The alternate effects of anti-TNFα therapeutics and their role in mycobacterial granulomatous infection in Crohn’s disease

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ABSTRACT

Introduction: Crohn’s disease is an inflammatory bowel disease that has been debated to be associated with bacterial triggers such as Mycobacterium avium subspecies paratuberculosis (MAP). Standard treatment of Crohn’s disease (CD) patients includes a family of immunomodulators and biologics such as Anti-Tumor Necrosis Factor alpha (Anti-TNFα). This cytokine in particular has been known to play vital roles in fighting microbial infections through formation and maintenance of granulomas. Areas covered: This perspective is focused on elucidating the negative effects of using Anti-TNFα therapeutic agents as a treatment option in CD patients who are more likely suspected to have MAP infection, and the role of other immunomodulators in MAP infection. Expert commentary: While treatment with Anti-TNFα is beneficial to reduce inflammation and to provide short term relief to the patients, it also compromises the immune system causing susceptibility to microbial infection. More than 50% of CD patients have shown no response to Anti-TNFα treatment which indicates a demand for introducing novel CD treatment in combination with antibiotics as a future CD treatment plan.

1. Introduction

Crohn’s disease (CD) is a chronic relapsing form of inflammatory bowel disease (IBD) affecting the digestive tract. Patients diagnosed with this disease are suffering from abdominal pain, persistent diarrhea, and malnutrition. The prevalence of CD has been rapidly increasing in North America and other countries adapting western lifestyle over the recent decades [1]. Studies involving human genetics, animal models, and clinical trials have indicated new insights into CD pathogenesis [2]. We propose and support the hypothesis that dysregulated immune responses against microbial environmental triggers in genetically susceptible subjects lead to development of CD (Figure 1). Among the most debated and accepted microbial triggers in CD pathogenesis is Mycobacterium avium subspecies paratuberculosis (MAP) [3–5]. However, current standard treatment guidelines for CD do not primarily include antimycobacterial therapy. The ultimate goal is to suppress the abnormal inflammatory immune response by using several anti-inflammatory drugs and biologics, none of which have any meaningful effect to eradication of microbial triggers such as MAP. In some circumstances, CD patients might be prescribed with combinational therapy which includes a biologic and an immunomodulator. As with all therapy, there are many reported adverse effects of CD medications, especially those related to multiple infections [6]. It is also essential to maintain a good nutritional status since CD patients have reduced ability to absorb proteins, carbohydrates, fat, water, vitamins, and minerals.

2. Therapeutic context of targeting cytokines in CD and mycobacterial infection

2.1. Contradicting role of cytokines in mycobacterial infection

Overreactive immune response in CD patients includes significant elevation in tumor necrosis factor alpha (TNFα). In normal state, TNFα is produced by numerous cells, including macrophages, CD4+ and CD8+ T-cells, B-cells, neutrophils, endothelial cells, natural killer cells, smooth muscle cells, fibroblasts, and osteoclasts, where its concentration is undetectable (<10 fg/ml) [7]. In CD, macrophages secrete high level of TNFα (>200 pg/ml) [8,9] which causes upregulation in interferon gamma (IFNγ), interleukin (IL)-6, IL-8, IL-1β, and granulocyte-macrophage colony-stimulating factors (GM-CSFs), leading to cell recruitment and formation and maintenance of granuloma [10] (Figure 2). The latter is an alternative approach selected by the immune system to overcome infection by isolating and neutralizing invasive microorganisms such as MAP [7,11]. Lower TNFα level leads to impairment of the immune system in its effort to eradicate infection. Several studies demonstrated that granulomatous infection has increased significantly in TNFα-deficient animals [12,13]. GM-CSF may suppress the growth of M. avium and M. tuberculosis by activating human macrophages [14]. The opposite is true; GM-CSF might also stimulate the growth of some other intracellular parasites [15]. In vitro studies have also shown that IL-6 increases the human macrophage susceptibility to M. avium infection which could explain susceptibility of patients with
human immunodeficiency virus to mycobacteria and others [16]. Collectively, cytokines might have bidirectional effect on intracellular microbial infections, and this could be by either stimulating or suppressing the macrophages activity directly, or it could affect granuloma formation and maintenance leading to isolation of microbes from the whole system.

