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The Emerging Role of Leptin Antagonist as Potential Therapeutic Option for Inflammatory Bowel Disease

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Abstract

Inflammatory bowel disease (IBD) is a chronic relapsing immune-mediated inflammatory disorder that affects millions of people around the world. Leptin is a satiety hormone produced primarily by adipose tissue and acts both centrally and peripherally. Leptin has been shown to play a major role in regulating metabolism, which increases during IBD progression. Leptin mediates several physiological functions including elevated blood pressure, tumorigenesis, cardiovascular pathologies and enhanced immune response in many autoimmune diseases. Recent development of a leptin mutant antagonist that blocks leptin activity raises great hope and opens up new possibilities for therapy in many autoimmune diseases including IBD. To this end, preliminary data from an ongoing study in our laboratory on pegylated leptin antagonist mutant L39A/D40A/F41A (PEG-MLA) treatment shows an inhibition of chronic colitis in IL-10^{-/-} mice. PEG-MLA effectively attenuates the overall clinical scores, reverses colitis-associated pathogenesis including a decrease in body weight, and decreases systemic leptin level. PEG-MLA induces both central and peripheral leptin deficiency by mediating the cellular immune response. In summary, after blocking leptin activity, the correlative outcome between leptin-mediated cellular immune response, systemic leptin levels, and amount of adipose tissue together may provide new strategies for therapeutic intervention in autoimmune diseases, especially for intestinal inflammation.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

Keywords

Crohn's disease (CD); inflammation; inflammatory bowel disease (IBD); leptin antagonist; pegylated leptin; ulcerative colitis (UC)

INFLAMMATORY BOWEL DISEASE

Crohn's disease (CD) and ulcerative colitis (UC), two major forms of Inflammatory bowel disease (IBD), are chronic immune-mediated inflammatory condition of intestine. IBD affects millions of people globally [1]. The precise mechanism and pathogenesis of IBD remains unclear, but accumulating evidence suggests a multifactorial nature with immunological, environmental and genetic contributions making systematic studies difficult. Genetic and/or familial history factors also play major roles in the development of IBD. Further, in the past, ethnic disparities related to the frequency of IBD have also been reported [2]. The causes of IBD remain unknown, but are reported to be associated with food intake, increased energy expenditure and reduced absorption of nutrients [3]. To this end, IBDs, especially CD, are commonly characterized by body weight loss, anorexia and increased energy expenditure during acute stages of intestinal inflammation [4–7]. Further, studies also suggest that enhanced or aberrant immunologic responsiveness to normal gut flora may result in the induction of IBD, and an overall autoimmune dysregulation and imbalance may also enhance the progression of IBD [8–11]. In recent studies, it has been suggested that leptin may also play a role in anorexia associated with IBD. Typically, anorexia, malnutrition and altered body composition are well-known features of IBD.

LEPTIN

The adipocyte-derived hormone leptin is also known as a pro-inflammatory cytokine, primarily recognized for the control of appetite and obesity, and for having a potent role in mediating many autoimmune diseases [12]. Leptin is a satiety hormone produced primarily by adipose tissue [13, 14]. It is a modulator of feeding behavior as well as fat stores and provides signals to feeding centers of the brain regarding nutritional status, fat mass, appetite and energy expenditure [15, 16]. Leptin receptors (LRs) are widely distributed throughout the body, and its mRNA is known to be expressed in hematopoietic cells and lymphocytes [17]. In addition, LRs are highly expressed in the hypothalamus, an important regulator of body weight, as well as in T lymphocytes and vascular endothelial cells. Its level is directly related to the amount of adipose tissue. Leptin has also emerged as a potential mediator of inflammatory status and a positive modulator of IL-1 α , TNF- α and IL-6 secretion [18]. TNF- α partially regulates leptin levels at inflammatory sites during inflammation and IL-1 α levels correlate with leptin during tumor progression [19, 20]. Leptinemia is also under control of IL-1 α and TNF- α interactions [21]. Interestingly, both leptin and TNF- α decrease food intake and regulate other aspects of energy metabolism [16, 22]. These studies clearly support the idea that leptin modulates pro-inflammatory cytokines (IL-1 α and TNF- α) that mediates the induction of inflammation.

