturation in children under the age of eighteen. Researchers have investigated the potential of other drugs such as human growth hormone, conjugated linoleic acid, rifaximin [35,43,64,66], antibodies targeting pro-inflammatory cytokines [19–22] and T-cells [24,25,57–63], and anti-inflammatory cytokines [26–31] in enhancing the quality of life in Crohn's patients, and have illustrated promising results. However, further research both *in vitro* and *in vivo* is essential in order to determine the best treatment regimen for patients with Crohn's disease. Future studies should focus on identifying what specific cellular mechanisms (i.e. inducing apoptosis in leukocytes, or targeting TNF-alpha) are required to allow Crohn's patients to successfully achieve remission and what undesirable side effects can be associated with these treatment options. By elucidating these mechanisms, researchers and clinicians will be better able to treat the symptoms of Crohn's disease by targeting specific cellular pathways at a molecular level.

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