Review Article

Mechanism of Action of Anti-TNF Therapy in Inflammatory Bowel Disease

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Abstract

Several anti-tumour necrosis factor [TNF] blocking strategies have been evaluated in patients with Crohn’s disease. Compounds that have been tested included the full monoclonal IgG1 antibodies infliximab and adalimumab, the pegylated anti-TNF F[ab']2 fragment certolizumab, an IgG4 anti-TNF CDP571 with reduced affinity for the Fc receptor, the soluble TNF receptor I oneccept, and the TNF receptor II-Fc fusion protein etanercept. The endpoints of these studies suggest that not all methods of blocking TNF are equal. Here we will review the differences in the clinical, biochemical, and endoscopic endpoints of the major clinical studies. Collectively the data suggest that only IgG1 monoclonal antibodies have the ability to induce complete clinical, biochemical, and endoscopic remission. We discuss the potential multiple modes of action that may contribute to the response to full IgG1 anti-TNFs, focusing on the rapid induction of lamina propria T cell apoptosis and Fc receptor-dependent induction of M2-type wound-healing macrophages. We discuss how novel insights into the mechanism of action of anti-TNFs in Crohn’s disease may contribute to the development of novel anti-TNFs with improved efficacy.

Key Words: Crohn’s; anti-TNF; infliximab; adalimumab; certolizumab; mucosal healing; wound healing; macrophage; T cell; apoptosis

1. Introduction

The breakthrough in the treatment of patients with inflammatory bowel disease [IBD] that resulted from the introduction of monoclonal antibodies to anti-tumor necrosis factor α [TNFα] has firmly established the dogma that TNF is a major pathological cytokine in this disease. There are indications however that TNF is more than just a driver of the acute phase response and may in fact play a protective role in the intestinal mucosa.1 Also, several new approaches to block TNF that were successful in rheumatoid arthritis have failed in clinical trials in Crohn’s disease. This indicates that there may be mechanisms of action other than the mere neutralisation of soluble TNF in the treatment of Crohn’s disease. In this review we will briefly review the biology of TNF, discuss the results of the clinical trials with TNF blocking agents in Crohn’s disease in terms of clinical, biochemical, and endoscopic outcomes, and finally discuss the different mechanisms that could contribute to the response to anti-TNF that have been investigated to date.

2. The biology of TNF, more than just a pro-inflammatory cytokine

TNF is produced as a 26 kD transmembrane protein [mTNF]. The extracellular domain of TNF is subsequently cleaved off by TNF-alpha converting enzyme [TACE] to generate soluble TNF. TNF has two receptors, TNFRI and TNFRII, with distinct intracellular signalling pathways.2,3 TNFRI mainly binds soluble TNF, whereas TNFRII binds mTNF. TNFRI can both activate a caspase-8 dependent cell death signalling pathway and result in the activation of the NF-kB transcription factor family, the key activating switch of the innate immune response that can also induce an important anti-apoptotic response. The cellular response to TNFRII signalling therefore depends on the balance between these two opposing signalling pathways. In contrast, TNFRII does not activate caspase-dependent death signalling pathways but instead exclusively activates pro-survival and pro-inflammatory signalling pathways [see Kalliolias and Ivashkiv for an excellent recent overview].2

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TNF signalling outcome therefore not only drives the expression of acute phase proteins but also influences important cellular behaviour such as cell migration and proliferation and cell death in a highly context-dependent manner. The role of TNF as a cytokine was first clearly described by Cerami and colleagues who found that TNF secreted by macrophages was responsible for the severe cachexia that can be observed in parasite-infested animals [the authors initially named TNF cachectin].\(^5\)\(^6\)\(^7\) The critical role of TNF in the acute phase response was subsequently discovered in endotoxin-induced models of septicemia in mice\(^8\) and chimpanzees.\(^9\) The endotoxemia experiment in chimpanzees by Van der Poll et al. demonstrated that, although rapid TNF release was responsible for subsequent production of IL-6 and IL-8, there was no effect of anti-TNF on important parameters of septicemia such as neuophilia and lymphopenia. In fact, treatment with anti-TNF specifically inhibited fibrinolysis, raising fears of an increased risk of thrombosis in already high-risk septic patients.\(^10\) This was reason for this group to abandon the further development of anti-TNF for sepsis; however, some of the members of the group were subsequently the first to report the successful use of anti-TNF [infliximab] for Crohn’s disease in 1993.\(^11\)