LEPTIN IN THE GUT

Leptin's mRNA and protein have been found in the chief cells of human gastric and rat fundic mucosa. It has been shown that leptin levels in the stomach are affected by nutritional state and the administration of coelchistokinin (CCK). In addition, the biogenesis and production of gastric leptin involve a 19 KD leptin precursor that is not involved in leptin secretion in adipose tissue and the level of leptin is lower after a meal than during fasting conditions. Rat gastric leptin is decreased by starvation, but does not change significantly from the fasted state. However, refeeding of fasted rats decreases gastric leptin to its 2/3 levels in 15 minutes and induces a small increase in plasma level. Leptin is free and stable in the stomach juice and increases under the stimulation of hormone secretion and pentagastrin, and is secreted by the endocrine cells of the stomach. Granules of P cells in the basal area of glands stain positively for leptin [23]. LR has also been detected in the human fundic mucosa and jejunum, suggesting that the gut is a direct target of gastric leptin [24]. Leptin over-expression is closely correlated with gastric cancer (GC) invasion [25]. However, further research is needed to study the role of leptin in the pathogenesis in patients with CD of gastric origin. Leptin has been examined in experimental rodent models of intestinal inflammation as well as in IBD patients. It has been shown that dextran sodium sulfate (DSS) induced colitis in mice delayed the puberty in male mice out of proportion to change body weight and serum leptin level [26]. In rats, trinitrobenzene sulfonic acid (TNBS) induced colitis results in increased leptin concentration and is associated with decreased food intake and weight loss [27]. Further, severity of colitis in these rats is correlated with elevated plasma leptin concentration and is associated with anorexia and loss of body weight. There are several possibilities for the discrepancy in decline of leptin level during severe inflammation after TNBS induction that has also been noticed in many studies in IBD patients. In experimental mice, body weight is reduced suggesting a reduction in adipose tissue mass that might diminish leptin levels. Further, reduced food intake due to inflammation increases fasting time, which might lower leptin levels. This is also true for human IBD patients, where a drop in plasma leptin occurred in nonobese humans subjected to fasting or obese subjects on a low caloric diet [28, 29]. This observation is supported by a study in IL-2^{-/-} mice that shows leptin concentration is lower in the fed state than in either pair-fed or freely fed controls, and that systemic leptin concentration during inflammation may not reflect fat mass [30]. Correspondingly, in IBD patients, systemic leptin level increases compared to normal healthy donors [31–33]. In a recent study, it has been shown that expression and release of leptin increases in UC patients with infectious diarrhea [34]. These results are in contradiction with some earlier studies on both human and experimental models of obesity not associated with inflammation, which suggest that leptin level correlates with percent of body fat, and percentage of leptin is increased during weight gain and decreased during weight loss [35]. This notion was also supported by other obesity-related studies that show systemic leptin concentration correlates with body weight and decreases in association with weight loss [16, 36, 37].

These perceptions are further supported by reports showing that during body weight loss, leptin and TNF- α expression correlates with percent body fat and both molecules increase during weight gain and decrease during weight loss [35, 38]. This is in contrast with reports

that show that in CD patients TNF- α levels are elevated in tissue and secretory fluids; correspondingly, there are increased numbers of TNF- α producing lamina propria (LP) cells [39, 40]. Further, anti-TNF- α also leads to and attenuates the development of colitis in certain murine models of IBD [41]. Further to this, there are reports that leptin secretion is regulated both *in vitro* and *in vivo* by TNF- α post-translationally [42]. It is reasonable to assume that both leptin and TNF- α are involved in inflammation not necessarily associated with body weight gain or loss after certain points of inflammation and it may be possible that TNF- α induces leptin during early inflammation. The other possibilities are that leptin and/or TNF- α dissociate from feeding centers of the brain resulting in this discrepancy of body weight during acute/chronic inflammation. In the present study, the majority of IBD patients have normal body mass index (BMI) due to the disease course. Hence, the variability of leptin levels in subjects within a group is minimal.

