

Anti-Tumor Necrosis Factor-Alpha (TNF- α) Treatment Strategies in Crohn's Disease

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Abstract: Crohn's disease is a complex multifactorial disorder characterized by the alternation of a cytokine-driven T-lymphocyte-depending inflammation of the intestinal mucosa, and "off" periods, where patients are completely asymptomatic. Although all the causative factors have not been clearly identified, the continuously growing understanding of the major abnormalities of the inflammatory and immune response leading to the often debilitating symptoms reported by Crohn's disease patients, improves our capacity to characterize new potential therapeutic targets with the subsequent hope to discover new (more efficient and less toxic) drugs. Saying that, in the recent years, tumor necrosis factor-alpha undoubtedly emerges as a key cytokine involved in Crohn's disease pathogenesis, and constant efforts have been made to control tumor necrosis factor-alpha deleterious effects in Crohn's disease. This review schematically summarizes the current understanding of tumor necrosis factor-alpha's role in Crohn's disease pathogenesis as well as the present and the future treatment strategies which may be helpful in patients by inhibiting tumor necrosis factor-alpha production and effects. Beside drugs under investigation, several original approaches are described or mentioned, most of them leading to recent patents such as polyclonal anti-TNF-alpha antibodies from avian origin, allowing potentially oral administration, or combination strategies such as vitamin D and anti-TNF-alpha antibodies or methotrexate and anti-TNF-alpha antibodies, or decoy oligodeoxynucleotides interfering with the binding of nuclear factor- κ B to its target genes promoters.

Keywords: Crohn's disease, tumor necrosis factor- α , tumor necrosis factor- α receptors, monoclonal antibodies, fusion proteins, vitamin D, kinase inhibitors, nuclear factor- κ B inhibitors, phosphodiesterase-4 inhibitors.

INTRODUCTION

Crohn's disease (CD) is a chronic granulomatous transmural inflammatory process of the digestive tract, belonging, with ulcerative colitis, to the inflammatory bowel disease (IBD) family. As CD can affect any part of the alimentary tract, actually, it is most commonly localized at the terminal ileum, the colon, and/or the perianal region. Usually, CD clinical picture is an alternation of phases where disease is quiescent (remission phases) and of acute relapses (active disease phases), responsible for general and intestinal disabling symptoms (e.g. fatigue, weight loss, abdominal pain, diarrhea, recurrent sub occlusive episodes, complete obstruction requiring surgery, etc.), and subsequent alteration of quality of life and/or repeated hospitalization. In the high incidence countries (North America and Europe), IBD affects approximately 2 million people, and it is noteworthy that in the last few decades, CD incidence continues to increase in these countries, and progresses in regions where it has been less commonly described until now. Although its etiology has not yet been fully addressed, increasing knowledge of its pathogenesis has prompted remarkable progress in the development of new therapeutic strategies [1,2].

Whereas normally, intestinal mucosal inflammatory and immune response (IIR) is tightly regulated, keeping a balance between immune effectors and regulators resulting in the maintenance of gut homeostasis, by contrast, in CD, the aggregate effect of genetic predisposition and environmental factors [3,4] lead to an aberrant IIR [2]. It is beyond the purpose of this article to review the numerous abnormalities of the mucosal IIR reported in CD, but schematically, CD-associated intestinal IIR is dominated by a predominant type 1 helper (CD4+) T-lymphocyte (T_H1) cytokine profile with increased production of interferon-gamma (IFN- γ), interleukin (IL)-12, and IL-2 [5-7]. This T_H1 cytokine profile is considered to play a pivotal role in disease onset and/or perpetuation, resulting in an excessive release of other pro-inflammatory cytokines (such as tumor necrosis factor-alpha [TNF- α], IL-1, or IL-6, or chemokines (e.g. IL-8)) in the serum, stool, and intestinal mucosa [8-15], inducing edema, thrombosis, intestinal epithelial barrier dysfunction and/or destruction, etc. explaining the intestinal lesions and the subsequent clinical symptoms.

TARGETING TNF- α PRODUCTION

Crohn's Disease Conventional Therapy

In CD, the two main therapeutic objectives are (i) to induce remission (or at least significant reduction of symptoms: i.e. clinical response) and, (ii) to maintain remission (prevention of relapse). In clinical trials, response to

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treatment or clinical remission are determined using tools measuring disease activity, the most widely applied being CD activity index (CDAI) [16]. Classically, CD patients are considered in clinical remission when their CDAI is below 150, while mild to moderate disease is defined by a CDAI value between 150 and 350, and severe disease by a value > 350. A 70 or 100 points decrease of the CDAI score (depending on studies) compared to the value at inclusion (baseline CDAI) defines clinical response, if the post-therapeutic CDAI value remains higher than 150. A CDAI value below 150 defines disease remission regardless of the magnitude of the CDAI decrease compared to the basal value. In patients with fistulae, remission usually signifies that all fistulae that were draining at baseline are closed after treatment at two consecutive visits, whereas response or improvement is defined as a decrease at two consecutive visits by more than 50% of draining fistulae compared to baseline. Several recent reviews or consensus statements have proposed therapeutic indications for active CD or maintenance treatment [17,18]. Conventional drugs used in IBD, usual dosage and main indications are detailed in references 17 and 18.

The Rationale for the Development of Anti-TNF- Strategies in Crohn's Disease

Tumor necrosis factor- is a 157-amino acid pro-inflammatory cytokine predominantly produced by monocytes, macrophages, and T-cells. Tumor necrosis factor- binding to its specific receptors (p55 TNF receptor also designed as TNFR1 and p75 TNF receptor called TNFR2) activates a range of transduction pathways such as nuclear factor kappa B (NF- B) or Janus kinase pathways, thereby inducing diverse biological effects, all playing, at least in part, a role in CD pathogenesis. These include (i) transcription of genes coding other inflammatory mediators involved in the IIR (e.g. pro-inflammatory cytokines such as IL-1 or IL-6, arachidonic acid metabolites - i.e. prostaglandins and leukotrienes, nitric oxide, reactive oxygen species, etc.), (ii) increased intestinal epithelial or endothelial expression and secretion of adhesion molecules and chemokines playing a major role in the recruitment of other inflammatory cells to the diseased mucosa, (iii) induction of matrix metalloproteinases (which have been shown to play a role in damaging intestinal epithelium), (iv) activation of the coagulation cascade, (v) induction of the expression of major histocompatibility complex class II antigens on colonic epithelial cells, (vi) as well as granuloma formation, etc. [1,2,5].

The similarity of these "physiological" properties of TNF- and several particularities of the IBD-associated intestinal mucosal IIR, taken together with the observation of (i) elevated TNF- serum levels in CD patients (and their correlation with clinical activity) [8,19], (ii) increased TNF- levels in the stool of IBD patients [9], (iii) enhanced TNF-mRNA expression in colonic mucosa from ulcerative colitis or CD patients [20], and, (iv) high TNF- concentrations in culture supernatants of colonic biopsies from IBD patients [10-13], lead to consider TNF- as a key mediator for CD pathogenesis, and subsequently, as a potential key therapeutic target. This hypothesis has been reinforced by animal studies showing that anti-TNF- antibody therapy (despite the fact that some used models resemble more ulcerative colitis than CD), alone or combined to IFN-

neutralization, significantly improve experimental colitis [21-24].

How Can TNF- Production be Targeted?