The success of anti-TNF in treating chronic intestinal inflammation is not as straightforward as it may seem. In fact, TNF signalling can promote epithelial wound healing,\(^1\) and several experiments in mice have demonstrated that, depending on the context, TNF may even be required to suppress intestinal inflammation. For example, neutralisation of TNF with a monoclonal antibody aggravated the severity of acute colitis when the epithelial layer was damaged with dextrane sulphate sodium [DSS].\(^8\) Similarly, TNF knock-out mice are highly sensitive to DSS-induced acute colitis.\(^12\) Even in chronic models of colitis, the role of TNF can depend on the context. For example, absence of TNF aggravates the severity of colitis in IL-10 knock-out mice.\(^13\) Similarly, in the T cell transfer model of colitis, disease worsens if the transferred pathogenic T cells lack TNFRII,\(^14\) and regulatory T cells require TNFRII to exert their protective effects in this model.\(^15\)\(^16\)

3. Indications that not all anti-TNF blocking strategies are equal in IBD

The first description of the successful treatment of a patient with Crohn’s disease with the chimeric monoclonal IgG1 anti-TNF antibody infliximab was in 1993.\(^11\) Clinical efficacy was subsequently demonstrated in placebo-controlled trials for both Crohn’s disease and ulcerative colitis.\(^17\)\(^18\)\(^19\) The successful incorporation of infliximab into clinical practice stimulated the development of new anti-TNF agents. These new anti-TNF agents were developed structurally different from infliximab [see Figure 1], often in an effort to reduce side effects or prolong half-life. Anti-TNF agents tested in clinical trials for IBD included two additional IgG1 monoclonal antibodies: the humanised anti-TNF antibodies adalimumab and golimumab. An alternative strategy that was developed was to neutralise TNF with a soluble TNF receptor I [onectein] or a TNF receptor II-Fc fusion protein [etanercept]. One important difference with the anti-TNF antibodies is that the soluble receptors are not specific for TNF, as both also bind lymphotoxin-alpha. Others attempted to reduce side effects by preventing interaction with the body’s Fc receptors such as in certolizumab, a pegylated anti-TNF F[ab]’2 fragment [lacks the Fc portion] or the IgG4 anti-TNF CDP571 [IgG4 has strongly reduced affinity for the Fc receptor]. These different anti-TNF agents have all been demonstrated to effectively neutralise the biological activity of TNF. All different TNF-blocking agents have shown efficacy in clinical trials for rheumatoid arthritis with the exception of onercept [which was not tested]. However, as we will see below, not all anti-TNF agents are [equally] effective for the treatment of Crohn’s disease. This suggests that there may be one or more mechanisms besides neutralising the biological activity of soluble TNF involved in the efficacy of anti-TNF agents in Crohn’s disease. Effector mechanisms that are independent of neutralisation of TNF may relate to the important structural difference between the different TNF blockers. In this review, we will first summarise different aspects of the clinical efficacy of different anti-TNF agents in inflammatory bowel disease [IBD]. As only IgG1 monoclonal antibodies have been tested in ulcerative colitis, no conclusions can be drawn about the mechanism of action of anti-TNFs in ulcerative colitis. We will therefore restrict this review, and our discussion of the way structural differences in anti-TNF agents may influence clinical efficacy, to Crohn’s disease.

4. Efficacy of anti-TNFs: the major clinical trials in Crohn’s disease

Making a comparison between clinical trials is difficult due to different designs of the studies, and strong conclusions can only be made if head-to-head studies are performed. However, there are some noticeable differences between the different anti-TNFs regarding the different endpoints, which will be discussed separately below. We will only briefly discuss data on clinical trials with infliximab and adalimumab, as many excellent overviews exist or the results of the studies with these compounds.\(^18\)

5. Clinical response and remission

Most clinical trials evaluating symptomatic efficacy of anti-TNF therapy in Crohn’s disease have relied on symptomatic response as defined by a reduction in the Crohn’s Disease Activity Index [CDAI]. The CDAI depends on symptoms such as abdominal pain, diarrhoea and overall well-being. The significant overlap with symptoms of irritable bowel syndrome [IBS], that affect a large proportion of patients with Crohn’s disease, makes the CDAI an unreliable score to measure active inflammation and mucosal damage in these patients. Indeed, it was already shown in 1990 that a very poor correlation existed between the CDAI and endoscopic data in a study of patients treated with prednisolone,\(^19\) and this was recently confirmed in a large study of patients treated with infliximab.\(^20\) It has been firmly established that both infliximab and adalimumab can induce and maintain clinical response and remission based on the CDAI.\(^15\)\(^16\)\(^17\)\(^21\)

