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Dupilumab to target interleukin 4 for inflammatory bowel disease? Hypothesis based on a translational message

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ABSTRACT

Dupilumab is a human monoclonal antibody that has recently been approved for the treatment of mild-to-severe atopic dermatitis. Dupilumab inhibits the interleukin (IL)-4/IL-13 signalling by binding specific subunits of the receptors. IL-4 is involved in the regulation of both innate and adaptive intestinal immune system, thus its possible role in the pathogenesis of inflammatory bowel disease (IBD) has been investigated. Several polymorphisms of IL-4 have been found in patients with IBD. Furthermore, the crucial role of IL-4 has been demonstrated in Th2-driven models of colitis as well as in Th1-related models of this disease. Finally, many studies have shown the increased production of IL-4 in two models of experimental colitis: the dextran sulphate sodium – related colitis and the oxazolone-related colitis. In the latter, a dual antagonist of IL-4/IL-13 has proved to ameliorate the course of the disease. In conclusion, IL-4 antagonists might play a role in the treatment of IBD, especially in ulcerative colitis, which shows a classic pattern of Th2-related disease.

Keywords: Crohn's disease; Dupilumab; inflammatory bowel disease; interleukin-4 antagonists; interleukin-13; ulcerative colitis

Introduction

The burden of autoimmune disorders is progressively increasing worldwide. The first data about the epidemiology of autoimmune diseases were published in 1997 by Jacobson et al., and showed a prevalence of 3.2% between 1967 and 1997 in the United States.[1] Subsequently, one Danish study performed by Eaton et al. estimated a prevalence of 5.3%, between 1977 and 2001, based on national hospitalization registry data.[2] The most recent data, which cover 20 years, from 1989 to 2008, are extrapolated from a review conducted by Cooper et al., which showed a worldwide prevalence of 7.6-9.4%.[3]

As a consequence of the increased prevalence of autoimmune disorders, new drugs, specifically monoclonal antibodies, which target critical cytokines of the signalling pathways involved in the mechanisms of disease, are progressively studied. Interestingly, clinical trials contribute to translational message from basic research to the ultimate understanding of the functioning of targeted molecules with the hope to optimize and perhaps personalize treatments.[4] Thanks to monoclonal antibodies, it has become clearer which are the sites of human body (i.e. intestinal mucosa and skin) where the targeted cytokines mainly work, and which is their role in the pathogenesis of each specific disease.

On the basis of already known pathogenetic models of inflammatory bowel diseases (IBD), this narrative review describes the recent development of the new drug dupilumab, targeting interleukin-4 (IL-4), explaining the reasons of its potential application in the treatment of IBD.

The role of dupilumab in atopic dermatitis

Atopic dermatitis is a widespread chronic inflammatory skin disease. It is characterized by a damaged skin barrier function, with an increased transepidermal water loss and inflammation, a reduction of long chain fatty acid in the lipid bilayer and a lympho-histiocytic infiltration.[5-7] Although its causal agents remain unknown, in the last years also gastrointestinal microbiota alterations, with consequences in the development of the host's immune system, have been involved as potential mechanisms of damage.[8,9] These processes are mainly Th2-cell mediated and therefore Th2-related cytokines, such as IL-4 and IL-13, are increased in the

involved skin, as well as dendritic epidermal cells. Conversely, Th1-related cytokines, like interferon (INF)-γ and IL-2, are decreased.[10-12] IL-4 and IL-13 induce spongiosis of the skin and reduce filaggrin gene expression in keratinocytes, leading to a damage of the barrier function.[13,14] Furthermore, IL-4 stimulates B-cell differentiation and an immunoglobulin (Ig) subclass switch, resulting in IgE production.[15]

Dupilumab is a human monoclonal antibody approved by the Federal and Drugs Adiministration on March 28, 2017 for the treatment of adult patients with moderate-to severe atopic dermatitis which is not adequately controlled with topical therapies.[16] It inhibits the IL-4 and IL-13 signalling by binding the IL-4R-α subunit[17], which is part of both IL-4R and IL-13R. It thereby downregulates the JAK-STAT downstream signalling pathway.[18]

In patients with atopic dermatitis, many genes are regulated through the JAK-STAT pathway[19]: its activation in fact, leads to the downregulation of skin barrier proteins, such as loricrin, involucrin and filaggrin[14,20] and it promotes the production of chemokines such as CCL3L1, CCL8, CCL24, CCL25, CCL26, CXCL6 and CXCL16 by keratinocytes.[21] Moreover, it stimulates the Th2-cell differentiation, resulting in an increased production of IL-3, IL-4, IL-5, IL-6, IL-10 and IL-13[22], and causes the B-cell activation which is involved in the IgE production.[23,24] Hence, by blocking the IL-4 and IL-13 signalling pathways, dupilumab acts on the critical disease mechanisms of atopic dermatitis.