Activation of a variety of intracellular transduction pathways by a broad range of extracellular antigens (in the case of CD, luminal antigens, those involved in its pathogenesis remaining until now to be precisely characterized), regulates TNF- mRNA transcription, and after additional steps, TNF- membrane anchorage and TNF- release as a soluble protein; Fig. (1). Several transduction pathways have been suggested to activate TNF- production in CD, e.g. transcription factor NF- B activation [25], mitogen-activated protein kinases (MAPK) activation - the most relevant being p38 MAPK [26,27], extracellular signal-regulated kinase (ERK), and Jun N-terminal kinase (JNK; Janus kinase pathway) activation -, or activation of other intracellular transduction pathways such as intracellular cyclic adenosine monophosphate (cAMP) formation through phosphodiesterase-4 activation (PDE-4) [28]. Accordingly, blockade of either NF- B or MAPK, or increasing intracellular cAMP might be considered of interest for TNF- downregulation. Interfering with TNF- mRNA translation through a variety of mechanisms [29] might also be effective. Finally, after translation, cytosolic pro-TNF- is transformed into TNF- which first binds to cell membrane (mTNF- : membrane-bound TNF-) before being cleaved by a specific metalloproteinase named TACE (TNF- -converting enzyme) and released as a soluble cytokine (sTNF-). These steps might also serve as anti-TNF- targets (e.g. using TACE inhibitors). Finally, biological effects of TNF- can be counteracted by molecules specifically interfering with TNF- interaction with its receptors on effector cells.

ANTI-TNF- ANTIBODIES

The interest for antibodies as potential therapeutic tools growth up after the fundamental article by Köhler and Milstein published in Nature in 1975 [30], and recently emphasized by Margulies [31]. As Margulies stated, initial work by Köhler and Milstein, although representing the culminating result of the interaction of numerous years of integrating new knowledge and technical efforts in domains as different as biochemistry, cell culture, immunology, somatic cell genetics, etc., clearly represents the foundation stone of a new era in both diagnostics and therapeutics, finally leading, first to the generation of human-mouse chimeric or humanized monoclonal antibodies (mAbs), followed by fully human mAbs with minimal immunogenicity, and/or finally (until now) mAbs combination with chemical modifications, such as PEGylation - developed at least in order to enhance the half-life of these therapeutics [31].

The Infliximab Story in CD: From a Hypothetical Molecular Target to Another Potential Therapeutic Target

Infliximab (Remicade[®]; Centocor Inc./Schering-Plough) is the first and until now most extensively studied anti-TNF-mAb. It is approved by the Food and Drug Administration (FDA) for CD treatment (and more recently treatment of active ulcerative colitis). Infliximab is a human-chimeric

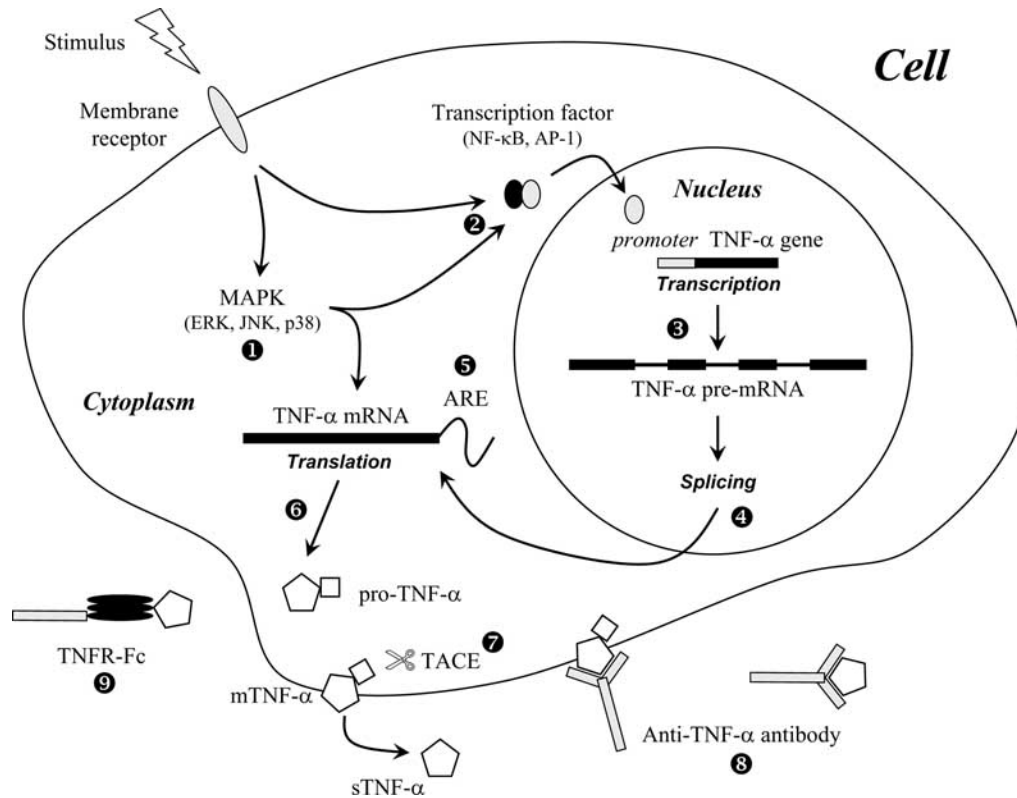


Fig. (1). This figure summarizes several pathways leading to tumor necrosis factor-alpha (TNF- α) production, from induction of transcription to TNF- α release. Each pathway represents a potential target for inhibiting TNF- α (shown in circled numbers). MAPK, mitogen-activated protein kinase; JNK, Jun N-terminal kinase; ERK, extracellular signal-regulated kinase; NF- κ B, nuclear factor kappa B; AP-1, activating protein-1; mRNA, messenger ribonucleic acid; ARE, adenosine uracil rich enhancer; TACE, tumor necrosis factor-alpha converting enzyme; mTNF, membrane-bound TNF- α ; sTNF, soluble TNF- α ; anti-TNF Ab, anti-TNF- α antibody; TNFR-Fc, TNF-receptor Fc fusion protein. Adapted from reference [29].

mAb composed of a complement-fixing human IgG1 constant region (75% of the mAb) and a murine-derived variable region (25% of the molecule) containing the high affinity regions which recognize and bind to both soluble and mTNF- α . Considering that antibody blockade of TNF- α prevents granuloma formation in a variety of non-IBD disease models, van Dulleman *et al.* [32], in a single centre, open pilot study, first suggest that anti-TNF- α antibodies may be suitable as a treatment for CD patients. These authors reported 4 weeks after a single 10 mg/kg of body weight anti-TNF- α chimeric monoclonal antibody intravenous (IV) infusion (at that time named cA2 but when commercialized, becoming infliximab), a normalization of the CDAI and a healing of the mucosal ulcerations in 8 out of 10 steroid-refractory active CD patients with an average response to treatment of 4 months [32]. Thereafter, Targan *et al.* for the Crohn's Disease cA2 Study Group [33], showed in a Phase II, multicentre, double-blinded, placebo-controlled, randomized study, that a single 5 mg/kg IV infusion, in patients with moderate-to-severe active CD, resulted 4 weeks later in a clinical response (decrease in CDAI > 70 points from baseline value) or a clinical remission (CDAI < 150) in 81% and 48% of patients compared to 17% and 4% in the placebo group respectively. Higher dosages (i.e. 10 mg/kg or 20 mg/kg) were not shown to be more efficient [33]. Unfortunately, the effect of such a single infusion did not last longer than 12 weeks [33]. Therefore, Rutgeerts *et al.* [34] ex-

amined the usefulness of repeated infusions to maintain remission. In a randomized, double-blind, multicentre placebo-controlled trial of 73 CD patients from the study of Targan *et al.* [33], they observed an overall significant superiority of infliximab (10 mg/kg administered IV every 8 weeks) for remission maintenance at week 44, compared to placebo (remission in 52.9% versus 20% respectively, $p = 0.013$) [34]. These results have been confirmed by the large scale ACCENT-I [35] and ACCENT-II [36] studies [37], as well as the beneficial effects of infliximab in CD patients with perineal (fistulous) disease [36,38] or in patients with ulcerative colitis [39], both for treating disease relapse [38,39] and as maintenance treatment [36,39].