In contrast, compounds with reduced or absent interaction with the Fc receptor seem to be less efficient in inducing clinical remis- sion. Certolizumab failed to demonstrate superiority over placebo for induction of clinical remission in three independent clinical trials, including the PRECISE-1 where patients were stratified according to C-reactive protein [CRP] levels at baseline.\(^22\)\(^23\)\(^24\) However, in those patients that do show a response, maintenance therapy does result in significantly higher clinical remission rates compared with placebo in the longer term [42\% for certolizumab vs 26\% for placebo at Week 26, \(p = 0.01\)].\(^24\) Although the initial studies suggested that CDP571 might have at least some clinical efficacy, the largest randomised study showed no evidence of activity.\(^21\)

In the clinical trials performed with the soluble TNF receptors etanercept and onercept, no efficacy was seen for the treatment of Crohn’s disease at all. In a randomised placebo-controlled trial, etanercept was not effective compared with placebo for either
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6. Biochemical response

The acute phase protein C-reactive protein is secreted into the circulation by the liver in response to systemically circulating mediators of inflammation. The correlation between CRP and the severity of mucosal inflammation as assessed by endoscopic scoring is modestly better than the CDAI. One of the limitations of the use of CRP in Crohn’s disease is that the CRP is not elevated in all patients with objective signs of intestinal inflammation, and that CRP can be elevated for causes unrelated to Crohn’s disease [e.g., a respiratory infection]. However, in those patients in whom CRP is persistently elevated, it can be a useful tool to monitor disease activity as it is easily obtained and can be measured at multiple time points. In many of the clinical trials, consecutive measurements of CRP have been performed.

Treatment with infliximab and adalimumab results in a rapid reduction of the level of CRP. Certolizumab similarly reduced CRP levels as early as Week 2 after the start of treatment. CDP571 reduced CRP despite the fact that no clinical remission was observed with CDP571. No CRP levels were reported in the study that examined the efficacy of etanercept in Crohn’s disease. In the dose-finding study for onercept, no significant trend was found across dose groups for the study population as a whole or in the patients with increased CRP. Thus, the systemic biochemical response to mucosal inflammation in the liver as measured by CRP can be reduced by both IgG1 monoclonal antibodies and compounds with absent or reduced binding to the Fc receptor. This indicates that neutralisation of TNF by these strategies reduces the systemically circulating inflammatory mediators, consistent with the central role

Figure 1. Schematic representation of the different anti-TNF blocking compounds that have been tested in Crohn’s disease.

response or remission at 4 weeks [response: 39% vs 45%, \(p = 0.763\); remission: 9% vs 20%, \(p = 0.39\)] and 8 weeks [response: 30% vs 30%, \(p = 1.0\); remission: 13% vs 25%, \(p = 0.44\)]. Onercept was evaluated in a phase II randomised placebo-controlled dose-finding trial. There was no significant effect of onercept on clinical remission across the different dose groups in this study. It should be noted that it is unclear if the dose of onercept used in the patients results in clinically relevant neutralisation of TNF. For etanercept however, we know that the dose used in the clinical study [25 mg twice weekly] is at least the same as the dose that is effective in rheumatoid arthritis. It was noticeable that the rate of remission in etanercept-treated patients was in fact only half that observed in the placebo group at both Week 4 and Week 8, although this was not statistically significant.

IgG1 anti-TNF antibodies

- Infliximab
- Adalimumab
- Golimumab

anti-TNF Fab fragment

- Certolizumab

IgG4 anti-TNF antibody

- CDP571

TNF-receptors

- Etanercept
- Onercept

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of TNF in systemic inflammation that has been observed in models of endotoxaemia.