In human IBD patients, increases in leptin levels are associated with UC [31, 32]. Further, overexpression of leptin mRNA in mesenteric adipose tissue in IBD patients has been shown [43]. Colonic leptin induces epithelial wall damage and neutrophil infiltration that represent characteristic histological findings in acute intestinal inflammation [44]. In a recent study, it was shown that children with IBD have significant under nutrition and lower leptin levels than controls [45]. The role of leptin in IBD has been studied, but the results are conflicting and therefore further investigation is required [46, 47]. Despite strong evidence for the role of leptin in autoimmunity, the precise mechanism and its activity has been controversial and both direct and indirect mechanisms have been described [48, 49]. Leptin can directly affect numerous immune cell types of both innate and adaptive systems and stimulate pro-inflammatory cytokines.

LEPTIN INDUCES CELL-MEDIATED IMMUNE RESPONSE

Studies in mice have demonstrated that leptin deficiency affects both the innate and acquired immune systems [50]. The ob/ob mouse shows a decrease in sensitivity of T cells to activating stimuli and mice show atrophy in lymphoid organs with a decrease in circulating T cells and increasing monocytes, suggesting a role for leptin in cell-mediated immune responses [48, 51]. The attention in this area began to widen after a report showed that LRs are expressed on T lymphocytes and mediate chronic intestinal inflammation in mice [52]. After this, several studies published in this area showed a close relationship between frequency of LR expression and immune response. The leptin receptor Ob-Rb is expressed by B and T cells, suggest a direct intercession to immune responses [53]. In addition, leptin intervenes with the immune system by regulating hematopoiesis [54] and lymphopoiesis [51]. Leptin also increases IFN- γ -producing 31 polarized cells [48, 55] and interestingly persuades dendritic cells that are employed for antigen presentation to induce a 31 response [56]. This induction of 31 response seems to be mediated by stimulation of IL-2, IL-12 and inhibition of IL-4, IL-10 production [57]. It has been shown that leptin exerts its bioactivity at developmental, proliferation and activation levels [58] and indirectly activates human neutrophils via TNF- α induction *in vitro* [59]. Leptin also enhances the proliferation of T cells after concanavalin A (Con A) activation [60]. The other function of leptin is to promote survival of T cells and jurkat lymphocytes [61] by modulating anti-apoptotic protein in stress-induced apoptosis [62]. A report also indicated that leptin treatment in an obese

patient due to leptin deficiency reversed the body weight and T cell response to mitogen activation *in vitro* [63]. In summary, these studies overall clearly support the notion that leptin mediates the cellular immune response that might intercede with progression of inflammation.

LEPTIN CONNECTION WITH REGULATORY T CELLS (TREGS)

The critical protective role of Tregs in numerous autoimmune diseases and inflammation including IBD has been well established. Rosa et al., put forward the key piece of the puzzle regarding leptin's direct link with Tregs anergy and hyporesponsiveness [64]. Mice with genetic deficiency of leptin have a higher percentage and absolute numbers of circulating Tregs and treatment of wild-type mice with leptin neutralizing antibody produced an expansion of Tregs [65]. In a recent study, an increase of leptin in multiple sclerosis (MS) patients correlated with reduced number of Tregs is shown [66]. Such reductions are likely to be a direct consequence of leptin binding to receptors on Tregs. The Tregs are normally anergic and hyporesponsive to T cell receptor (TCR) signaling, but the specific neutralization of leptin combined with TCR signaling reversed anergy and hyporesponsiveness of these cells [64]. Thus, during inflammation, increases in other cytokines like IL-6 and TNF- α might intervene with leptin levels or *vice versa* to induce chronic inflammation. Taken together, these studies support the notion that elevated systemic leptin concentration correlates with the severity of inflammation in general. It has been proposed that leptin could control immune self-tolerance by affecting Tregs responsiveness and function. There are reports showing that Foxp3 plays an important role in the control of intestinal inflammation [67] and naturally arising CD4⁺ CD25⁺ Tregs have been shown to prevent or even cure colitis in the T cell transfer model [68, 69].