Nevertheless, the use of infliximab has been associated to several - sometimes severe (leading in a few patients to death) -, secondary effects such as (i) infectious complications, (ii) a debated effect related to risk of congestive heart failure, (iii) hypersensitivity reactions, and (iv) more commonly, adverse events such as headache, nausea, cough, etc. Finally, the immunomodulatory nature of infliximab (and more generally anti-TNF- α therapeutic strategies) raised concerns regarding the risk of development of new cancers [40,41], a question recently emphasized by the report of 6 cases of hepatosplenic T-cell lymphomas in young adolescents and children concomitantly treated by 6-mercaptopurine or azathioprine, despite previously reported reassuring data from the TREAT registry [41].

Most infections considered as adverse events of infliximab involve the respiratory tract. Pharyngitis, sinusitis and bronchitis are the most common, although serious infections, including pneumonia, abscesses, sepsis, legionellosis, etc., and finally, tuberculosis, have been reported [42-45]. The reactivation of tuberculosis - over 70 cases have been reported, usually occurring early after infliximab introduction, in countries with a low incidence of tuberculosis -, lead to screening recommendations for tuberculosis in all patients prior to initiation of infliximab treatment (and by extend of other anti-TNF- therapeutic molecules) [46].

Initially, Phase II studies of infliximab - as a potential treatment for congestive heart failure in patients not suffering from CD -, suggested a higher incidence of hospitalization and mortality by worsening heart failure in patients previously known to suffer from congestive heart failure (New York Heart Association class III and IV) treated with infliximab at 10 mg/kg doses. Nevertheless, no association between infliximab treatment and *de novo* heart failure has been found [47,48].

Hypersensitivity reactions can be divided into acute or delayed reactions. Acute infusion reactions occur during the infusion or within 2 hours of the completion of the infusion, and are believed to be non-IgE-mediated, anaphylactoid-like reactions, probably IgG-related. Acute infusion reactions variably associate breath shortness (rarely real dyspnea), chest pain, palpitations, flushing, headache, and in some patients, urticaria and/or hypotension. In the later, the possibility of a true IgE-mediated anaphylactic reaction has to be considered. These reactions are usually treated by slowing down or temporarily stopping the infusion (depending on the intensity of the reported symptoms). If the reaction is not life-threatening and resolves promptly, then it is often possible to restart the infusion at a lower rate. Medication with acetaminophen or diphenhydramine can be used, and pre-medication with these drugs (or with IV steroids as suggested by several authors [49]) may be helpful in those patients needed to be treated by infliximab if there is a history of prior mild infusion hypersensitivity reaction. The delayed hypersensitivity-like syndrome typically occurs 5 to 9 days after an infusion and includes symptoms of pruritus, headache, hand, face or lip swelling, myalgias, back pain, arthralgias (which might include unusual locations, e.g. the temporomandibular joint, the jaw, or the neck), fever, skin rash or leukocytosis. Although these symptoms can be quite severe, requiring in rare patients hospitalization and IV steroids administration, most of the time, delayed hypersensitivity-type reactions are self-limited, resolving after 24 to 72 hours. These reactions occur in 2-2.8% treated patients. An association between their occurrence and the development of antibodies to infliximab (ATI), - formerly named human antichimeric antibodies (HACA) -, has been clearly noted [49,50]. In fact, the chimeric (human-mouse) nature of infliximab, makes it immunogenic, leading to the formation of ATIs (or HACAs), which in turn lead to loss of efficacy and acute infusion reactions [49,50]. Therefore, discovery of less (but as effective) immunogenic alternatives to infliximab in the future (e.g. humanized or fully human anti-TNF- antibodies, etc.; see below) appears worthwhile.

Such an alternative has been tested using etanercept (Enbrel®, Immunex/Wyeth), a genetically engineered fusion

protein combining two identical chains of recombinant human TNF- p75 receptors to an Fc domain of a human IgG1 antibody. By using recombinant human TNF- p75 receptors, the resulting so called fusion protein is fully human, and, as a consequence, less immunogenic than the chimeric mAb infliximab. Furthermore, clinical efficacy of etanercept in rheumatoid arthritis patients, either as a primary anti-TNF- treatment or as an alternative to infliximab in patients intolerant to- or not responding to- infliximab, has proven suitable [51,52]. Unfortunately, despite etanercept raises some promise in CD treatment after an open clinical trial in 10 patients with active disease [53], in a subsequent randomized study on 43 patients with moderate-to-severe CD, its subcutaneous injection (25 mg twice-a-week, an effective dose in rheumatoid arthritis patients), although safe, was not shown to be better than placebo [54]. This result highlighted at least one important point for further development of TNF- -targeting strategies in CD, as it strongly suggests that sTNF- neutralization is not sufficient to obtain clinical efficacy. In fact, by contrast to etanercept which has a high "off rate" after binding to TNF- receptor, indicating that it poorly binds to target cells that express mTNF- (in particular monocytes/macrophages and T-lymphocytes), infliximab binds to mTNF- , thereby inducing programmed cell death (apoptosis) of the target cells [55]. This pro-apoptotic effect has been clearly demonstrated and participates undoubtedly to its therapeutic action [56-61], a biological property all the more interesting as immune cells apoptosis has been reported to be decreased in CD compared to its normal physiological level [62,63]. Therefore, evaluating the pro-apoptotic properties of new anti-TNF- molecules appears as a new potential therapeutic target for screening suitable future therapeutic molecules in CD [64] and/or, as suggested recently by Van den Brande *et al.* [65], to predict *in vivo* treatment success of anti-TNF- strategies.

Other Anti-TNF- Antibodies

CDP571 (Humicade®, Celltech)

CDP571 is a humanized anti-TNF- antibody where the murine domains not binding to TNF- have been replaced by parts of a human IgG4 molecule in order to attempt to reduce immunogenicity. The resulting molecule is a chimeric human-mouse mAb, called "humanized" antibody, as the human-mouse ratio is of 95%-5%. By contrast to infliximab, CDP571 is administered subcutaneously (SC). Two controlled studies have evaluated its clinical efficacy [66,67]. The first enrolled 106 CD patients with moderate-to-severe disease receiving either a single dose of 10 mg/kg or 20 mg/kg of CDP571, or placebo, followed by re-treatment every 8 or 12 weeks with CDP571 or placebo. The clinical remission rates at week 24 in patients receiving CDP571 were not significantly different from patients receiving placebo regardless of CDP571 dose used and/or interval between two SC injections [66]. A second randomized, double-blind, placebo-controlled, multicentre trial, evaluated the efficacy and tolerability in 396 patients with moderately to severely active CD treated by CDP571 10 mg/kg SC every 8 weeks or placebo: despite a significantly higher clinical response observed at weeks 2 and 4 in patients receiving CDP571, difference in response rates at week 28 compared

to placebo did not reach statistical significance [67]. Although clinical response at week 28 was significantly higher with CDP571 compared to placebo in a subgroup of patients with a baseline C-reactive protein (CRP) concentration greater than 10 mg/L (28.7% versus 12.1%; $P < 0.018$) (post-hoc analysis), its modest short-term benefit in induction therapy, its relative failure to maintain a long-term effect, and finally, its incapacity to show a steroid-sparing effect in steroid-dependent patients [68], lead to discontinue its clinical development in CD treatment.