7. Mucosal healing

In the initial clinical trials performed with anti-TNF therapy, the primary endpoints were symptomatic parameters. However, different studies have demonstrated that there is a poor correlation between symptoms and the actual severity of inflammatory disease as assessed by endoscopy.\(^\text{25,26}\) Consensus has therefore developed among inflammatory bowel disease clinicians that the primary aim of treatment should be objective complete resolution of inflammatory activity and ulceration as determined by endoscopy.\(^\text{25}\) In Crohn’s disease, the definition of complete endoscopic resolution is the complete absence of ulcerations, a condition termed ‘complete mucosal healing’. It has been shown in Crohn’s disease that complete mucosal healing induced by anti-TNF therapy correlates with increased steroid-free remission rates, increased incidence of sustained clinical remission, and reduced incidence of surgery and hospitalisation.\(^\text{35,34,37}\)

Mucosal healing induced by treatment with infliximab was investigated in a subset of patients in the ACCENT1 study, where mucosal healing was a secondary endpoint.\(^\text{36}\) In this study, 0/17 [0\%] patients achieved complete mucosal healing at Week 10 after a single-dose induction compared with 10/32 [31\%] after a three-dose induction \(p = 0.01\). At Week 54, 1/14 [7\%] patients in the placebo group showed mucosal healing compared with 13/26 [50\%, \(p = 0.007\)] of patients treated with infliximab maintenance. Endoscopic response with adalimumab was examined in the EXTEND study which had mucosal healing as a primary outcome.\(^\text{21}\) At Week 12, 8/61 [13\%] patients achieved mucosal healing on induction therapy followed by placebo compared with 17/62 [27\%] adalimumab maintenance \(p = 0.056\). At Week 52, the rate of complete mucosal healing was 0/61 [0\%] of patients on placebo vs 15/62 [24\%] on adalimumab maintenance therapy \(p < 0.001\).

No placebo-controlled study has been performed to examine endoscopic response in patients treated with certolizumab. However, recently an uncontrolled endoscopic study was reported in 89 certolizumab-treated patients, the MUSIC study.\(^\text{37}\) In this study, the rate of complete mucosal healing was 4/89 [4\%] patients at Week 10 and 7/89 [8\%] at Week 54. Thus, rates of mucosal healing seem to be relatively low with certolizumab and it is not clear if this is significantly superior to a placebo. The final and perhaps most important conclusion from the clinical trials is that Fc-mediated interactions may play a role in anti-TNF induced mucosal healing.

In summary, only the IgG1 monoclonal antibodies have so far been shown to be able to achieve all relevant endpoints, that is to induce clinical remission, reduce systemic inflammation as measured by CRP, and especially to induce complete mucosal healing.

8. Clinical efficacy: patients on etanercept can paradoxically develop new-onset Crohn’s disease

Several investigators have made the observation in both individual cases and large cohorts,\(^\text{38,39,40,41,42}\) that patients with juvenile idiopathic arthritis [JIA], ankylosing spondylitis, and rheumatoid arthritis can develop new-onset Crohn’s disease while being treated with the soluble TNFRII receptor-Fc fusion protein etanercept. This indicates that etanercept is not only ineffective in active Crohn’s disease [see above] but also does not act to prevent new-onset Crohn’s disease. Intriguingly, many of the patients that develop Crohn’s disease while on etanercept achieve clinical remission when switched to a monoclonal anti-TNF antibody [infliximab or adalimumab].\(^\text{43,44}\) The incidence of IBD in a European registry for JIA patients treated with etanercept was 362 per 100 000 patient-years, about 43 times higher than in the general paediatric population.\(^\text{45}\) Of course the increased risk observed in this population may be related to an increased risk of IBD in patients with JIA or the extensive use of non-steroidal anti-inflammatory drugs [NSAIDs] in this population, but the incidence seems very high. A study by Braun et al. evaluated a total of 1514 patients in nine clinical trials in which patients received either infliximab \(n = 366\), adalimumab \(n = 295\), etanercept \(n = 419\), or placebo \(n = 434\) for ankylosing spondylitis. No new-onset IBD was found in either the placebo- or the infliximab- and adalimumab-treated patients compared with five cases of new-onset IBD in the patients treated with etanercept.\(^\text{46}\) Although these numbers are too small to draw any firm conclusions, this contributes to the indication from the JIA registry that etanercept may even [slightly] increase the risk to develop IBD. Biologically there is an important difference between the anti-TNF antibodies and etanercept, which provides a rationale for the indications that treatment with etanercept may result in an increased risk for new-onset Crohn’s disease and aggravate established disease. As etanercept is a soluble TNFRII receptor, it is not specific for TNF but also inhibits signalling by the alternative TNFRII ligand lymphotoxin-\(\alpha\). Lymphotoxin-\(\alpha\) is a key cytokine in the regulation of the mucosal immune system, which contributes to the induction of intestinal lymphoid structures, an adequate IgA repertoire, and generation of tissue-protective IL-22 producing type III innate lymphoid cells.\(^\text{47}\)