2.2. Dual effect of anti-TNFα and other immunomodulators in CD

There are several drugs being prescribed to CD patients which are mainly designed to lower the TNFα in order to reduce inflammation and achieve remission. Etanercept, infliximab, adalimumab, and certolizumab pegol are just examples of anti-TNFα IgG monoclonal antibodies which are widely used for the treatment of CD patients [11]. Anti-TNFα therapeutic agents indirectly inhibit the production of IFNγ produced by activated T-cells [17]. A contradicting effect is expected in cases associated with microorganisms such as MAP, since lowering IFNγ reduces T-cell response, formation of granuloma, and isolation of infecting pathogens [18].

Recent in vitro culture studies reported that some drugs used in CD standard treatment have shown anti-MAP growth activity [19–21]. Altered MAP growth in culture treated with azathioprine (AZA), 6-mercaptopurine (6-MP), cyclosporine A, tacrolimus, and rapamycin has been reported [19,20]. Methotrexate (MTX) inhibited MAP growth in higher potency than 6-MP [21]. MTX could potentially affect MAP growth and survival since it reduces folate generation which interferes with bacterial DNA replication. MTX in lower doses is also known to downregulate pro-inflammatory cytokines which results in clinical improvement in CD patients [22,23]. As shown in Table 1, other drugs used in CD standard treatment have contradicting effect on MAP. Aminosalicylates (5-ASA) intensified MAP growth in culture when the bacteria were exposed to concentrations higher than 25 µg/ml [19]. The negative effect of some of these drugs on MAP growth suggests that response of CD patients to treatment with AZA, 6-MP, cyclosporine A, tacrolimus, and rapamycin may be due, in part, to the detrimental effect of the drugs on MAP in these patients. On the other hand, the favorable effect of 5-ASA on MAP growth is alarming to CD patients treated with 5-ASA. However, these in vitro direct effects might be different from how the drug might interfere with MAP survival under physiological conditions.

While treatment of CD with anti-inflammatory and biologics is necessary to control inflammation and to provide short-term benefit to the patients, a strategy to eradicate infection in cases associated with microorganism such as MAP should be included before selection of treatment. The anti-MAP effect of these nonantibiotic drugs should not be misleading. Bacteriostatic or bactericidal effects of any drug require optimum doses at or above the minimum inhibitory concentration of the drug. Therefore, an alternative combined therapy plan should be considered for long-term remission and possibly a cure from this disease. Basically, an alternative strategy should combine targeted immunotherapy and effective antibiotics where together they can block the source and the signs of inflammation. Effective antibiotic treatment includes selection of specific anti-MAP drugs, the necessary treatment duration, and enforced compliance by the patients. RHB-104 is an investigational anti-MAP formulation consisting of clarithromycin, rifabutin, and clofazimine which is currently being used in an US FDA-approved phase III international clinical trial to treat...
Adalimumab is similar to infliximab in its pharmacokinetics, but the incidence of developing TB has been reported to be greater with infliximab use [7]. This could be justified by differences in the bioavailability and the peak concentration since infliximab is given intravenously while adalimumab is administered as a subcutaneous injection. Anti-TNFα agents increased the risk of granulomatous infection, especially M. tuberculosis in a dose-dependent manner [36]. For instance, patients receiving higher dosages of adalimumab (40 mg/week) have developed active TB [39]. Other granulomatous infections are listed in Table 2.

4. Anti-TNFα treatment is linked to cytotoxicity and autoimmunity

4.1. Cytotoxicity of anti-TNFα therapeutics

The long-term consequences of anti-TNFα medications and their effects at cellular level need further investigation. Infliximab had no effect on human monocytic cell line (THP-1) cell apoptosis in vitro at concentrations as high as 100 µg/ml; however, etanercept and pirfenidone showed a significant reduction in cellular viability at 0.5 and 300 µg/ml, respectively [43].

Treating animal models with monomeric or synthetic dimeric soluble TNF receptors increased serum TNFα after Lipopolysaccharides (LPS) stimulation in comparison to LPS alone [44]. Grattendick et al.’s in vitro study agrees with this result since etanercept but not infliximab elevated cell-associated TNFα up to sixfolds in THP-1 cells following LPS treatment compared to cells treated with LPS alone at 0.1 µg/ml [43]. However, the amount of secreted TNFα was neutralized in the same study following treatment with infliximab and etanercept at 0.1 and 0.01 µg/ml, respectively [43]. Pirfenidone is capable of inhibiting TNFα synthesis at the translational level, and it showed significant reduction of

3. Anti-TNFα therapeutics increase granulomatous infection

The potential role of anti-TNFα therapeutic agents in developing granulomatous infections varies among different treatment options. This might be attributed to several factors, such as differences in their pharmacokinetics. Etanercept for instance has a shorter serum half-life than infliximab or adalimumab, which results in an extended suppression of TNF following treatment with TNF antagonists with longer half-lives [35]. Studies have shown that the incidence of developing tuberculosis (TB) infection is higher after using infliximab in comparison to etanercept [36–38].