Dupilumab has been approved for subcutaneous administration, starting from an initial dose of 600 mg, followed by a weekly dose of 300 mg.[17] Steady-state concentrations are obtained after 16 weeks of treatment.[17] This drug was firstly tested in patients with persistent allergic asthma and eosinophilia, in whom it improved lung function and reduced exacerbations.[25] Since allergic asthma and atopic dermatitis are genetically linked and share the same immunopathological mechanisms[26], randomized clinical trials with dupilumab for the indication of atopic dermatitis were subsequently conducted.[27] The safety profile of dupilumab was superior to conventional immunosuppressive drugs, like cyclosporine or methotrexate.[27] The most common side effects were erythema or edema at the injection-site and conjunctivitis.[28]

The role of IL-4 in the intestinal immune system

IBD are distinguished in two main phenotypes, Crohn's disease (CD) and ulcerative colitis (UC), both characterized by chronic inflammation of the intestinal mucosa and variable course [29]

While the etiology of IBD remains unclear, the hypothesis of a multifactorial genesis supposes that an antigenic stimulus may act as a "trigger" for an abnormal activation of the immune reaction towards colorectal mucosa. The intraluminal antigenic stimulation would facilitate the loss of the immunotolerance towards indigenous bacterial flora and consequently activates the gut-associated immune system. [30,31] Pharmacological management of IBD, partly reflects our limited understanding of the pathogenesis of the disease, and its complex phenotypic presentation.[32-35]

IL-4 has also been investigated as a possible pathogenetic factor in the onset of IBD. Changes in cytokines' expression have been frequently found in patients with IBD,[36] and several speculations about the role of the intestinal lymph cells have been made. Th17-cells, which produce IL-17, IL-21 and IL-22, are thought to cause inflammatory and autoimmune reactions.[37] Conversely, T-reg cells, which produce IL-10, and transforming growth factor (TGF)-beta), are known for their regulatory effects on immune responses.[38]

IL-4 and IL-5, produced by Th2-cells, have been classically considered as anti-autoimmune cytokine by inhibiting Th17-cells.[39] Thus, IL-4 and IL-10 have always been thought to be protective cytokines in the pathogenesis of IBD.

Nonetheless, IL-4 directly regulates intestinal epithelial cell functions: it modulates the growth of epithelial cells, can alter the intestinal mucosal barrier function (a key element in the IBD damage[40], and stimulates the adherence and migration of neutrophils to the epithelium.[41] Therefore, overproduction of IL-4 may subvert the immunological homeostasis between the intestinal immune system and the environmental antigens[42], including the gut microbiota[43], by promoting enhanced translocation of luminal pathogens and recruiting inflammatory cells at the very beginning of the disease.

The possible role of IL-4 polymorphisms in the genesis of IBD

To support of the potential involvement of IL-4 alterations in the pathogenesis of IBD , a recent study conducted by Ebrahimi Daryani et al. investigated the presence of polymorphisms that may predispose to the onset of this disease. They found that all genotypes studied were correlated to IBD: C-allele of IL-4-590 polymorphism and T-allele of IL-4-1098 polymorphism were found in the whole group of IBD patients.[44]

Another study pointed out the importance of the above-mentioned polymorphisms.[45] The gene of IL-4 maps on the long arm (q23-31) of chromosome 5.[46] A C→T exchange has been identified in position -590, which correlates with an increased transcriptional activity and secretion of IgE.[47] It has been suggested a correlation between this promoter region polymorphism and allergic asthma, atopic dermatitis and rheumatoid arthritis in US and Japanese families, despite this evidence has not been confirmed in UK and Australian families.[48-51] Another C→T substitution has been subsequently discovered at position -34 in the untranslated region which is in linkage disequilibrium with the position -590 in the promoter region.[52]

The IL-4R, instead, maps on the short arm (16p12) of chromosome 16. A G→A substitution at position 1902 (change from glutamine to arginine at amino acid 576) leads to an enhanced signalling activity and is commonly found in patients with atopy.[53,54]

Since the pericentromeric region of chromosome 16, which contains the IL-4R gene[55] and the long arm of chromosome 5, which contains the IL-4 gene[56], have been previously identified as susceptibility loci for CD, this study examined the possible role of the above-mentioned polymorphisms in the onset of the disease. The authors found that both variant IL-4 and the association between variant IL-4 and variant IL-4R are related to CD, suggesting a role of IL-4 in the pathogenesis of CD.[45]

T-cell receptor (TCR) α chain-knockout mice as IL-4-driven model of Th2-related colitis.