9. Mechanisms of resolution of inflammation and mucosal healing

From the clinical studies in Crohn’s disease summarised above, it has become clear that not all anti-TNF agents have equal clinical efficacy—in contrast to other chronic inflammatory conditions such as rheumatoid arthritis, where most TNF-blocking strategies seem to work. This suggests that in Crohn’s disease one or more effector mechanisms other than the neutralisation of soluble TNF are involved in resolution of inflammation and mucosal healing. Two major alternative modes of action have emerged from the research into the mechanism of action of anti-TNFs in past years: the induction of lamina propria T cell apoptosis and Fc region-dependent induction of M2-type wound-healing macrophages [Figure 2]. Below we will review the research on the mechanism of action of anti-TNF, focusing on these two mechanisms.

10. Induction of lamina propria T cell apoptosis

The apoptotic death of neutrophils, T lymphocytes, and myeloid cells is a fundamental aspect of the resolution of an inflammatory infiltrate and one of the key mechanisms that prevent the development of chronicity of inflammation.\(^\text{48}\) As discussed below, several authors have noted that treatment with anti-TNF results in the apoptosis of leukocytes in the lamina propria of treated patients. It should be realised however, that the detection of apoptotic cells in the lamina propria of anti-TNF treated patients does not mean that this apoptosis is directly induced by anti-TNFs. Since apoptosis is a normal aspect of resolution of an inflammatory infiltrate, it could be a downstream, indirect consequence of other effector mechanisms of anti-TNF. As we will see below however, there are several experiments that suggested that induction of apoptosis is direct.
The first observation of anti-TNF rapidly induced leukocyte apoptosis was made by Ten Hove et al.\textsuperscript{41} The investigators performed an elegant study in which 10 consecutive patients underwent endoscopy just before the administration of infliximab and 24 h after the infusion. A specific induction of TUNEL-positive [apoptotic] cells was observed, and staining in adjacent sections suggested that the apoptotic cells were mainly CD3 positive T cells. The same group later again examined apoptosis induction at 24 h after the administration of infliximab, by administering technetium-labelled annexin V to infliximab-treated patients, allowing the visualisation of apoptotic cells in vivo using single-photon emission computer tomography.\textsuperscript{46} In this study, it was found that annexin V uptake was specifically enhanced in the diseased intestine 24 h after the administration of infliximab. Also, uptake of annexin V correlated with clinical response as determined by the CDAI. No endoscopies were performed to examine mucosal healing in this study. Atreya et al.\textsuperscript{45} examined apoptosis in the lamina propria at 4 weeks after the start of infliximab or adalimumab in six patients, and confirmed the notion of anti-TNF induced T cell apoptosis by showing a significant induction of staining for active caspases in the lamina propria, predominantly in positive CD4+ T cells.\textsuperscript{47} Together these studies suggest that anti-TNF monoclonal antibodies can induce a rapid \textlesssim 24 h\textgreater induction of T cell apoptosis that is sustained for at least 4 weeks.

Several hypotheses have been advanced to explain the potential direct induction of T cell apoptosis by anti-TNFs. All these theories centre around the observation that anti-TNFs can bind to activated T cells and monocytes. We will therefore first discuss this aspect of apoptosis induction. It is generally assumed that this binding means that the anti-TNFs bind to the membrane-bound form of TNF [mTNF]. It should be noted, however, that in most experiments it cannot be excluded that compounds actually bind to soluble TNF bound to its receptor. Perhaps these two possibilities can explain some of the discrepant binding of different TNF-blocking agents to cells, reported in the literature. Van den Brande and colleagues were the first to examine binding of infliximab and etanercept to lamina propria T cells from patients with Crohn’s disease.\textsuperscript{46} The authors observed binding of activated T cells by infliximab but not etanercept. The authors concluded that, in contrast to infliximab, etanercept does not bind mTNF. We later made similar observations in CD3/CD28-activated T cells, where we found that infliximab, adalimumab, and certolizumab showed efficient binding to activated T cells compared with only very weak binding by etanercept.\textsuperscript{48} When we recently examined activated human primary T cells for mTNF on western blot however, we failed to detect mTNF whereas we readily detected mTNF in LPS-stimulated THP-1 cells that were used as a positive control [unpublished observations]. This raises the suspicion that instead of binding to mTNF, anti-TNF monoclonal antibodies may bind to soluble TNF when bound to its receptor on activated T cells. This may also explain the failure of etanercept [the soluble receptor] to bind to activated T cells, as the receptor-binding surface of TNF would already be occupied. Alternatively, infliximab and adalimumab may prevent processing of mTNF to soluble TNF, whereas etanercept may not. Indeed, in experiments in which a non-cleavable form of mTNF is artificially stably over-expressed in cells, efficient binding of etanercept to these cells can invariably be observed and is similar to that of both infliximab and adalimumab.\textsuperscript{49,50,51,52,53}

