TNF-α: A POTENTIAL THERAPEUTIC TARGET FOR INFLAMMATORY BOWEL DISEASE

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ABSTRACT

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), represents a group of chronic disorders characterized by inflammation of the gastrointestinal tract, typically with a relapsing and remitting clinical course. Cytokines play a key role in the development, recurrence and exacerbation of the inflammatory process in IBD. Advances in the knowledge of pathophysiology of IBD have highlighted the importance of cytokines mainly tumor necrosis factor alpha (TNF-α) in the inflammatory process and thus targeting TNF-α is a rational approach for the treatment of IBD. There exist several TNF antagonists in various phases of investigation, including the monoclonal antibody CDP571, the fusion peptide etanercept, the phosphodiesterase inhibitor oxpentifylline, and thalidomide. Despite some immunological side effects, anti-TNF-α therapeutic strategies represent an important breakthrough in the treatment of inflammatory diseases including IBD. This review mainly focuses on the present understanding of TNF-α-mediated biology and the current therapies in clinical use. Also, some of the new therapeutic approaches with small-molecule inhibitors have been discussed.

Keywords: Inflammatory Bowel Disease (IBD), Tumour Necrosis Factor-α (TNF-α), Infliximab, CDP571, Etanercept.

INTRODUCTION

Inflammatory bowel disease (IBD) comprises two forms, Ulcerative colitis (UC) and Crohn's disease (CD). Currently, the pathogenesis of UC and CD is not completely understood, although the chronic relapsing inflammation is thought to be result from a dysregulated, aberrant immune response to interstitial flora[1] and its development is influenced by genetic, environmental and immunological factors (Fig. 1)[2]. The inflammatory reaction in IBD is mediated by multitude of factors derived from a number of immune cells[3,4]. Manifestations of the disease can be severe and lead to long term therapy with a variety of medications and/or surgery[5]. Apporoximately 70% of individuals suffering CD require surgery; 20-25% of individuals suffering from UC ultimately come to surgical intervention[3].Standard medical therapy consists of agents that either treat suppurative complications or modulate the inflammatory cascade in a nonspecific manner. Many specific chemokine and cytokine effectors that promote intestinal inflammation have been identified. Such work has led to experimental clinical trials with a variety of cytokine antagonists. Compounds directed against one such cytokine, tumor necrosis factor α (TNF-α or TNF); have demonstrated the greatest clinical efficacy to date[5].

Fig. 1: Hypothesis of etiology and pathogenesis of inflammatory bowel disease[6].

Cytokines are key signals in the intestinal immune system, and are known to participate in the disruption of the normal state of controlled inflammation (physiological inflammation of the gut)[7]. They are small peptide proteins produced mainly by immune cells that facilitate communication between cells, stimulate the proliferation of antigen specific effector cells and mediate the local and systemic inflammation in an autocrine, paracrine and endocrine pathways[8]. TNF-α is a monocyte and T cell-derived cytokine with pleisotropic biologic effects[9]and was first identified in 1975 as an endotoxin-induced glycoprotein[10].

It is produced by many cell types. The main sources in vivo are stimulated monocytes, fibroblasts and endothelial cells. Macrophages, T-cells, B-lymphocytes, granulocytes, smooth muscle cells, eosinophils, chondrocytes, osteoblasts, mast cells, glial cells and keratinocytes also produce TNF-α on stimulation[11].

TNF-α has been implicated in diverse range of inflammatory, infectious and malignant conditions, and its importance in inflammation is highlighted by efficacy of anti-TNF antibodies or administration of soluble TNF receptors (TNFRs) in controlling disease activity in various inflammatory conditions[12]. Several studies reported increased TNF-α and messenger RNA levels in mucosal biopsies from IBD patients, particularly in Crohn's disease[13,14].

TNF-α and TNF Receptors

TNF-α is a protein of 185 amino acids glycosylated at positions 73 and 172[11]. It is produced predominantly by activated macrophages and T-lymphocytes as a 26 kDa protein (pro-TNF), which is expressed on the plasma membrane where it can be cleaved in the extracellular domain by the matrix metalloproteinases, which results in the release of a soluble 17 kDa soluble form. Both membrane-associated and soluble TNFs are active in their trimeric forms. TNF-α converting enzyme (TACE) mediates the release of TNF-α from cell surface which acts on ubiquitously expressed TNF-α receptors 1 and 2[12,15].