It has been shown that leptin affects generation and proliferation capacity of Tregs, which are well known for their central role in control of peripheral immune tolerance [64]. In mice, the deficiency of leptin and its receptor have been shown to increase the absolute number, percentage and functional activities of Tregs [70] with a resistance to autoimmune disease [71], and its replacement returns Tregs levels back to that found in normal mice. It has been shown that an increase in leptin level correlates with disease severity in human IBD and MS patients and inversely correlates with circulating Tregs [66]. The increasing percentage of Tregs in wild-type mice after LR fusion protein treatment ameliorates experimental autoimmune encephalomyelitis EAE [66]. Interestingly, Tregs have been shown to be an important source of leptin, with both secreted leptin and expressed LRs. During an ongoing investigation in our laboratory, we noticed a significant increase in systemic leptin in IBD patients and an experimental mouse model of colitis. Leptin levels correlate with a decrease in circulating Tregs and an increase in disease severity of colitis. However, the initial study from our laboratory suggests that PEG-MLA treatment reverses the disease severity by inducing mucosal and systemic circulatory Tregs. The ongoing study in our laboratory will open an avenue for future research on leptin involvement in immune regulation and inflammation. A close association is reported between obesity and high leptin levels in inflammatory conditions like rheumatoid arthritis (RA), and starvation in mice delayed the onset of EAE disease. Interestingly, reduced leptin level ameliorates symptoms of inflammatory conditions, which supports our observation. This review expands and supports

the concept about the efficacy of leptin-induced Tregs to modulate inflammatory responses therapeutically.

LEPTIN IN AUTOIMMUNE DISEASE: ITS ANTAGONIST AS A NEW THERAPEUTIC APPROACH

It is well known that adipose tissue, placenta, gut, other tissues and Tregs secrete leptin [72, 73]. One major pharmacological challenge to blocking the LRs responsible for transferring leptin through the blood brain barrier is the effect on both the hypothalamus and peripheral effector organs. Past reviews [74] clearly suggest many ways to neutralize leptin; by soluble LPs that bind free circulating leptin, developing a leptin antagonist that binds LPs or specific monoclonal Abs that bind to receptors and stop the binding of leptin. Previous studies clearly linked leptin with 31 induction and enhanced susceptibility-induced autoimmune disease including CD, RA, MS, Type-1 diabetes (T1D), EAE and antigen-induced arthritis (AIA) [75]. Leptin-deficient (ob/ob) mice showed 72% reduction in colitis severity and leptin treatment eliminates resistance against experimentally induced colitis [52]. In another study, ob/ob and db/db mice were partially protected from toxin A-induced intestinal inflammation, and leptin administration reversed this protection [76]. It has been shown that in RA leptin antagonist mediates the severity of disease at early stage sensitive to leptin as compared to chronic stage [77]. Similarly in other autoimmune diseases, increased leptin worsened the EAE disease [70] by increasing IFN- γ release and IgG2a production [78]. In lupus erythematosus serum, leptin levels were reported to be higher as compared to control counterparts [79]. However, polymorphism in genes encoding leptin and their receptors does not contribute substantially, but modest effects cannot be ruled out in the pathogenesis of MS patients [80]. It has been shown that leptin also promotes the development of T1 diabetes through 31 response [81]. In a recent review, it was also clearly shown that leptin antagonism therapy works for the prevention and treatment of immunity-related disorder in mice [82]. It has been shown that LR antagonists abrogate the effects of long-term maternal hypoxia by governing the key enzyme StAR, and other factors play a role in modulating cortisol synthesis in these fetuses [83]. As mentioned above, leptin mediates several physiological functions, such as regulation of energy metabolism and reproductive function, and induces autoimmune diseases by enhancing immune response, tumorigenesis, elevated blood pressure and cardiovascular pathologies. These studies clearly indicate that leptin induces inflammation, and blocking leptin activity by leptin antagonism has been proposed in this review through our preliminary work and others as an immunotherapeutic approach for the treatment of autoimmune diseases, specifically for IBD. It has been shown that administration of anti-tumor necrosis factor-alpha (TNF- α) antibody or soluble TNF- α receptor inhibits severity of colitis [84]. Our laboratory has shown that antibody therapy directed toward a chemokine, CXCL10, is successful at impeding IBD development and abrogates colitis in a murine model of IBD [85]. However, all available treatments so far have a number of side effects, are cost ineffective and in many cases relapses occur after withdrawal of treatment. These results usually offer an expansion for the number and variety of drugs that target the inflammatory process for prolonged periods of time with minimal or no harmful side effects. Taking all of these possibilities into account and our laboratory's focus on IBD, we explored the new possibilities for their use in research by blocking leptin