Membrane-bound TNF has a short intracellular tail that can become phosphorylated at three different serine residues, which is believed to result in cellular signalling events. This autocrine signalling by mTNF is sometimes referred to as outside-to-inside or reverse signalling. Mitoma and colleagues stably transfected the Jurkat T cell line with TACE resistant [uncleavable] mTNF, and observed that infliximab but not etanercept induced apoptosis in these cells.\textsuperscript{54} The apoptosis induction critically depended on the presence of the serine residues on the intracellular tail, suggesting that apoptosis was dependent on reverse signalling. The authors later showed that adalimumab and golimumb behaved similarly to infliximab in these cells.\textsuperscript{55} In the same follow-up work, it was found that certolizumab reduced viability of transfected Jurkat T cells, but this effect was independent of caspase-mediated apoptosis.\textsuperscript{56} It should be stressed that this anti-TNF induced reverse signalling by mTNF has only been observed in Jurkat T cells stably transfected with an uncleavable mTNF, and that there is little evidence that activated T cells actually express mTNF as mentioned above.

\begin{figure}[h]
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\caption{Two of the major alternative mechanisms of action of anti-tumour necrosis factors [TNFs]. A. There is an anti-apoptotic signal by mTNF expressed on monocytes to the TNFRII expressed on CD4+ T cells. This mechanism is inhibited by anti-TNFs, resulting in apoptosis of CD4+ cells irrespective of the presence of the Fc part of the antibody. B. Anti-TNFs bind to mTNF on activated T cells; the Fc part of the antibody is recognised by Fc receptors expressed by monocytes, triggering their differentiation to an M2-like alternative or wound-healing macrophage fate. This mechanism is dependent on the presence of the Fc region of the antibody and is therefore absent in the case of certolizumab.}
\end{figure}
An alternative mechanism of action of anti-TNF induced T cell apoptosis has recently been achieved by Arteya et al. Arteya and colleagues investigated the effect of infliximab, adalimumab, certolizumab, etanercept on apoptosis in peripheral blood mononuclear cells (PBMCs) and lamina propria cells from IBD patients. The authors found that when lamina propria CD4+ T cells expressing TNF-R2 are co-cultured with mTNF-positive CD14-positive macrophages, infliximab, adalimumab, and certolizumab induced high levels of T cell apoptosis, in contrast to etanercept. The failure of etanercept to induce significant apoptosis in lamina propria T cells confirmed previous findings by Van den Brande et al. who originally made the observation that infliximab, but not etanercept, induced apoptosis in lamina propria T cells.

The induction of T cell apoptosis was dependent on inhibition of TNF-R2 signalling in the CD4+ T cells. The authors propose that mTNF-expressing CD14 macrophages protect CD4+ T cells from apoptosis by activating TNF-R2 signalling, and that this viability signal is interrupted by compounds that bind mTNF. It is not entirely clear why etanercept would not induce apoptosis in this scenario, as etanercept binds mTNF with a very similar affinity as adalimumab and infliximab. Again, binding of anti-TNF compounds to cells does not necessarily mean binding to mTNF, as monoclonal antibodies [but not etanercept] may bind soluble TNF bound to its receptor and may act to prevent mTNF processing and therefore stabilise mTNF, which would normally be rapidly processed to soluble TNF. This may be a confounding factor in many of the published experiments. The difficulty of working with lamina propria mononuclear cells is that the effect of the drugs on apoptosis induction that was observed [in the 5–10% range] is small relative to the baseline apoptosis that is present in cultures of lamina propria mononuclear cells [20–45%]. This was also observed in our own unpublished experiments, and made it difficult to obtain easily reproducible results.