TNF-α and its specific receptors TNFR1/TNFR2 are the major members of a gene superfamily of ligand and receptors that regulates essential biologic functions. The extracellular domains of TNFR1 and TNFR2 are homologous and manifest similar affinity for TNF-α, but the cytoplasmic regions of the two receptors are distinct and mediate different downstream events. Signalling via TNFR1 is the major mechanism responsible for the effects of TNF-α. Soluble TNF-α receptors (sTNFR) derived directly from proteolytic cleavage of membrane TNFR1 and TNFR2 and are present at low
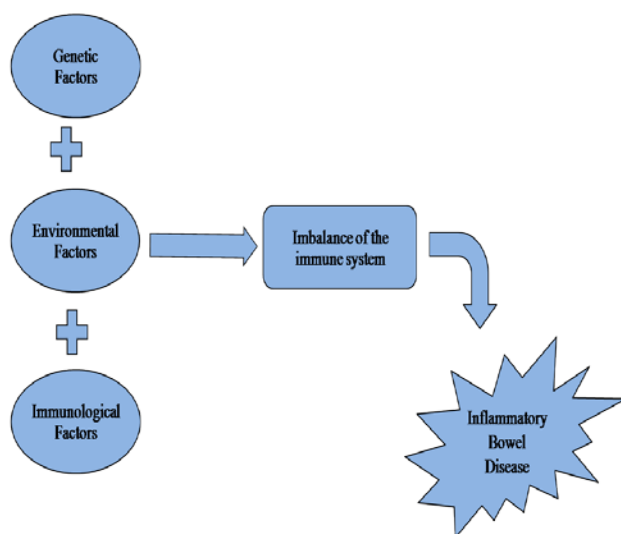
concentrations in the plasma of healthy individuals. Elevated circulatory levels of the these soluble forms may reflect ongoing inflammation[17,18].
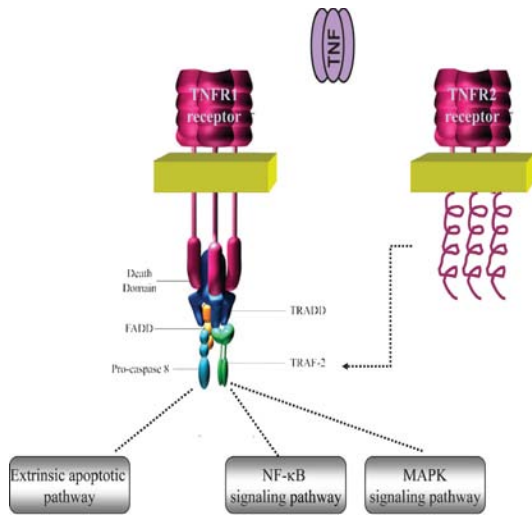

Fig. 2: **TNF-α receptors[16]**

Receptor activation by TNF family ligands causes recruitment of various adaptor proteins with subsequent activation of downstream signalling pathways. TNFR superfamily can be classified in three major groups according to specific intracellular sequences and to signalling adaptors recruited[16]. The first group include receptors, such as TNFR1 (p55 or 55-kD TNFR), Fas and various others, that share a highly conserved sequence of about 80 amino acids in the cytoplasmic region called the death domain.

Activation of these receptors leads to homotypic interactions with adaptor proteins containing death domains such as Fas-associated death domain (FADD) and TNFR-associated death domain (TRADD)[19,20,21]FADD is the proximal transducer of apoptosis initiated by the FasL/Fas interaction, whereas TRADD is the proximal transducer of apoptosis triggered by the interaction between TNF-α and TNFR1.

The latter signaling pathway requires an interaction between TRADD and FADD, which in turn interact with caspase-8[22], and occurs only when protein synthesis is blocked. However, recruitment of TRADD can also trigger downstream events related to inflammation[23]through further adaptor proteins including TNF receptor associated factors (TRAFs), receptor interacting protein (RIP) and mitogenactivated kinase-activating death domain (MADD)[19]. Therefore, activation of TNFR1 may induce apoptosis as well as inflammation.

The balance between these two pathways is extremely delicate and regulated at numerous levels. The second group include receptors, such as TNFR2 (p75 or 75-kD TNFR), CD30, CD40 and others, that contain in their cytoplasmic region specific amino-acid sequences called TNFR-associated factors (TRAFs) interacting motifs (TIMs). However, the recruitment of TRAFs seems necessary for TRADD activation and TNFR-1-mediated downstream events.

Thus, it has been speculated that TNFR2 may serve to increase TNFR1 signalling through TRAFs recruitmentor, alternatively, as a counter-regulatory to suppress TNF-α mediated signalling. All nucleated cells express TNF receptors, although their distribution varies with cell type. TNFR1 is expressed constitutively on most cell types, whereas expression of TNFR2 can be induced. In addition, TNFR2 is restricted to certain cell types and can discriminate TNF-α from different species[16].

The receptor-ligand interaction causes intracellular signaling without internalization of the complex, leading to phosphorylation of IκBα and thus activation of nuclear factor κB (p50-p65) heterotrimer, which then interacts with the DNA chromatin structure to increase transcription of proinflammatory genes such as IL1B, IL6, IL8 and TNFA. The response to TNF-α activation is

balanced by shedding of extracellular domain of the TNF-α receptors. The schematic representation of mechanisms involved in TNF-α biology and signaling is shown in Fig. 3.
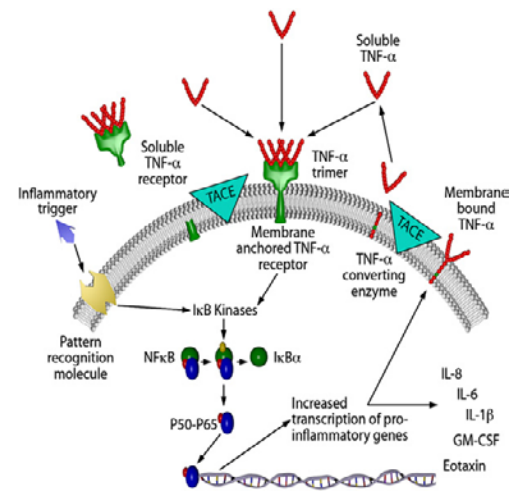

Fig. 3: Summary of TNF-α biology and cascade of TNF-α signalling[15]

TNF-α and Inflammation

Under physiological homeostatic conditions the biological functions of family of cytokines encompasses beneficial and protective effects in both innate immunity and haematopoieses and also plays crucial role in organogenesis[24,25]. TNF-α is known to have numerous biological properties relating to inflammation, proliferation, differentiation and cancer growth.