Adalimumab (Humira[®], Abbott Laboratories)

Adalimumab is a fully humanized recombinant human anti-TNF- IgG1 mAb. Like infliximab, it binds both to soluble TNF- and to mTNF-, thereby inducing apoptosis in TNF- -expressing cells [69,70]. As it contains no mouse peptide sequences, it is expected to be less immunogenic and subsequently more tolerable than infliximab or other chimeric or humanized mAbs. Previous open studies of adalimumab's efficacy and tolerance in CD patients who had lost responsiveness or developed intolerance to infliximab [71-73] have been published before the results of the CLASSIC (Clinical Assessment of Adalimumab Safety and Efficacy Studied as an Induction Therapy in Crohn's) study became available [74]. In the CLASSIC study, 299 patients with moderate-to-severe CD, naïve for anti-TNF- therapy were randomized to receive adalimumab (SC) at weeks 0 and 2, either 40 mg/20 mg, 80 mg/40 mg, 160 mg/80 mg, or placebo [74]. Remission rates at week 4 were 18% ($P = 0.36$ compared to 12% in the placebo group), 24% ($P = 0.06$), and 36% ($P = 0.001$) respectively, and clinical response rates 37% for the 80 mg/40 mg dose and 49% for the 160 mg/80 mg dose compared with 23% in patients receiving placebo [74]. It is interesting to note that a proportion of patients who had either lost previous response to infliximab, or were intolerant to infliximab, can achieve clinical response or remission with adalimumab [75,76]. This has been shown in an open label study [75], but also in the GAIN (Gauging Adalimumab Efficacy in Infliximab Nonresponders) study, a Phase III, double-blind, placebo-controlled study of patients with moderate-to-severe active CD where patients were randomized to receive either adalimumab (160 mg SC at week 0 and 80 mg SC at week 2; $n = 159$) or a placebo ($n = 166$) [76]. At week 4, 21% of patients on adalimumab achieved remission (7.2% in the placebo group, $P < 0.001$), 38% had a clinical response with a CDAI decrease ≥ 100 points (CR100: 25% in the placebo group, $P < 0.008$), and 52% a decrease of ≥ 70 points from CDAI baseline value (but less than 100 points: CR70) compared to 34% in the placebo group ($P < 0.001$) [76]. Data from the CHARM study (Crohn's trial of the fully Human antibody Adalimumab for Remission Maintenance), a large scale Phase III double-blind, placebo-controlled, multicentre study in moderately to severely active CD patients, showed that this effect was maintained after 26 and 56 weeks of treatment by adalimumab 40 mg SC administered every week or every other week (eow) [77], regardless of history of previous anti-TNF- therapy [78], and of baseline CRP concentration [79]. In a series of abstracts presented at the 14th United European Digestive Week (UEGW) in October 2006, adalimumab treatment has also been shown to result in prolonged (≥ 90 days) steroid-free remission at week 56 (in approximately

20% of responders), and to be useful in inducing and maintaining healing of draining CD fistulas. Despite data on adalimumab's side effects in CD patients are limited, current information from studies in CD patients, as well as the experience of adalimumab's use in rheumatoid arthritis, indicate a good tolerance. For example, in clinical trials of adalimumab in the treatment of rheumatoid arthritis, no difference compared to placebo, in terms of incidence of adverse effects in general or serious adverse events in particular, regardless of the infectious nature of the reported adverse events, has been observed. Compared to infliximab, as adalimumab is a fully human antibody, the development of antibodies against adalimumab (AAA) has been expected to be significantly reduced. Actually, AAA can still develop. In the CLASSIC study, 2 patients developed AAA (i.e. 0.04%) after 4 weeks of treatment. Experience in patients with rheumatoid arthritis noted AAA in 5% of patients (1% for patients taking concomitantly methotrexate [80], 12% in those not treated by methotrexate [84]). Hypersensitivity reactions appear rare.

Certolizumab pegol (Cimzia[®], UCB Pharma)

Certolizumab pegol (CDP870) is a polyethylene-glycolated FAB' fragment of a humanized anti-TNF- mAb. Engineered FAB' immunoglobulin fragments represent a promising alternative to therapeutic use of whole IgG antibodies. In fact, their elimination half-life and exposure are reduced compared with the parent antibody, but these pharmacokinetic particularities can be improved by site-specific addition of polyethylene glycol, resulting in an increase in half-life and a decrease in distribution volume and clearance [82]. This has been proven for certolizumab pegol in humans and primates, where pegylation resulted in sustained SC absorption, high bioavailability, low clearance, and prolonged elimination half-life [82]. Certolizumab pegol binds to both soluble and mTNF-, and an *in vitro* study showed that sTNF- was not detectable in supernatants of human peripheral blood monocytes pre-incubated for 1 hour with either one of these 3 anti-TNF- antibodies before being activated by bacterial lipopolysaccharide and incubated 4 hours more after extensive washing [83]. By contrast to other anti-TNF- antibodies, the same group reported that certolizumab pegol was not able to induce peripheral blood mononuclear cells apoptosis *in vitro* [84], suggesting that TNF- neutralization and induction of apoptosis might not be the sole mechanisms of action of anti-TNF- antibodies. Nevertheless, and despite its suggested inability to induce apoptosis, certolizumab pegol has a proven efficacy in both inducing and maintaining remission or clinical response in CD. In a Phase II placebo-controlled, randomized, dose-finding study, 291 CD patients with moderately to severely active disease were randomly allocated to be treated either by a 100, 200, 400 mg of certolizumab pegol SC administration, or placebo, at weeks 0, 4 and 8 [85]. There were no significant differences between the treatment and the placebo groups at week 12 [85]. However, in the patients subgroup with CRP concentrations ≥ 10 mg/L (post-hoc analysis), clinical response was significantly higher in those treated by the 400 mg/dose (53.1%) compared to patients receiving placebo (17.9%, $P = 0.005$) [85]. In a subsequent Phase III trial (PRECiSE 2: Pegylated Antibody Fragment Evaluation in Crohn's Disease: Safety and Efficacy), 668

patients with moderate-to-severe active CD disease received a 6 week open-label induction therapy with certolizumab pegol (400 mg SC/dose eow). Thereafter, the responders were randomized to receive either placebo (n = 212) or certolizumab pegol (n = 216, 400 mg/dose every 4 weeks) as maintenance treatment, and followed for a total of 26 weeks. Clinical response rate (CR100) at week 26 in the overall intention-to-treat (ITT) population was 62.8% for certolizumab pegol compared to 36.2% for placebo ($P < 0.001$), and the clinical remission rate (ITT) 47.9% compared to 28.6% for placebo ($P < 0.001$), regardless of baseline CRP concentration [86]. Patients mainly reported mild to moderate adverse effects, the most common being headache (12.6% during induction: 6.9% in patients receiving certolizumab pegol and 6.6% in those receiving placebo) [86]. Local injection reactions were low (2.8%), and less frequent in the certolizumab pegol group. Serious non-CD-related infections occurred in 3 times in certolizumab treated patients and 2 times in CD patients receiving placebo [86]. Another series of abstracts presented at the UEGW 2006, reported that recent onset CD patients show higher remission rates and durability of response, and that certolizumab pegol is effective in patients previously treated by infliximab (although remission and response rates seem lower than in naïve patients). Noteworthy, ATI in patients with CD do not cross-react with certolizumab pegol. Finally, treatment with certolizumab pegol improves quality of life (UEGW 2006), as well as work productivity and the ability to carry out daily activities. Regarding immunogenicity, the incidence of antibodies against certolizumab pegol has been of 12% in the Phase II dose-finding study [85].