The murine models have been surprisingly useful for understanding the genesis of IBD, in which the cytokines' cascades are tightly connected and the role of each signal pathway receptor and the cytokines involved are barely understood. Table 1 shows murine models regarding the role of IL-4 and IL-13 in experimental colitis.

It has been shown that TCR-α chain-deficient mice developed IBD, and the colitis was associated with increased number of Th2-cells producing eminently IL-4.[57-59] Iijima et al. showed that approximately 60% of these mice, including those treated with mock (inactive) anti-IL-4 antibodies and those left untreated, spontaneously developed IBD. Conversely, those mice treated with anti-IL-4 antibodies did not express any clinical or histological signs of IBD, with a decrease of Th2 related cytokines and an increase in INF-γ expression.[60] These findings suggest that aberrant mucosal Th2-cells are involved in the development of IBD

and the treatment with anti-IL-4 antibodies altered the cytokines profile from Th2 to Th1 types, as a result of the decrease of B-cells.[60]

A study, conducted by another group, gave direct evidence of the involvement of IL-4 in the disease processes, showing that double knockout murine models (TCR-α chain and IL-4 deficient) had a reduced incidence of colitis.[61]

Using the same model of colitis, Mizoguchi et al. have studied the possible role of IFN-γ, rather than IL-4, for the onset of the disease. They created double-mutant mice which were negative for TCR-α, and negative for either IL-4 or IFN-γ. The lack of IL-4 was confirmed to suppress the onset of colitis, whilst the lack of IFN-γ did not interfere with the onset of colitis, which resulted as similar as the colitis developed in the single TCR-α mutant mice.[61]

The role of IL-4 in the T cell transfer model of colitis.

Although IL-4 is involved in Th2 immune responses[62], it can also interfere with the Th1 responses. The latter are evocated when antigen presenting cells (APC) release IL-12, inducing the differentiation of CD4+ T cells, which produce IFN-γ and IL-2.[62] CD shows a typical Th1 pattern of disease, with a large amount of IFN-γ and TNF-α.[63] IL-4 is classically known to suppress Th1-type inflammatory response.[64]

However, there is increasing evidence that IL-4 can worsen Th1 related immune responses.[65] In a study conducted by Fort et al.[66], the effect of IL-4 treatment was tested in the CD4(+)CD45RB T cell transfer model of colitis, an established model of Th1-related disease.[67] IL-4 treatment was shown to exacerbate Th1-colonic inflammation in this model and did not switch the colitis to a Th2-type immune response.[66] As the receptor of IL-4 is expressed in many types of cells other than T cells (i.e. B cells, monocytes, intestinal epithelial cells)[68-71], in this study the effect of IL-4 treatment was also tested using CD4(+)CD45RB T cells from mice deficient for α chain receptor of IL-4 (IL-4R-α). The treatment was able to exacerbate colitis in this model, suggesting that IL-4 has a proinflammatory effect in both T cells and non-T cells.[66] To support this evidence, it has been shown that IL-4 therapy for human cancers leads to diarrhea and abdominal pain, related to mucosal ulcerations which resemble the early lesions of CD.[72] On biopsies obtained from ileal resection it has been found an increase of IL-4 m RNA.[73]

The role of IL-4 in the dextran sulphate sodium-induced colitis and in the oxazolone-induced colitis.