Also it plays central role in shock due to sepsis and wasting syndromes due to variety of cancer[3,4]. Transcription of TNF-α gene in activated monocytes, macrophages, platelets, adipocytes and T cells results in secretion of TNF-α[26]. Circulating soluble TNF-α binds to two TNF-α receptors mediating multiple biologic effects, including activation of macrophages, further augmentation of T cell response, expression of adhesion molecules, recruitment of neutrophiles to local site of inflammation and induction of granuloma formation[27].

Although TNF receptors are differentially expressed on a wide range of cells and tissues, many of the proinflammatory effects of TNF can be explained on the basis of TNF's effects on vascular endothelium and endothelial leukocyte interactions. In response to TNF, endothelial cells promote inflammation by displaying, in a distinct temporal, spatial and anatomical pattern, different combinations of adhesion molecules for leukocytes, including E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).

In combination with the release of chemokines[28], these responses lead to recruitment of different populations of leukocytes independent of antigen recognition. In addition, many of the classical features of inflammation can be produced by local effects of TNF on endothelial cells. TNF-induced expression of cyclooxygenase 2 can increase production of vasodilatory PGI_2 resulting in vasodilatation[29], causing 'rubor' and 'calor' through increased local blood flow. 'Tumour' can result from TNF-mediated increased vascular permeability, allowing the increased trans-endothelial passage of fluid and macromolecules to create oedema[30].

In vivo administration of bacterial lipopolysaccharide (LPS) induces high level of TNF-α production in animal models. Also a high level of TNF-α is observed in human subjects when bacterial endotoxin was administered. Numerous in vitro and in vivo studies indicate that high level generation of TNF-α leads to exacerbation of inflammatory and prooxidative responses that are important in the pathogenesis of many diseases including artherosclerosis, rheumatoid arthritis, inflammatory bowel disease, and various pulmonary conditions[31].

Role of TNF-α in Inflammatory Bowel Disease

TNF-α mediates multiple proinflammatory signals, that play a central role in pathogenesis of IBD, including neutrophil recruitment to local site of inflammation, activation of both coagulation and fibrinolysis and induction of granuloma formation[32]. The biological effects of TNF-α are schematically represented in the Fig. 4.

TNF-α has been found in the serum, stool and intestinal tissue of patients with IBD. The specificity remains unclear and may be comparable to other forms of inflammation. Cells that express TNF-α can be found in the gut mucosa and lamina propria of CD patients[33]. In CD tissues, TNF-α positive cells have been found deeper in lamina propria and in the submucosa, whereas TNF-α immunoreactivity in UC is mostly located in subepithelial macrophages[14].

Nuclear factor kappa beta (NF-κβ) is a pivotal transcription factor that increases the expression of many cytokines, enzymes, and adhesion molecules. TNF-α also prolongs inflammation by activating NF-κβ-dependent pathways, which contribute to ulceration and degradation of the mucosa through the release of matrix metalloproteinases[34,35].

Increased TNF-α production and NF-κβ nuclear translocation have been noted in lamina propria mononuclear cells derived from both CD and UC patients[36]. Lipopolysaccharide-induced TNF-α factor, a transcription factor that binds to the TNF-α gene and promotes TNF-α expression in human macrophages, was discovered in the colonic tissue and macrophages in both patients with CD and UC[37]. TNF-α upregulates other proinflammatory mediators such as IL-6 and IL-1β thus amplifying the early sequences of the inflammatory cascade[38].
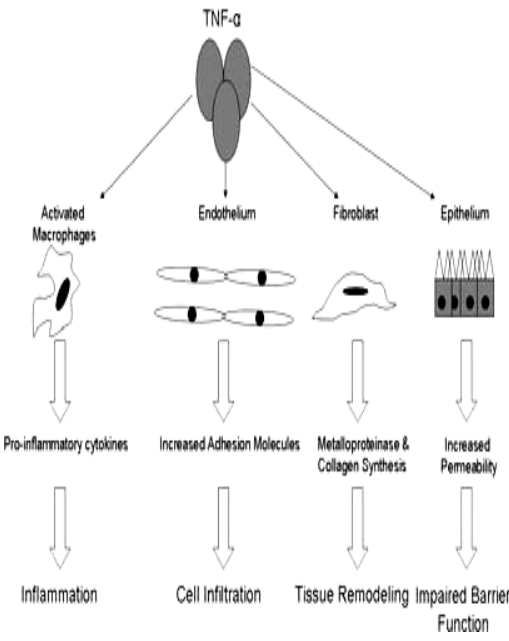

Fig. 4: Biological effects of tumor necrosis factor alpha (TNF-α) [27]

In Fig. 5, the potential inflammatory effects of mucosal overexpression of TNF are summarized. Activation of mucosal immune cells (such as macrophages [Møs]) leads to secretion of TNF. Bacterial products are an example of one such secretory stimulus. (1) TNF induces the production of matrix metalloproteinases (MMPs) from stromal cells that contribute to tissue injury. (2) TNF directly activates endothelial cells (EC) by upregulating adhesion molecules. (3) It also induces the secretion of cytokines and chemokines from both endothelial cells and mucosal mononuclear cells. (4) TNF upregulates interferon g (IFN-g) production by mucosal T cells. (5) Epithelial cells secrete chemokines in response to TNF and contribute to the recruitment of inflammatory cells to the epithelium and submucosa. (6) TNF in combination with IFN-g may lead to apoptosis of epithelial cells with a resultant "leaky" mucosal barrier and perpetuation of inflammation. (7) TNF activates and mobilizes immature dendritic cells (IDC) to the regional lymph nodes. (8) These lymph nodes initiate an adaptive immune response. (9) Monocytes and activated T cells are recruited to the mucosa by the cascade of inflammatory signals and mediators, ultimately leading to granuloma formation.
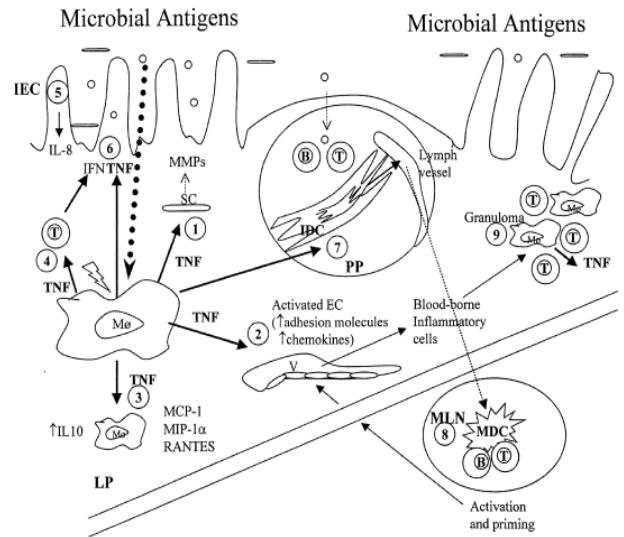