PERSPECTIVES

Despite their proven benefit in CD treatment, in particular in patients with severe and/or steroid resistant or steroid dependent disease, beside clinical questions which have to be resolved in the coming years (should anti-TNF- antibodies be used earlier in CD treatment? how long may (can) they be used? is concomitant immunosuppressive therapy with 6-mercaptopurine, azathioprine, or methotrexate necessary? etc.), the growing use and development of anti-TNF- antibodies opens a range of more “pharmacological” questions such as: (i) can anti-TNF- antibodies be engineered for oral or local (rectal) administration? (ii) may anti-TNF- antibodies be used alone or in combination with other anti-cytokine antibodies or molecules? etc. The first question has been addressed, leading to the development of specific avian polyclonal antibodies directed to TNF- , but also to other pro-inflammatory cytokines (i.e. IL-6, IL-12) or chemokines (i.e. IL-8) [87]. The used method is based on hen immunization against the target cytokine(s) or chemokine. Immunoglobulins against these cytokines are extracted from egg yolk (named chicken yolk immunoglobulins: IgY) following a two-step method performed according to a modification of procedure described by Polson *et al.* [88]. Among other presented results, the inventors provide several interesting data concerning (i) determination of anti-TNF- IgY neutralizing ability (in comparison to infliximab’s neutralization capacity) in a cell-based neutralization assay determining the effectiveness of the tested molecule to prevent murine L929 TNF- -induced cells death, and, (ii) the evaluation of the anti-TNF- IgY in

the TNBS (trinitrobenzene sulfonic acid)-induced colitis in rats [87]. In the first series of experiments, the neutralization activity of the anti-TNF- IgY antibody has been shown to be significantly better than that of infliximab, with a two-fold difference between the ND50’s (neutralization dose preventing cell death in 50% of the L929 cells) of anti-TNF-

IgY on one hand, and infliximab on the other hand, and hundred-fold difference between the ND90’s of the two tested anti-TNF- antibodies [87]. *In vivo*, in the rat TNBS-induced colitis, anti-TNF- IgY also appears effective. Acute treatment studies were performed by administering orally 7.5, 30 or 120 mg/day of anti-TNF- IgY 24 hours before TNBS challenge, or 48 hours after TNBS rectal administration [87]. To summarize: (i) dose response studies show a significant improvement of rats body weight, and a significant positive effect on total colon weight, histology score, and colonic myeloperoxidase (MPO) activity, with the maximum effect at the highest (120 mg/day) dose in animals treated 48-hours after TNBS challenge, (ii) anti-TNF- IgY at 120 mg/day, in the same experimental conditions, are significantly superior (considering all the studied parameters) to sulphasalazine, (iii) beginning anti-TNF- IgY (120 mg/day) administration 24 hours before induction of colitis, shows roughly comparable results, (iv) after pre-challenge treatment with vehicle, dexamethasone, and anti-TNF- IgY, dexamethasone reduces total colon weight at the same level than anti-TNF- IgY, compared to placebo, but in comparison to anti-TNF- IgY, has no significant impact on morphological damage score, histological score, or colonic MPO concentration [87]. Chronic treatment studies show also an improvement of the studied parameters [88]. Finally, the authors of this patent determined the location of the anti-TNF- IgY in colonic sections (immunohistochemistry), observing a predominant location in the mucosa and submucosa, near ulcerated areas [87].

The question of associating anti-TNF- antibodies to other molecules (either other anti-cytokine antibodies, or other drugs) is of interest considering potential improvement of therapeutic efficacy and/or reduction of adverse events, in particular immunogenicity. One can imagine a combination of anti-TNF- IgY to anti-IL-6, IL-8, or IL-12 IgY which have been produced using the same method than anti-TNF- IgY [87]. Unfortunately, neutralization ability of anti-IL-6 IgY, anti-IL-8 IgY, and particularly anti-IL-12 IgY are not very convincing at this time [87]. Nevertheless, an association of anti-TNF- antibodies and anti-IL-12 antibodies, may be of potential interest, as IL-12 has been recognized as an important mediator in CD pathogenesis [6, 89, 90], and, as anti-IL-12 treatment has been suggested to be beneficial both in treating experimental colitis [91-96], and finally, active CD in humans [97]. Such a strategy has been proven effective in a murine model of induced-arthritis, a result encouraging for potential development in CD [98]. In order to increase treatment efficacy, other combinations with anti-TNF- antibodies have been suggested such as those with TNF-TNFR family members [99-101], vascular endothelial growth factor antagonists [102], other antiangiogenic compounds [103], or vitamin D [104-107]. This last combination may have two main objectives: (i) to improve bone mineral status in CD patients (often recognized to have osteopenia or osteoporosis [104]), and, (ii) to add the anti-inflammatory

effects of vitamin D or related compounds to anti-TNF-antibodies, for example [105]. In the recent years, anti-inflammatory and immune properties of vitamin D or related compounds have been more and more reported [106]. These data lead to propose biologically active vitamin D compounds (e.g. calcitriol) alone [105] or in combination with other therapeutic molecules [107] in CD treatment and/or prevention. Interesting results in animal models of experimental colitis have been recently reported [105,107], opening a new field for investigators.

Finally, Feldmann and Maini published a patent associating infliximab to methotrexate [108], both in order to improve therapeutic efficacy and potentially to reduce the occurrence of ATI, as it has been demonstrated in CD for concomitant immunosuppressive therapy (mainly 6-mercaptopurine or azathioprine) [49,50] and described in rheumatoid arthritis with methotrexate [109].

TNF- RECEPTORS

Tumor necrosis factor- exerts pro-inflammatory effects by binding to its membrane receptors TNFR1 (p75) and TNFR2 (p55). These receptors belong to a family of membrane receptors including for example FAS antigen, CD27, CD30 (Ki-1), CD40 (gp50) or OX 40, sharing together a repetitive pattern of cystein-rich domains in their extracellular portions. Proteolytic cleavage of the extracellular fraction of TNFR1 and TNFR2 resulted in generation of soluble forms of TNF- receptor. These soluble receptors for TNF- provide another option for its neutralization. As stated above, etanercept (Enbrel[®], Immunex/Wyeth), a fusion protein incorporating a human IgG1 Fc "backbone" linked to a soluble recombinant human TNF receptor p75, has been shown ineffective in the treatment of moderate-to-severe CD [54]. As for etanercept, oncept (developed by Serono International S.A.), a recombinant form of the human soluble p55 TNF- receptor (binding both to soluble and mTNF-), has first been suggested to be beneficial in a small sized Phase I open-label pilot study (12 patients with moderate-to-severe CD randomized to receive either oncept 11.7 or oncept 50 mg three times weekly for 2 weeks) [110]. This study shows that CDAI decreased rapidly in both treatment groups with five complete remissions and two CR100 at week 6, an effect sustained during 2 to 4 months after stopping oncept [110]. Considering these results, a larger (n = 207), dose-finding, double-blind placebo-controlled trial has been performed, randomizing CD patients with acute or chronic moderately to severely active disease to receive either SC oncept (10,25,35, or 50 mg) or placebo, three times weekly for 8 weeks [111]. Unfortunately, although there were no differences in the incidence of adverse events, this study failed to demonstrate superiority of oncept (regardless of the used dose) compared to placebo, in achieving the primary endpoint which was remission induction [111]. Therefore, at this time, TNFR treatment of CD with moderate-to-severe disease appears of no benefit.