### Table 2. Other reported causative agents of granulomatous infections after using antitumor necrosis factor alpha therapeutics.

<table>
<thead>
<tr>
<th>Granulomatous infection causative agent</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Histoplasma capsulatum</td>
<td>[40]</td>
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<tr>
<td>Listeria monocytogenes</td>
<td>[41]</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>[35]</td>
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<tr>
<td>Coccidioides immitis</td>
<td>[35]</td>
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<tr>
<td>Aspergillus fumigatus</td>
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secreted TNFα at 33 µg/ml and cell-associated TNFα at 100 µg/ml in THP-1 cells stimulated by LPS [43,45].

4.2. Autoimmunity and anti-TNFα therapeutics

The immunogenicity of TNF inhibitors is crucial since unintended consequences such as autoimmunity might occur [46]. Various factors influence the rates of immunogenicity such as age, gender, genetics, route of administration, drug dose, and clearance rate. In clinical trials, more than 35% of IBD and rheumatoid arthritis (RA) patients have developed resistance or side effects to anti-TNFα therapeutic agents due to the development of neutralizing antibodies which antagonize the therapeutic effects of these medications [46,47]. Since infliximab has been used for a long time, it is the most extensively studied anti-TNFα therapeutic agent. Infliximab is a chimeric antibody with 25% murine sequence which makes it more susceptible to induce immune reactions characterized by secretion of anti-chimeric antibodies known as human anti-chimeric antibodies (HACAs) [48]. Those antibodies target infliximab by neutralizing its ability to inhibit TNFα and by enhancing its clearance due to immune precipitation, which prevents the drug from reaching the sites of inflammation [49]. HACAs therefore will be able to hinder the clinical efficacy of infliximab by affecting its pharmacokinetics, pharmacodynamics, and bioavailability [50]. Antibodies against etanercept have been detected in less than 5% of patients receiving this treatment; however, those antibodies unlike infliximab antibodies are non-neutralizing [49]. Although adalimumab has a humanized structure, antibodies against it were detected in 12% of treated patients [51]. Furthermore, patients treated with certolizumab pegol has developed antibodies against the drug, but the clinical aspect remains unknown [48]. Researchers have evaluated the presence of specific types of antibodies by quantifying IgM and IgG produced against anti-TNFα therapeutic agents [51]. IgM antibodies were more common in patients treated with infliximab, etanercept, and adalimumab; however, the clinical response was not correlated with the level of antibodies produced, suggesting that the type of antibody (IgG, IgM, or IgA) plays a significant role in developing adverse reactions or altering the therapeutic effects [52].

5. Reported adverse effects of anti-TNFα therapeutics

As previously reported, the most common problem with anti-TNFα therapy is frequent infections [6]. There is a strong affiliation between mycobacterial infection and anti-TNFα therapeutic agents, since the risk for developing TB is higher compared to placebo controls [53]. TNFα-deficient animal models were more susceptible to develop mycobacterial infections in comparison to wild-type controls, although they had no difference in survival rate in a healthy environment [54]. TNFα might have a critical role in the immune defense against mycobacterial infections; however, the link between blocking TNFα and macrophage mycobacterial activity needs further investigation. Indeed, other types of infections have been reported after prescribing anti-TNFα agents, such as meningitis, sepsis, histoplasmosis, and pneumonia [55–57].

Infusion-site reactions have been reported after the use of anti-TNFα agents. It could happen at the infusion time or within 1 h of discontinuation; however, delayed hypersensitivity reactions were reported within 3–12 days after infusion in CD patients, but it was infrequent [6]. Other reported side effects include malignancy, heart failure, neurologic disorders, and diabetes mellitus in young individuals [6]. Most recently approved humanized monoclonal antibodies targeting integrins for CD treatment such as vedolizumab have shown lower incidence of adverse effects compared to other traditional anti-TNFα therapeutics, although natalizumab carries a significant risk of leukoencephalopathy [58].