Fig. 5: The potential inflammatory effects of mucosal over expression ofTNF[5]

B: B lymphocyte; IEC: intestinal epithelial cells; IL: interleukin; LP: lamina propria; MCP-1: monocyte chemotactic protein 1; MDC: mature dendritic cells; MIP-1a: macrophage inflammatory protein 1a; MLN: mesenteric lymph node; PP: Peyer's patch; RANTES: regulated on activation, normal T cell expressed and secreted; T: T lymphocyte; V: venule.

TNF-α and T cell Apoptosis

The T cell is central figure in the immunopathogenesis of CD, and recent evidence suggests that T lymphocytes isolated from patients with CD are resistant to apoptotic (programmed cell death) signals. This observation is thought to be because of an imbalance of cellular concentrations of Bcl-2 (an antiapoptotic protein) and BAX (a proapoptotic protein)[39]. Initiation of apoptosis can be signalled through two major pathways, which cross-talk abundantly: the extrinsic pathway is promoted by transmembrane death receptors such as TNFR, whereas the intrinsic pathway seems to be critically regulated by alterations in mitochondrial permeability[16].TNF-α antagonism may specifically augment apoptosis of these long-living and proliferating T cells[40]. In contrast, TNF antagonism does not necessarily lead to apoptotic cell death in intestinal epithelial cells[41]. Clinical observations support the central importance of T cells in CD. Decreased disease activity has been noted in some CD patients who develop weakened T-cell function[42] and increased disease activity has been noted in CD patients who have stimulated T-cell function[40].

Anti-TNF therapy in IBD

Initially, UC was felt to be solely mediated by a T helper 2 (Th-2) response, which would not involve TNF-α in its pathogenesis. These conceptions led to delays in studies of anti-TNF agents. A better understanding of the immune and inflammatory mechanisms involved in IBD has led to development of biologic therapies that target key proinflammatory molecules such as TNF-α. Abundant evidence demonstrated the importance of TNF-α in IBD, providing a rationale for the use of biologic anti-TNF-α treatments. There are several ways of inhibiting TNF-α, including binding and neutralizing the molecule by monoclonal antibodies, blocking TNF-α production and secretion either directly or indirectly, and inhibiting soluble TNF-α receptors[43].

Mechanisms of TNF-α blockade

Early efforts at understanding how anti-TNF biologies work in immune-mediated disease centered on the ability of anti-TNF agents to neutralize soluble TNF-α or to block TNF receptors from binding to their ligands[44]. More recent models postulate that anti-TNF biologies may work by affecting intracellular signaling, with the end result being a hastened cell cycle arrest, apoptosis, or suppression of cytokine production[45]. These in vitro and few in vivo studies that have given some insight into possible mechanisms by which anti-TNF agents may improve disease are summarized in Fig. 6. Following evidences support that anti-TNF biologies might correct some of the impairments.
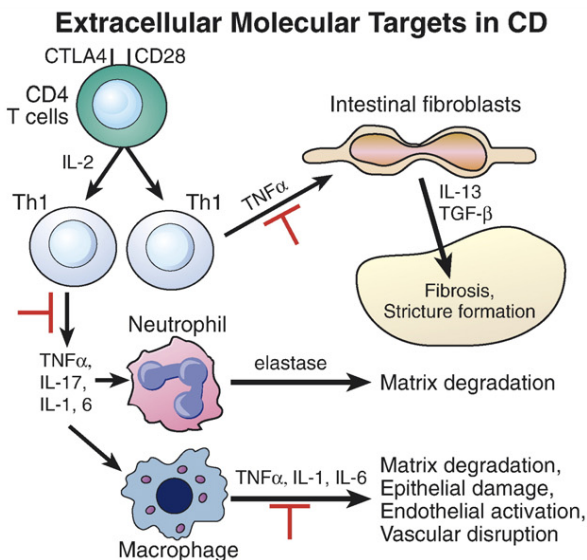

Fig. 6: Extracellular molecular targets of TNF antagonists in Crohn's disease (CD), pathways which TNF antagonists may block are shown by red symbol[46]

Effect on apoptosis

One of the best-described mucosal immune derangements in IBD is a resistance of lamina propria T cells to apoptosis. Anti-TNF agents are able to induce apoptosis of activated lymphocytes in inflamed biopsies or peripheral blood. One study performed colonoscopy with biopsy in 10 IBD patients before, and 10 weeks after a course of infliximab (administered at weeks 0, 2 and 6) and in 10 control subjects with functional diarrhea[46]. They found that patients treated with the infliximab had increasing number of TUNEL-staining apoptotic lamina propria T cells, approaching the number of apoptotic lamina propria T cells of those in the control group[47,48].