activity and developing a new platform for IBD therapy as well as for other autoimmune diseases (Figure 1).

POSSIBLE ROLE OF LEPTIN ANTAGONISM IN IBD

Leptin appears to act as metabolic switch, as lower leptin levels during starvation or blocking of leptin downregulates high energy-demand processes during an immune response. Due to the multiple diverse and opposite effects of leptin, the benefit of blocking leptin activity is real and timely. The practical means of inhibiting leptin activity is by directly blocking the LRs that are responsible for transferring leptin through the blood brain barrier, which affects both the hypothalamus and peripheral effector organs.

It has been well documented that molecular masses of hormones and those of leptin have very limited (8–30 min) half-lives. Increasing protein size more than 70 kDa by the attachment of PEG molecules increases the potency of leptin antagonists. PEG attachment to leptin resulted in reduced renal clearance and consequent prolongation of its half-life cycle. Recently, several PEG-conjugated medications have proven to be superior to their unmodified parent molecules and are now widely used in clinical practice. PEG-MLA has been confirmed by a previous study as a true antagonist with no agonistic activity [86]. Further, PEG-MLA has been shown to abolish the increased expression of genes that encode short form (LRA) and long form (LRb) angiotensin II and endothelin-1 [87]. PEG-MLA also prevents homeostatic downregulation following high fat enhanced feeding in five-month old rats [88]. Further, another study suggests that luminal addition of leptin can be completely blocked by ovine leptin L39A/D40A mutein [89]. It has recently been shown that the leptin antagonist (L39A/D40A/F41A) reversed the effects of exogenous and endogenous leptin on α -casein expression in mammary gland explants [90].

We have investigated the effect of PEG-MLA-mediated inhibition of chronic experimental colitis in IL-10^{-/-} mice. In these studies, we noticed that PEG-MLA effectively attenuated the overall clinical score by reversing colitis-associated pathogenesis including a decrease in body weight and systemic serum amyloid A (SAA), and leptin levels [91]. PEG-MLA also reduced systemic and mucosal inflammatory cytokine expression, increased insulin levels and enhanced systemic and mucosal Tregs in mice with chronic colitis. We also noticed that activation of STAT1 and STAT3 and the expression of Smad7 were reduced after PEG-MLA treatment in colitic mice [92]. Taken together, our study clearly links inflammation with leptin suggesting that nutritional status influences immune tolerance through enhanced frequency of Tregs. Inhibiting leptin activity through PEG-MLA might provide a new and novel therapeutic strategy for the treatment of IBD as well as other autoimmune diseases.

CONCLUSION

Leptin is one of the most important hormones secreted by adipocytes and connects nutritional status to immune response. The harmful effects of leptin mainly center in the area of autoimmune disease, heart failure, atherosclerosis, blood pressure and cancer progression. Therefore, blocking leptin activity might be a useful tool to provide therapy either alone or with some other drugs. The high affinity leptin antagonist PEG-MLA, we used in our

ongoing investigations, looks very promising at least for the amelioration of IBD severity. Similarly, PEG-MLA can be used in any autoimmune disease associated with increased secretion of leptin and production of pro-inflammatory cytokines.

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FIGURE 1.
Schematic diagram showing possible mechanism of pegylated leptin antagonist mediated abrogation of experimental colitis.