The effect of IL-4 in IBD has also been studied by inducing experimental colitis. A study, conducted by Stevceva et al.[74], used the dextran sulphate sodium to induce an experimental colitis, that resembles UC[74-79], in IL-4 deficient mice. The experimental colitis was significantly milder in IL-4 deficient mice, with lower histological activity score. In parallel, they found and increased amount of IFN-γ.[74] This result suggests a role of IL-4 in influencing the severity of the colitis. Another study investigated the role of IL-33 in the same model of colitis.[80] Through its receptor ST2, IL-33 acts in different ways according to the context.[81,82] It is expressed in the epithelium and endothelium, and increases in response to inflammatory stimuli or necrosis.[81,82] It is expressed in almost all innate immune cells[81-87] and can promote Th1/Th17 responses[88,89], as well as Th2 responses and increase of IL-5.[81-88] The study conducted by Pushparaj et al.[80], demonstrated that IL-33 and ST2 are the earliest genes induced in dextran sulphate sodium-related colitis and act as major pathogenic factor in the exacerbation of UC. Furthermore, they showed, for the first time, that the immune response led by IL-33 mandatorily requires the activation of IL-4 to be fulfilled.[80] Furthermore, one study conducted by Boirivant et al. used a haptenating agent, oxazolone, to induce a Th2-related colitis.[90] This colitis only involves the distal half of the colon and its pattern of disease resembles UC.[90] Due to its haptenating power, oxazolone has been shown to stimulate T cells, resulting in increased amount of IL-4, counterbalanced by an increase of TGF-β.[90] Oxazolone colitis is prevented by administering anti-IL-4 agents.[90] This colitis is immunologically similar to that colitis occurring in the TCR-α chain knockout mice described above.[57-59] The two kinds of colitis differ from the extension of disease: oxazolone-induced colitis only involves the distal colon and it is an acute, self-limited inflammation[94], whereas colitis occurring in TCR-α chain knockout mice involves the appendix and then involves the entire colon, persisting as chronic inflammation.[91,92] The difference may be related to the different expression of TGF-β among the two kinds of colitis.[90] Finally, Kasaian et al. have evaluated the possible therapeutic role of IL-4/IL-13 dual antagonist in oxazolone colitis.[93] Due to the pleiotropic activity of most cytokines[94-100], the authors developed a dual antagonist to strengthen the therapeutic activity.[93] IL-13, like IL-4, contribute to intestinal inflammation, as it causes a permeabilization of the epithelial barrier mucosa by inducing the apoptosis of epithelial cells and disruption of tight junctions.[97] By elevating the expression of claudin-2, a tight junction pore protein, IL-13 promotes ion flux across the barrier.[101,102]

This dual antagonist was proved to block both cytokines [93,103] and was shown to ameliorate the course of the disease.[93] Serum concentration of the IL-4/IL-13 antagonist was inversely proportional to disease severity, colon tissue expression of pro-inflammatory genes, and serum amyloid P concentration.[93] This result emphasizes the role of IL-4 and IL-13 in the onset of UC and suggests a possible role for IL-4/IL-13 antagonists in the treatment of UC.

Conclusions.

Many studies have provided evidence for a critical role of IL-4 in the immunopathogenesis of IBD . Specific polymorphisms of IL-4 have been found in crucial loci[44,45]; furthermore, the study of different models of colitis, both in knockout mice[60,61,66] and in experimentally-induced colitis[74,80,90,93], has permitted to understand the eminent proinflammatory activity of IL-4, acting either in the genesis of disease, or during the exacerbations. IL-4 is involved both in innate[41-43] and adaptive immune system[62,64,65]; it can influence the intestinal barrier function[41] and has been proved to stimulate Th2-related responses[62], therefore it seems to play a role in Th2-related disease, like bronchial asthma, atopic dermatitis and UC. Nonetheless IL-4 may interfere with Th1 responses, intensifying the inflammatory process, and may play a role in CD, which is eminently a Th1-related disease [61-62]. For these reasons we hypothesize that antagonists of IL-4, like dupilumab, that has been recently approved for the treatment of mild-to-severe atopic dermatitis[16,17], might play a role in the treatment of IBD and specific clinical trial, at least in UC, should be encouraged.

Conflict of interest

The authors declare that they have no conflict of interest.

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Fort MM et al[66]	IL-4 treatment in CD4(+)CD45RB(high) T cell transfer colitis
Stevceva L et al[74]	Dextran sulphate sodium induced colitis in IL4- and IL4+ mice
Pushparaj PN et al[80]	IL33 treatment in DSS induced colitis and DSS-induced colitis in ST2(-/-) BALB/c mice
Boirivant M et al[90]	Anti IL4, anti IL12 and anti TGF-β treatment in oxazolone induced colitis
Kasaian MT[93]	IL-4/IL-13 antagonist treatment oxazolone induced colitis

Table 1