Effect on intracellular signaling

Some of the anti-TNF agents act on several cellular targets, including proteins that regulate apoptosis, progression through cell cycle, and expression of cytokines. Infliximab binding transiently stimulates p38 MAP kinase both in vivo in patients with CD, as well as in vitro[49].

Effect on cytokine production

The immunopathogenesis of CD culminates in inflammation via interplay of several cytokines including not only TNF-α but also others including IL-1β and IFNγ. Anti-TNF agents exert its anti-inflammatory effects by acting on multiple constituents in cytokine network.

Effect on barrier function

The epithelial cells are prime players in innate immune response and they have been implicated as playing a major role in the dysregulated immune homeostasis of IBD. Foci of epithelial cell apoptosis may be the cause of increased ionic permeability in IBD. It is proved that patients with CD had a higher index of apoptotic epithelial cell foci than healthy control patients treated with anti-TNF agent had epithelial barriers that were restored to normal[46].

Anti-TNF agents

Different therapeutic approaches have been focused on inhibition of TNF-alpha in patients with IBD. Monoclonal antibodies such as; infliximab, adalimumab, certolizumab (CDP870) and CDP571; the p75 soluble TNF receptor fusion protein etanercept and p55 soluble TNF receptor onercept are most studied ones. Some of them have failed in clinical studies and different phases of clinical studies are still ongoing for some of them[50]. Two monoclonal antibodies (mAb) and a soluble receptor, which bind to soluble and membrane forms of TNF-α and can neutralize the pathological effects of TNF-α are licensed for clinical use[51].

Monoclonal antibodies and fusion proteins

Antibodies serve as adapters connecting antigens to effector molecules. Antibody binding alone may provide sufficient neutralization although cell lysis via complement fixation or antibody-dependent cellular toxicity is another potential mechanism to reduce cytokine production. Attempts to therapeutically administer murine monoclonal antibodies (100% murine antibody) created using hybridoma technology resulted in significant human immune responses due to the formation of human antimouse antibodies (HAMAs) directed against the constant region of the mouse antibody. With repeated treatment, formation of HAMAs caused both a shortening of the half-life for the murine monoclonal antibody (due to increased clearance of the antibody from the serum) and hypersensitivity reactions[33].

Protein engineering techniques that transplant antigen binding sites from murine to human antibodies have been used to decrease the amounts of murine protein in therapeutic monoclonal antibodies, thus reducing immunogenicity. The first generation of engineered monoclonal antibodies were simple chimeric monoclonal antibodies in which the variable domains of a mouse monoclonal antibody were transplanted to the constant domains of human antibodies. The resulting chimeric monoclonal antibody is approximately 75% human and 25% murine. While chimeric monoclonal antibodies are less immunogenic than murine monoclonal antibodies, they still result in the formation of human antichimeric antibodies (HACAs)[52] directed against transplanted variable domain from the mouse. However, these chimeric monoclonal antibodies appear to have better pharmacokinetics than murine monoclonal antibodies, with half-lives significantly extended in humans[33].

The second generation of engineered monoclonal antibodies were "humanized" by transplanting the antigen binding region (complimentarity determining regions) of murine variable domains to human antibodies. The resulting humanized monoclonal antibody is approximately 95% human and 5% murine. While expected to be less immunogenic than chimeric monoclonal antibodies, humanized monoclonal antibodies do result in formation of human antihuman antibodies (HAHAs) directed against both the variable regions (mouse and human) and the allotype (human) antibody structures[53,54,55]. Similar to chimeric monoclonal antibodies, these humanized monoclonal antibodies appear to have better pharmacokinetics than murine monoclonal antibodies, with significantly prolonged half-lives in humans[53,56].

The third generation of engineered antibodies are more properly termed "fusion proteins." Fusion proteins are created by linking DNA encoding a human protein receptor to DNA encoding the Fc portion of a human antibody, followed by expression of the DNA in a mammalian cell line. The resulting recombinant protein is a 100% human, immunoglobulin-like dimer. Even these completely human recombinant proteins have some immunogenicity and can result in the formation of HAHAs. The fusion of the Fc portion of an antibody to soluble protein receptors significantly prolongs the halflives of soluble receptors. Selection of different constant domains has implications for the effector function of chimeric and humanized monoclonal antibodies and fusion proteins[33].

Infliximab

Infliximab is a monoclonal antibody (25% murine, 75% human) directed at TNF-α and is indicated for the treatment of CD and rheumatoid arthritis. The mechanism of infliximab is not completely

understood; however, direct neutralization of TNF-α does not entirely explain its effect. Infliximab binds both to soluble and transmembrane TNF-α. In contrast, etanercept, which is a TNF-α inhibitor that only neutralizes soluble TNF-α, is not effective in treating IBD. Infliximab has been found to exert a proapoptotic effect on monocytesand T cells in the lamina propria of the gut. In T cells, the proapoptotic effect of infliximab may be exerted by inhibiting the production of granulocyte macrophage colony-stimulating factor, which is a growth factor that stimulates granulocyte growth and differentiation and activates neutrophils with enhanced adhesion[27].

ACCENT I[57] and ACCENT II[58] were two important multicenter, randomized, double blind, placebo controlled trials, upon which the approval of the drug for CD was based in 1998. In the ACCENT I trial, the aim was to assess the efficacy and safety of repeated infusions of infliximab in patients who improved after an initial infusion, also corticosteroid sparing effects and safety in a large number of patients. The results showed that patients with CD who respond to an initial dose of infliximab are more likely to be in remission at weeks 30 and 54, to discontinue corticosteroids, and to maintain their response for a longer period of time if infliximab therapy is maintained every six weeks. Maintenance infliximab treatment was safe and genarally well tolerated.