By contrast to the use of exogenously administered TNFR one might also attempt to increase the endogenous formation of TNFR. As stated above, production of soluble TNFR resulted from proteolytic cleavage of the intact membrane receptor which is called shedding. Considering this hypothesis, authors have suggested to use molecules

(named TNFR releasing enzymes) inducing endogenous TNFR shedding, thereby increasing the concentrations of endo-genous soluble TNFR and subsequently, decreasing the concentrations of sTNF- [112,113]. Although not yet tested in models of experimental colitis or in CD patients, such an approach appears potentially interesting.

TNF- RECEPTOR ANTAGONISM

Deleterious effects of circulating TNF- can also be counteracted by interfering with its binding to TNFR using TNFR antagonists. Until now, although several researchers developed proteins with TNFR antagonist activity [114-116], this strategy has not been tested in experimental colitis or in IBD patients. Nevertheless, and despite induction of apoptosis seems not absolutely necessary to obtain a clinical response in CD (as suggested *in vitro* for certolizumab pegol), the fact that the engineered TNFR antagonists significantly inhibit *in vitro* TNF- -induced apoptosis [114] is of importance, as it could be considered as a potential obstacle of their efficacy in CD patients.

MAPK INHIBITORS

Production of TNF- is highly controlled by the induction of TNF- gene transcription after cell activation. There are two predominant ways to change gene transcription after extracellular signaling. The first involves several intracellular self-propagated protein phosphorylations, finally resulting in the activation of resident (inactive) nuclear transcription factors, which modulate gene transcription after translocation from the cytoplasm to the nucleus and binding to the specific regulatory domain in the target gene promoter region. A typical example is MAPK activation [117]. A second major way is to directly activate a latent cytoplasmic transcription factor that accumulates in the nucleus after nuclear translocation, thereby driving transcription after binding to DNA [118]. A third option can be gene activation by increasing cAMP [119].

Mitogen-activated kinases are an evolutionary family of enzymes that form an integrated network required to achieve their multiple specialized functions in biology, strongly participating in cell proliferation and differentiation control, cell death, and IIR. As noted in the section *Targeting TNF-production*, three major groups of MAPK in humans have been characterized and have subsequently be considered, in some pathological conditions, as potential therapeutic targets [117]. The ERK module was the first to have been identified. Its activation results both in activation of transcription factors, - the most important being factors from the activating protein 1 (AP-1) family -, and in activation of several membrane proteins. To our knowledge only one report [120] using IV semapimod (a c-raf inhibitor) in active CD patients has been published, indicating clinical response or remission in 5/6 enrolled patients with concomitant decrease in phosphorylated-MEK (the substrate of Raf) expression in colon biopsies after treatment compared to its expression before. In parallel with the ERK MAPK pathway, JNK appears also essential for AP-1 expression. The two primary AP-1 transcription components that are phosphorylated (i.e. activated) by JNK are c-Jun and activating transcription factor 2 (ATF-2) [117]. Concerning JNK inhibition in CD, there are also only few studies on their potential therapeutic

properties. A first study by Hommes *et al.* [121] reported enhanced JNK (and p38 MAPK) activation in colonic biopsies from patients with severe CD (n = 12). Using a small molecule [122] - CNI-149, inhibiting both JNK and p38 MAPK activation -, they observed a significant decrease in CDAI in an open-label trial where patients have been randomized to receive either 8 or 25 mg/m² of CNI-1493. Clinical response (decrease in 120 points compared to baseline CDAI which should have been > 380) has been reported in 67% of patients at week 4 and 58% at week 8 following treatment, clinical remission (CDAI < 150) in 25% of patients at week 4 and 42% at week 8, endoscopic improvement in 11/12 patients, and steroid-tapering has been possible in 89% of patients [121]. More recently, the effects of a specific JNK inhibitor (SP600125) have been tested *in vitro* on inflammatory cytokines production by CD patients leukocytes and colonic biopsies [123], and *in vivo* in DSS-mice (an animal model of experimental colitis which seems more related to ulcerative colitis than CD) [123,124], showing promising results. Numerous potential JNK inhibitors are currently under development, either small molecules [125-127] or antisense oligonucleotides, which is another original and promising approach to block gene expression or translation [128]. Some of these molecules have been claimed to be useful in treating CD or other disorders where aberrant MAPK activity seems to play a role in pathogenesis. However, most of these small molecules or antisense oligonucleotides, are until now in the first phase of pharmacological development (i.e. analysis of *in vitro* JNK inhibitory activity), and sufficient and strong data are lacking for considering them as usable potential therapeutic molecules. Further work studying *in vitro* efficacy in cellular models, addressing questions related to pharmacodynamics, potential cellular toxicity, and if conclusive, *in vivo* animal studies is needed before putting some of them in the pipeline of potentially suitable therapeutic molecules in CD. Finally, p38 MAPK inhibition has been considered of interest in CD treatment. Initial studies have shown that p38 MAPK is activated in CD and linked to TNF- signaling in IBD [26]. Despite these findings, animals studies have been conflicting, ten Hove *et al.* [129] and Malamut *et al.* [130] reporting a dichotomous role of SB 203580 (a p38 MAPK inhibitor) in TNBS-induced colitis in mice, and Hollenbach *et al.* [131] using a different model of experimental colitis (DSS-induced in mice) showing an improvement of the clinical score, as well as histological alterations and a decrease in colonic pro-inflammatory cytokines production by the same inhibitor. In fact, SB 203580 seems not to inhibit only p38 MAPK activity, but interacts also with the activation of the NF- B pathway by strongly inhibiting the Rip-like interacting caspase-like apoptosis-regulatory protein kinase (RICK), a key component of a pathway leading to NF- B induction, also considered as an effector kinase of CARD15/NOD2, and thereby opening new directions for CD treatment [130]. Finally, a recent published randomized, double-blind, placebo-controlled clinical study in patients with moderate-to-active CD, treated with doses ranging from 10 to 60 mg twice daily for 8 weeks, of BIRB 796 - a p38 MAPK inhibitor - did not find any difference in clinical endpoints (safety, CDAI, CRP concentrations, quality of life) compared to placebo [132]. These negative results emphasize the importance of a better understanding of the

role of p38 MAPK in CD (or more generally in IBD) before evaluating other p38 MAPK inhibitors [133-137] for CD treatment. As suggested by the study of Hollenbach *et al.* [131], ulcerative colitis could be a better "target" than CD for using these molecules, and other strategies (i.e. RICK inhibition) might be more promising. Finally, a recent study by Beardmore *et al.* [138] has suggested that p38 , and not p38 , seems to be the major isoform (and subsequently the major potential target) involved in the immune response, a fact which should be taken into consideration for developing new p38 inhibitors.