6. Concluding remarks

Several factors and triggers are involved in CD development including a strong microbial association and genetic susceptibility. We propose and support the hypothesis that dysregulated immune response against MAP in genetically susceptible subjects leads to development of this disease. Current standard treatment guidelines for CD do not primarily include antimycobacterial therapy. They consist of anti-inflammatories, immunomodulators, and biologics which may significantly impair macrophages and formation of granulomas which are vital for isolating and limiting microbial infection. Therefore, alternative combined therapy plan should be considered for long-term remission and possibly a cure from this disease. Combining antibiotics with targeted immunotherapy together is expected to block the source and the signs of inflammation.

7. Expert commentary

Targeting TNF-α has initially shown that antagonizing single cytokine pathway may control the symptoms of CD or IBD in general in addition to other autoimmune disease such as RA. However, anti-TNFα therapy was insufficient to induce response in many CD patients, which indicates that there are other factors influencing CD pathophysiology. Consequently, anti-TNFα therapy could worsen the condition of these particular non-responding patients with inducing multiple infections, especially those affiliated with granuloma maintenance and several unwanted adverse effects [6]. Studies have reported that about 10–30% of IBD patients have shown no initial response to anti-TNFα, and almost half of the patients who showed an initial response have lost it over time [59]. Therefore, there is an extensive search for alternative therapeutic targets such as IL-6, IL-12, and IL-23, in addition to novel inhibition of selective pathways such as using antisense oligonucleotide to target SMAD-7 and small molecules inhibiting JAK-1/JAK-3 pathways [60].

Although many of these investigational medications seem to be promising candidates for effective treatment of CD, none of them is targeting MAP, the hypothesized causative pathogen of CD, except the combinational antibiotic treatment which is currently known as RHB-104 [24,25]. There is an increasing supportive evidence showing the role gut microbiota has in CD pathogenesis [61]. Using antibiotics such as metronidazole in combination with ciprofloxacin and rifaximin has been recommended for active luminal disease involving the colon in some CD patients [61]. Furthermore, one meta-analysis has suggested that combining broad-spectrum antibiotics with immunomodulators improves
clinical outcome of CD patients [62]. Another meta-analysis has demonstrated similar efficacy between using immunomodulators or antibiotics in fistulizing CD with lower incidence of severe adverse effects in patients receiving antibiotics [58]. In contrast, antibiotics might carry a risk for inducing microbial resistance, and before using them for CD treatment, MAP infection must be proven as a strong causative microbial agent affiliated with CD. In addition to that, CD patients must be informed for drug compliance in order to avoid antibiotic nonadherence.

8. Five-year view

For many decades, CD has been widely known as an autoimmune disorder which has been treated with anti-inflammatory and immunosuppressant drugs. Like other autoimmune disorders, multifactors have been associated with disease pathogenesis. There is a compelling evidence pointing to MAP as a major bacterial infectious agent involved in this disease. The progressive understanding of the immunopathogenesis of CD has opened more avenues into targeted therapy. However, two issues remain to be addressed:

- Screening programs should cover all CD patients in order to detect any persistent bacterial infection.
- Positive status for MAP infection should be eradicated with antibiotics targeting this pathogen.

It is likely that in the next 5 years, screening programs will be more efficient to detect MAP infection in the blood or intestinal tissues of CD patients. Once the patient is diagnosed with MAP infection, it is highly recommended to start him/her on anti-MAP therapy which is currently in phase III international clinical trial. Successful treatment of CD with antibiotics might induce disease remission instead of controlling chronic symptoms with immunomodulators, especially when combining them with new novel CD therapeutics targeting other cytokine pathways aside from TNFα.

Key issues

- Crohn’s disease (CD) pathophysiology involves aggressive immune responses in genetically susceptible subjects due to enteric bacterial infection, in addition to other environmental disease triggers.
- The most investigated and debated microbial causative agent involved in CD pathogenesis is Mycobacterium avium subspecies paratuberculosis (MAP).
- The current standard treatment guidelines for CD do not primarily consider a microbial association as possible cause for the disease pathogenesis, and the treatment is focused on a group of anti-inflammatory compounds, immunosuppressants and biologics which mainly target the tumor necrosis factor-alpha (TNFα).
- Antagonizing TNFα increased the risk of granulomatous infection, especially M. tuberculosis, which raises a concern about the urge of introducing antibiotics to CD treatment regimen with bactericidal effects such as the investigational medication RHB-104.

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Declaration of interest

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