ACCENT II trial also included adults with CD who had single or multiple draining abdominal or perianal fistulas, of at least three months duration. ACCENT II evaluated the efficacy and safety of repeated infusions of infliximab in maintaining closure of draining fistulas among patients who had a response to a three-dose induction regimen of infliximab. Among patients with fistulizing CD whose fistulas closed after infliximab induction therapy, continued infliximab infusions at fixed intervals maintained closure for a longer period than placebo infusions. Today infliximab has become the drug of choice in fistulizing patients.

There are few small studies and case reports of infliximab in patients with active ulcerative colitis that have showed conflicting results. Two randomized, doubleblind, placebo-controlled studies namely the Active UC Trials 1 and 2 (ACT 1 and ACT 2, respectively) evaluated the efficacy of infliximab for induction and maintenance therapy in adults with ulcerative colitis[50].

Clinical response or clinical remission with discontinuation of corticosteroids at week 30 in both studies and at week 54 in ACT 1, a clinical remission and mucosal healing at weeks 8 and 30 in both studies and at week 54 in ACT 1, and a clinical response at week 8 in patients with a history of disease refractory to corticosteroids were assessed. In each study, 364 patients with moderate-to-severe active ulcerative colitis despite treatment with concurrent medications received placebo or infliximab (5 mg or 10 mg per kilo gram of body weight) intravenously at weeks 0, 2 and 6 and then every eight weeks through week 46 (in ACT 1) or week 22 (in ACT 2).

Patients were followed for 54 weeks in ACT 1 and 30 weeks in ACT 2. In both studies, patients with moderate-to-severe active ulcerative colitis treated with infliximab at weeks 0, 2, and 6 and every eight weeks thereafter were more likely to have a clinical response at weeks 8, 30, 54 than those receiving placebo.

ACT 2 also helped us to understand the pathogenesis of UC. UC is believed to result from an immune response of type 2 helper T cells in the colonic mucosa, whereas CD is accepted as an immune disease of type 1 helper T cells, which would suggest that TNF-α is not a potent mediator in ulcerative colitis. The study showed that TNF-α plays an important role in the disease process and targeting this cytokine is an effective therapy for ulcerative colitis. The mechanism of action of infliximab in ulcerative colitis also includes the induction of apoptosis of inflammatory cells expressing membrane-bound TNF-α, as in Crohn's disease[48]. Based on the results of ACT 1 and ACT 2, infliximab has recently been approved for maintaining clinical remission and mucosal healing in patients with moderately to severely active ulcerative colitis, who have had an inadequate response to conventional therapy.

Infliximab has been associated with hypersensitivity reactions that include urticaria, dyspnea and hypotension, and usually occur within 2 hours of infusion. Serum sickness-like reactions were observed in some CD patients 3 to 12 days after therapy was reinitiated following an extended period without infliximab. Fever, rash, headache, sore throat, myalgias, polyarthralgias, hand and facial edema and dysphagia were also associated with a marked increase in antibodies to infliximab[16].

Adalimumab

Adalimumab is a fully human IgG$_1$ monoclonal antibody to TNF-α that is administered subcutaneously and has a long half life. It binds to human TNF-α, thereby interfering with binding to TNF-alpha receptor sites and subsequent cytokine-driven inflammatory processes. Most of the antibodies that develop against infliximab are presumably directed towards the murine portion of the molecule and will not cross react with the fully human adalimumab. The rate of formation of antibodies to adalimumab observed in clinical trials of Crohn's disease has been very low (approximately 3%)[50] Adalimumab side effects are similar to infliximab. Infusion reactions seen with infliximab do not occur with adalimumab, although the injections can be temporarily painful. Adalimumab has short-term benefits in CD and is tolerated and effective in patients who have lost their response to infliximab[59].

A previous phase 3 study (CLASSIC [Clinical assessment of Adalimumab Safety and efficacy Studied as an Induction therapy in Crohn's] I) demonstrated that adalimumab is effective for induction of clinical response and remission when administered as a loading dose in patients with moderately to severely active Crohn's disease who were naive to infliximab and other anti-TNF therapy. CLASSIC II was a subsequent pilot maintenance trial which demonstrated that adalimumab administered at doses of 40 mg every other week and 40 mg weekly was effective in maintaining remission in patients with adalimumab-induced clinical remission.

More recently, phase 3 double blind, placebo controlled trial–CHARM (Crohn's trial of the fully Human antibody Adalimumab for Remission Maintenance) study demonstrated the safety and efficacy of adalimumab administered 40 mg weekly vs every other week for maintenance of clinical remission in patients who had responded to induction therapy with adalimumab. Adalimumab was also found to be effective in sustaining clinical remission in CD regardless of whether patients received concomitant immunosupressive therapy with azathioprine, 6-mercaptopurine or methotrexate, and regardless of whether patients had previously been exposed to anti-TNF therapy with infliximab. Additional results of CHARM study showed that adalimumab is effective in the healing of draining fistulas in patients with CD. GAIN (Gauging Adalimumab efficacy in Infliximab Nonresponders) is the most recent double-blind placebo-controlled trial.