Nuclear factor- B activation has been shown to play an important role in CD [139]. In the normal intestine NF- B participates to several key functions including transcriptional control of various promoters of pro-inflammatory cytokines among which TNF- gene transcription [140]. Moreover, it has recently been suggested that monoclonal chimeric anti-TNF- antibody infliximab partly acts as an inhibitor of NF- B activation by enhancing mucosal levels of NF- B inhibitors I B and I B [141]. In resting cells NF- B forms a cytoplasmic complex with its natural inhibitors (I Bs), which prevents its translocation to the nucleus and the subsequent activation of its target genes. Schematically, after activation, I B is phosphorylated (through the activation of I B kinases [I K , and]), and after ubiquitination, degraded by the proteasome. This phenomenon allows NF- B nuclear translocation and activation of transcription. Nuclear factor- B can be divided in several families among which: (i) inhibitors of I B phosphorylation or ubiquitination, (ii) inhibitors of NF- B translocation, (iii) inhibitors of NF- B DNA binding. Numerous NF- B inhibitors have been patented during the last years [142]. In CD, the first attempt to inhibit NF- B has been reported in mice with TNBS-induced colitis or in the colitis model of IL-10-deficient (knock out mice) using a rectal administration of an antisense phosphorothioate oligonucleotide to the p65 NF- B subunit [27]. This experiment showed impressive improvement of experimental colitis [27], but no attempts to use such an approach in humans has been reported at our knowledge. Natural inhibitors of NF- B activation [143], e.g. sesquiterpenes derived from the *Boswellia serrata* tree (also known as *Salai guggul*), used in the Ayurvedic system of medicine, have also been suggested by *in vitro* studies [144], *in vivo* controversial experiments using animal models of colitis [145-147], or in small human studies [148], as potentially helpful for CD treatment. Nevertheless, not sufficient data are available to recommend these products for treating CD, some studies suggesting in addition potential hepatotoxicity [146]. More recent strategies used the so called DNazymes [149], molecules which have been developed to target mRNA molecules encoding the NF- B p65 subunit; although *in vitro* experimental data may be considered of interest, *in vivo* data are until now not available. Finally, some authors proposed to regulate NF- B activation by decoy oligodeoxynucleotides (ODN) [150,151]. Decoy ODN contain consensus sequences for the same binding site at the DNA promoter that the targeted molecule (i.e. NF- B). As a result, in our example, they act as "decoys" for NF- B, occupying its promoter region and preventing its access by NF- B and the subsequent molecular events [150]. Fichtner-Feigl *et al.* [151, 152]

reported recently their therapeutic efficacy as well as several resulting immunological modifications (e.g. suppression of pro-inflammatory cytokines IL-12, IL-23, IFN- γ , and concomitant increase in IL-10 from colonic *lamina propria* cells) in TNBS- or acetic oxazolone-induced colitis in mice.

PHOSPHODIESTERASE-4 (PDE-4) INHIBITORS

Phosphodiesterases (PDE) are a diverse family of enzymes that hydrolyze cyclic nucleotides. This results in an intracellular increase of either cyclic guanosine monophosphate or cAMP depending on the substrate, the type of the phosphodiesterase, and the cellular and molecular environment of the enzymatic reaction. Until now, 11 PDE isoenzymes have been discovered playing a role in a wide range of physiological processes. Phosphodiesterase-4 is a cAMP specific PDE. It is the predominant isoenzyme in the majority of inflammatory cells (except platelets) [119]. It is well known that increase in intracellular cAMP concentrations results in down-regulation of the transcription of several genes coding pro-inflammatory cytokines including IFN- γ , IL-2, but also TNF- α or IL-6 [153-155]. Thus, PDE-4 inhibitors appear as potential candidates in CD treatment especially considering their anti-TNF- α properties. In fact, this has been suggested by *in vitro* studies [28, 155], as well as *in vivo* studies in experimental colitis [156-159]. Unfortunately, clinical use of non-specific PDE-4 inhibitor pentoxifylline failed to show any benefit [160], and more recently developed PDE-4 inhibitors (most of them being developed for treating asthma) have not been tested in CD patients [161]. In addition, one of the major difficulty to bring PDE-4 inhibitors into clinics are some of their adverse effects, in particular nausea and vomiting. Nevertheless, potential advances may allow to overcome these side effects, such as (i) the identification of the PDE-4 isoform responsible for inducing emesis (which would allow to design molecules only targeting the isoform(s) that control(s) inflammation and immune response), and/or, (ii) the design of compounds not absorbed by the portal circulation after oral administration or undergoing extensive first-pass metabolism (like the corticosteroid budesonide), two strategies which would avoid systemic exposure to PDE-4 inhibitors and their related unwanted effects [153].

CURRENT & FUTURE DEVELOPMENTS

The significant advances in the understanding of CD pathogenesis lead to the identification of major therapeutic targets and to the subsequent development and use of biological therapies that target specific disease mechanisms. In CD, anti-TNF- α antibodies (but not soluble TNF- α receptors by contrast to other TNF- α -driven diseases: e.g. rheumatoid arthritis) have clearly proven their clinical benefit, by their ability to induce clinical response, and in a significant proportion of patients, remission, in clinical situations where conventional therapy has been shown ineffective or inducing unacceptable side-effects. These benefits concern both induction therapy and maintenance treatment, luminal and/or complicated disease (e.g. fistulizing disease or severe extraintestinal CD manifestations), and often allow to stop corticosteroids. Mucosal healing seems also more frequent than by using other more conventional medication. Nevertheless, several important questions remain to be resolved, including (i) that of the potential immunogenicity

of the currently used antibodies (even those being fully human antibodies) which can lead to increase the incidence of hypersensitivity reactions or be associated to a progressive decrease in therapeutic efficacy, (ii) the increased risk of severe infections (e.g. tuberculosis) despite following the adequate recommendations, and finally, (iii) the question of potential risk of increasing at long-term the risk of cancer in general, and lymphoma in particular, despite current data seem reassuring.

From a practical point of view, with the more and more extended use of anti-TNF- α antibodies other questions became of importance, in particular those related to pregnancy and lactation during anti-TNF- α treatment. If these are resolved for other classical immunosuppressant used usually in CD treatment (methotrexate is strictly contraindicated whereas purine derivatives can be considered to have no harmful effect during pregnancy, but not lactation where they are forbidden), the debate concerning anti-TNF- α antibodies remains, at this time, unresolved, despite there is more and more evidence that anti-TNF- α treatment is not contraindicated during pregnancy [162-166]. Nevertheless, considering the relative short time of the use of these molecules, caution must be taken and definitive conclusions cannot be drawn as yet. If the currently available anti-TNF- α antibodies seem not to be significantly different in terms of efficacy (however, there is no head-to-head study comparing one antibody to the other(s)), and general and infectious complications, the question of immunogenicity remains open and needs further observational studies.

These potential drawbacks as well as several other unanswered question related to anti-TNF- α antibodies: mode of administration (IV *versus* SC *versus* orally?), optimal strategy of their use (i.e. early use in a "top-down" strategy or use in case of failure of conventional treatment i.e. "step up" strategy?) association to other immunosuppressants? duration of treatment? etc., and the absence of response to anti-TNF- α antibodies (roughly in 30% of patients, presumably due to variability factors of genetic origin or not [167]), lead to attempt to inhibit TNF- α production or effects using other molecular targets. If some of them seem promising or if considering some new approaches really exciting from a scientific point of view, until now, and probably for the several coming years, anti-TNF- α antibodies represent probably the most efficient and promising treatment of CD patients not responding to conventional therapy. Nevertheless, research has to go on and more and more new molecules targeting as yet known targets for TNF- α inhibition or new actors in the TNF- α production process provide good hope for the future.