This was conducted to determine the efficacy and safety of adalimumab in the induction of response and remission in patients with Crohn's disease who had previously responded to therapy with infliximab and then lost response or became intolerant (secondary failure). Induction of clinical remission at week 4 was significantly higher in the adalimumab-treated group as compared to placebo-treated group. According to the results of three randomized, doubleblind, placebo-controlled trials–GAIN, CLASSIC and CHARM–FDA has granted priority review to adalimumab. Adalumimab is certainly one of the most promising drugs for the near future for the treatment of IBD[50].

Certolizumab

Certolizumab is a polyethylene glycolated (PEG) Fab′ fragment of a humanized anti-TNF monoclonal antibody made by microbial fermentation with *Escherichia coli*. PEG increases its circulating half-life to approximately 14 days, which is that of a whole antibody, and it is much longer than the half-life of unconjugated Fab′ fragments. This antibody has been developed to address the concerns that some toxicity associated with infliximab and adalimumab might be due to Fc-associated effects on complement activation and antibody dependent cytotoxicity[46].

In contrast to the IgG$_1$ antibodies, certolizumab does not appear to induce apoptosis. A previous phase 2 study demonstrated that certolizumab pegol may be efficious in the induction of clinical

response and remission when administered at a dose of 400 mg at weeks 0, 4 and 8 in patients with moderately to severely active Crohn's disease.

Although all certolizumab doses produced significant clinical benefit over placebo at all time points, clinical response rates were highest for 400 mg doses when compared 100 and 200 mg doses. Therefore, at week 12, no significant statistical benefit was found over placebo. PRECISE (Pegylated antibody fragment evaluation in Crohn's disease safety and efficacy) 1 is the phase 3 study to evaluate the efficacy in induction of response. PRECISE 2 was conducted to evaluate the maintenance response. The results showed that clinical response and clinical remission rates were significantly higher than placebo[50].

CDP571

A humanized monoclonal antibody to human TNF-α initially named CDP571 was constructed by transplanting the complementarity determining region of a mouse antihuman TNF monoclonal antibody to human IgG$_4$ with k light chains. CDP571 has a strong binding affinity to soluble trimers of TNF-α (kd 100 pM)[33] and is believed to bind to the transmembrane form of TNF-α. CDP571 and other investigational versions of humanized IgG$_4$ anti-TNF antibodies neither fix complement nor mediate antibody-dependent cytotoxicity. Thus, the in vivo effect of CDP571 is believed to result from the binding of soluble trimers of TNF-α and membrane bound TNF-α[60].

A small, randomized, double-blind, placebocontrolled, multi-center Phase IIa trial with CDP571 for CD has been reported. The trial was comprised of 30 patients with mildly to severely active CD refractory to medical therapy[61]. Patients were treated with a single dose of placebo or CDP571 5 mg/kg and followed for 8 weeks.

The primary endpoint for the study was a decrease in the median Crohn's Disease Activity Index (CDAI) score at 2 weeks. After receiving CDP571, the median CDAI score decreased significantly from 263 points at baseline to 167 points at week 2, in contrast, there was no significant decrease in the median CDAI score of the placebo treated group. These results suggested that CDP571 5 mg/kg may have short-term efficacy in mildly to severely active CD refractory to medical therapy. The optimal dose and dosing interval for CDP571 therapy for improvement, induction of remission, and maintenance for patients with CD remain to be determined[33].

A Phase I, open-label trial with CDP571 for UC has been reported from a single center[62]. Fifteen patients with mildly to moderately active UC, some of whom were refractory to medical therapy, were treated with a single dose of CDP571 5 mg/kg and followed for 8 weeks. The primary endpoint was a decrease in the mean Powell Tuck score over the course of the study. The mean Powell Tuck score decreased significantly from 6.7 to 4.6 points at week 1, and there was a trend towards a decreased score at week 2 but not at weeks 4 or 8. These results suggest a possible short-term benefit from CDP571, 5 mg/kg (up to 2 weeks) in mildly to moderately active UC. Additional studies are needed to prove efficacy and to determine the optimal dose and dosing interval[63].

Natalizumab

Natalizumab, a humanized monoclonal antibody to alpha-4 integrin, is administered intravenously. It had been approved for use in the treatment of multiple sclerosis and has been evaluated for treatment of Crohn's disease[50,64]. The report of 3 cases of multifocal leucoencephalopathy caused by the human polyoma JC virus in patients with multiple sclerosis and Crohn's disease treated with this agent, led to an estimated risk of 1:1000[50]. As a result marketing of the drug was suspended.

Soluble TNF-α receptors

Soluble TNF receptors bind to and inactivate TNF-α, effectively lowering the amount of TNF-α that is available for binding to membrane-bound receptors[46]. Unlike rheumatoid arthritis, soluble TNF-α receptors have not proven effective in the treatment of IBD[27].

Etanercept

Etanercept is a dimeric fusion protein consisting of two extracellular domains of the human p75 (75 kDa) TNF receptor (sTNFRII), linked to the Fc portion of a type 1 human immunoglobulin (IgG1). The Fc portion helps to retain the molecule in the circulation. By competitive inhibition, the two sTNFRII arms bind two of the three receptor-binding sites on the TNF trimer. TNF binding to the cell surface receptors is prevented, signal transduction is checked, and hence TNF-α-induced proinflammatory activity is inhibited. It is found that low concentrations of soluble TNF receptors stabilize the structure of TNF-α, but higher concentrations inhibit TNF-α binding to TNFRI and TNFRII on target cells[46]. As etanercept is a total human protein and it has less immunogenic effect in comparison with infliximab[16]. Although etanercept is a TNF-alpha blocker, it is not approved and marketed for inflammatory bowel disease. A randomized, controlled trial showed that etanercept was no better than placebo in inflammatory bowel disease[65].