ABBREVIATIONS

AAA	=	Antibodies to adalimumab
AP-1	=	Activating protein-1
ATI	=	Antibodies to infliximab
cAMP	=	Cyclic adenosine monophosphate
CD	=	Crohn's disease
CDAI	=	Crohn's Disease Activity Index

CR70	=	Clinical response (i.e. decrease of the CDAI 70 points but < 100 points compared to baseline CDAI)	[4]	Eckburg PB, Relman DA. The role of microbes in Crohn's disease. <i>Clin Infect Dis</i> 2007; 44: 256-62.
CR100	=	Clinical response (i.e. decrease of the CDAI 100 points compared to baseline CDAI)	[5]	Monteleone G, Fina D, Caruso R, Pallone F. New mediators of immunity and inflammation in inflammatory bowel disease. <i>Curr Opin Gastroenterol</i> 2006; 22: 361-64.
CRP	=	C-reactive protein	[6]	Peluso I, Pallone F, Monteleone G. Interleukin-12 and Th1 response in Crohn's disease: pathogenic relevance and therapeutic implications. <i>World J Gastroenterol</i> 2006; 12: 5606-10.
DSS	=	Dextran sulfate sodium	[7]	Breese E, Braegger CP, Corrigan CJ, Walker-Smith JA, MacDonald TT. Interleukin-2 and interferon gamma secreting cells in normal and diseased human intestinal mucosa. <i>Immunology</i> 1993; 78: 127-31.
eow	=	Every other week	[8]	Reimund JM, Duclos B, Sapin R, Derlon A, Chamouard P, Baumann R. Systemic tumor necrosis factor in Crohn's disease: relationship to disease activity and circulating acute phase reactants. <i>Eur J Gastroenterol Hepatol</i> 1992; 4: 919-24.
ERK	=	Extracellular signal-regulated kinase	[9]	Braegger CP, Nicholls S, Murch SH, Stephens S, MacDonald TT. Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. <i>Lancet</i> 1992; 339: 89-91.
FDA	=	Food and Drug Administration	[10]	Reinecker HC, Steffen M, Witthoef T, <i>et al.</i> Enhanced secretion of tumor necrosis factor-alpha, IL-6, and IL-1 by isolated <i>lamina propria</i> mononuclear cells from patients with ulcerative colitis and Crohn's disease. <i>Clin Exp Immunol</i> 1993; 94: 174-81.
HACA	=	Human antichimeric antibodies	[11]	Reimund JM, Wittersheim C, Dumont S, <i>et al.</i> Mucosal inflammatory cytokine production by intestinal biopsies in patients with ulcerative colitis and Crohn's disease. <i>J Clin Immunol</i> 1996; 16: 144-50.
IBD	=	Inflammatory bowel disease	[12]	Reimund JM, Wittersheim C, Dumont S, <i>et al.</i> Increased production of tumour necrosis factor-alpha, interleukin-1 beta and interleukin-6 by morphologically normal intestinal biopsies from patients with Crohn's disease. <i>Gut</i> 1996; 39: 684-89.
IFN	=	Interferon	[13]	Schreiber S, Nikolaus S, Hampe J, <i>et al.</i> Tumour necrosis factor alpha and interleukin 1beta in relapse of Crohn's disease. <i>Lancet</i> 1999; 353: 459-61.
I K	=	I B kinase	[14]	Mitsuyama K, Toyonaga A, Sasaki E, <i>et al.</i> IL-8 as an important chemoattractant for neutrophils in ulcerative colitis and Crohn's disease. <i>Clin Exp Immunol</i> 1994; 96: 432-36.
IL	=	Interleukin	[15]	Reimund JM, Duclos B, Dumont S, <i>et al.</i> Interleukin-8 is an important inflammatory mediator in ulcerative colitis and Crohn's disease. <i>Gastroenterol Clin Biol</i> 1997; 21: 131-37.
IV	=	Intravenous	[16]	Best WR, Beckett JM, Singleton JW. Rederived values of the eight coefficients of the Crohn's Disease Activity Index (CDAI). <i>Gastroenterology</i> 1979; 77: 843-46.
IIR	=	Inflammatory and immune response	[17]	Travis SPL, Stange EF, Lémann M, <i>et al.</i> for the European Crohn's and Colitis Organisation (ECCO). European evidence based consensus on the diagnosis and management of Crohn's disease: current management. <i>Gut</i> 2006; 55 (Supplement 1): i16-35.
ITT	=	Intention to treat	[18]	Büning C, Lochs H. Conventional therapy for Crohn's disease. <i>World J Gastroenterol</i> 2006; 12: 4794-806.
JNK	=	Jun N-terminal kinase	[19]	Murch SH, Lamkin VA, Savage MO, Walker-Smith JA, MacDonald TT. Serum concentrations of tumour necrosis factor alpha in childhood chronic inflammatory bowel disease. <i>Gut</i> 1991; 32: 913-17.
mAb	=	monoclonal antibody	[20]	Cappello M, Keshav S, Prince C, Jewell DP, Gordon S. Detection of mRNAs for macrophage products in inflammatory bowel disease by <i>in situ</i> hybridization. <i>Gut</i> 1992; 33: 1214-19.
mRNA	=	Messenger ribonucleic acid	[21]	Powrie F, Leach MW, Mauze S, <i>et al.</i> Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhiCD4+ T cells. <i>Immunity</i> 1994; 1: 553-62.
mTNF-	=	Membrane-bound tumor necrosis-alpha	[22]	Kojouharoff G, Hans W, Obermeier F, <i>et al.</i> Neutralization of tumour necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate-induced colitis in mice. <i>Clin Exp Immunol</i> 1997; 107: 353-58.
MAPK	=	Mitogen-activated protein kinase	[23]	Obermeier F, Kojouharoff G, Hans W, Scholmerich J, Gross V, Falk W. Interferon-gamma (IFN- γ) and tumour necrosis factor (TNF)-induced nitric oxide as toxic effector molecule in chronic dextran sulphate sodium (DSS)-induced colitis in mice. <i>Clin Exp Immunol</i> 1999; 116: 238-45.
MPO	=	Myeloperoxidase	[24]	Scheinin T, Butler DM, Salway F, Scallan B, Feldmann M. Validation of the interleukin-10 knockout mouse model of colitis: antitumour necrosis factor-antibodies suppress the progression of colitis. <i>Clin Exp Immunol</i> 2003; 133: 38-43.
NF- B	=	Nuclear factor kappa B		
PDE	=	Phosphodiesterase		
PEG	=	Polyethylene glycol		
RICK	=	Rip-like interacting caspase-like apoptosis-regulatory protein kinase		
SC	=	Subcutaneous		
TACE	=	Tumor necrosis factor-alpha converting enzyme		
T _H 1	=	Type 1 T-lymphocytes		
TNBS	=	Trinitrobenzene sulfonic acid		
TNF-	=	Tumor necrosis factor-alpha		
TNFR	=	Tumor necrosis factor-alpha receptor		
VEGF	=	Vascular endothelial growth factor		

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