Onercept

Onercept is a fully human recombinant soluble TNF p55 receptor administered subcutaneously. A clinical benefit was observed in a pilot study involving 12 patients with active Crohn's disease. However a subsequent placebocontrolled, phase 2 trial found no significant benefit for clinical response or induction of remission[50].

TNF-α Supressors/ Inhibitors

Certain drugs already in market can effectively reduce or block TNF production or its effects[46]. Small molecules that target signalling and synthesis pathways for TNF-α like thalidomide leads to inhibition of TNF-α. Large number of other small molecule agents are in various stages of preclinical and clinical development that inhibit synthesis of TNF-α[16].

Oxpentifylline

Oxpentifylline is an inhibitor of xanthine oxidase enzyme and is a strong suppressor of TNF in vitro by a variety of immune cell types. Oxpentifylline increases cellular cyclic adenosine monophosphate levels by inhibition of type IV phosphodiesterase activity, thus interfering with TNF gene expression[5].It was demonstrated that peripheral mononuclear cells and inflamed intestinal mucosa cells from patients with CD and UC secreted less TNF-α following in vitro treatment with oxpentifylline. Simultaneous administration of oxpentifylline and CDP571 in the DSS mouse model of colitis showed significant improvement and may enhance the therapeutic outcomes in IBD[27]. However, in an open-labeled study of 16 patients with corticosteroid dependent CD treated with oxpentifylline for 4 weeks, no significant clinical improvement was observed[66].

Thalidomide

Thalidomide inhibits TNF-α production in monocytes, T lymphocytes, and macrophages. In a rat model of experimental colitis, thalidomide effectively decreased colitis, probably through the inhibition of TNF-α in addition to its antiangiogenic properties. This mechanism of action was supported in human studies as well. Ten patients with IBD (1 with UC) received 300 mg oral thalidomide over a 12-week open-label study.

Among the 7 patients who experienced clinical improvement, both TNF-α and IL-12 levels decreased in a dose-dependent fashion, whereas IL-1β and IL-6 did not change significantly[67]. In addition, thalidomide caused marked clinical response in 8 of 9 patients with IBD, of which 2 of the 8 responders had UC. Furthermore, thalidomide caused complete clinical and histologic resolution of idiopathic colitis and proctitis in persons infected with HIV.

Other TNF-α Inhibitors:

OPC-6535 is a thiazole derivative that inhibits TNF-α production by monocytes in vitro and in vivo. The compound has shown promise in the treatment of UC, especially in patients with greater disease severity. RDP58 is a peptide composed of 12 d-amino acid residues that inhibits synthesis of proinflammatory cytokines by disrupting cell signaling transduction pathways.

It inhibits production of TNF-α, IFNγ, and IL-12 in vitro and in vivo. In a phase II trial of CD and UC, the compound decreased TNF-α mRNA levels and may have potential therapeutic implications in CD and UC[27]. In a double-blinded, placebo-controlled, randomized trial, 93 patients with mild to moderate active UC were randomized to 200 mg daily, 300 mg daily, or placebo. At both doses, 70% of patients receiving RDP58 had a significant clinical response compared to only 43% on placebo[68]. CNI-1493 is a guanylhydrazone that inhibits macrophage activation and the production of proinflammatory cytokines, including TNF-α, IL-1, and IL-6. Although CNI-1493 has shown significant clinical benefit in CD (including patients who failed treatment with infliximab), the compound has not been studied in UC[27].

Overexpression of NF-κβ was demonstrated in the colon of UC and CD patients. In preliminary studies, indirect inhibition of TNF-α by antisense therapy to NF-κβ p65 oligonucleotide led to clinical improvement in CD and UC patients[69]. However, these initial observations were not confirmed in a randomized controlled trial of antisense oligonucleotide to NF-κβ p65 delivered topically by enema to distal colonic inflammatory bowel disease[27].

CONCLUSION

The accumulated evidence strongly supports a central role for TNF-α in IBD. Biotechnologically manufactured TNF-α blockers are now firmly established in the treatment regimen of severe active forms of inflammatory bowel disease and are an effective treatment option for patients with IBD who do not respond adequately to conventional therapy. Infliximab, adalimumab and certolizumab all seems to be effective in CD. Infliximab is the only anti-TNF agent currently approved for the treatment of CD and UC. Adalumimab and Certluzimab Pegol seem like two most promising drugs for the near future.

Since inflammatory bowel disease is a chronic and life-long disorder, we need more long-term efficacy and safety data with maintenance therapy. Patients should be informed properly about important side effects and careful follow-up is mandatory. The success of TNF antagonist therapy has engendered a "biological" era in IBD. It is hoped that understanding of molecular mechanisms will lead to rational and specific approaches to restore normal immune regulation to the imbalance and immune dysregulation that characterizes IBD. Advances in scientific understanding and technology will lead to biological agents that are safe and have long term efficacy. Such agents are greatly anticipated and awaited.

Future Directions

Targeting TNF has been an important breakthrough in the management of inflammatory bowel disease. Following the discovery of novel cytokines and the role they may play in gut mucosal immunity, as well as the emergence of new concepts and changing paradigms in IBD pathogenesis, the roles of several cytokines have been elucidated and tested in both preclinical animal models and clinical trials of patients with IBD.

Complementary to this, proof of concept for new cytokine targets is rapidly developing, with the possibility of future cytokine-based therapies that may offer greater specificity and decreased toxicity for the treatment of IBD. In addition, further applications of cytokine-based therapies in human clinical trials and preclinical animal studies are ongoing. In future new targets in the pathogenesis of mucosal inflammation such as inhibition of cell adhesion, NF-kB and T cell activation should be evaluated.

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