Although there was an original report on infliximab-induced apoptosis in monocytes from patients with Crohn's disease, this was not reproduced by others and the relevance of this finding remains unclear. Work by Caprioli et al., however, showed that a decrease in the mRNA expression of macrophage marker CD68 correlated with an endoscopic response to infliximab at 6 weeks after the start of treatment, when the authors found evidence of TUNEL-positive CD68 macrophages in the lamina propria of these patients. Again, it is not clear if this effect is direct or the result of the resolution of inflammation that occurs in the responders to infliximab at this time point after the start of treatment.

In conclusion, the use of endoscopy and technetium-labelled annexin V has shown that lamina propria T cell apoptosis occurs within 24 h after the administration of infliximab. This can be a direct effect of infliximab on T cells or be a more downstream aspect of the biology of the resolution of an inflammatory infiltrate. Although reverse signalling though mTNF occurs in a T cell line that is stably transfected with an uncleavable form of mTNF, there is little evidence that this occurs in primary human T cells. An alternative theory proposes that anti-TNFs interrupt anti-apoptotic TNF-R2 signalling in CD4+ T cells, leading to apoptosis induction.

11. Fc-region dependent mechanisms

Several groups have investigated the contribution of Fc-dependent mechanisms in the response to anti-TNFs. The Fc region of an antibody can mediate several different effector functions. In complement-dependent cytotoxicity [CDC], an antibody binds to an epitope on a target cell and is subsequently bound by complement. The resulting activation of the complement cascade will result in the lysis of the target cell. In antibody-dependent cellular cytotoxicity [ADCC], the binding of a target cell results in the Fc receptor-mediated engagement of a leukocyte classically a natural killer [NK] cell) which kills the target cell by producing cell-lysing proteins such as granzymes. The role of Fc-dependent effects in therapeutic response has been described for other therapeutic antibodies such as trastuzumab and rituximab. In the case of rituximab, this has resulted in the development of the third-generation anti-CD20 antibody obinutuzumab in which the Fc region is modified to enhance the affinity for the Fc receptor.

12. Antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity

The capacity to induce CDC and ADCC by anti-TNF agents was evaluated by different research groups in vitro. Nesbitt et al. used cells which highly expressed mTNF to examine the ability of infliximab, adalimumab, etanercept, and certolizumab to induce CDC and ADCC. Infliximab and adalimumab both potently induced CDC and ADCC; the effect of etanercept was somewhat reduced, whereas certolizumab [which lacks the Fc] induced neither. Similar results were obtained by several other author groups, all in cells stably over-expressing mTNF. The reduced ability of etanercept to induce CDC may be related to the fact that etanercept lacks a region [the CH1 domain] that acts to bind the complement factor C3 which plays a central role in the complement system. It is not clear why many experiments have shown that etanercept has reduced ability to induce ADCC, as etanercept contains the domains required for Fcγ receptor-binding [the CH2 domain and hinge region of IgG1]. It could be speculated, however, that the antibodies but not etanercept are capable of forming immune complexes that may be a stronger trigger for Fcγ activation.

Although these in vitro studies demonstrate that some anti-TNF agents can induce ADCC and CDC, the clinical relevance is not clear as there are no in vivo data. Furthermore, these studies all used cell lines expressing high levels of mTNF; and the physiological relevance of these methods is uncertain. This was underscored by a study by Kaymackalan et al. These authors could also demonstrate that infliximab and adalimumab, but not etanercept, could induce CDC in mTNF-transfected cells. However, in an effort to confirm these data in a more physiological setting, they used PBMCs from healthy donors and stimulated them with a variety of activators of T cells and monocytes, but in this setting CDC could not be induced by any of the anti-TNF agents.

13. Fcγ-receptor mediated induction of wound healing macrophages

In the past we have extensively tried to characterise effects of anti-TNFs on activated human T cells. We have never been able to detect reproducible changes in either apoptosis or proliferation in T cells with any of the available compounds, working with heat inactivated serum [no complement activation]. We examined various ways of T cell activation such as the lectin phytohaemagglutinin, the superantigen staphylococcal enterotoxin B, and beads coated with CD3/CD28. We also used Jurkat T cells, T cells in PBMCs, and isolated primary CD4+ T cells [extensive unpublished data and Vos et al.]. The first time we observed reproducible effects of any TNF-neutralising compound in vitro was when we started to use a mixed
lymphocyte reactions method [MLR, in which the PBMCs of two
different donors are mixed].44 Infliximab and adalimumab, but not
etanercept or certolizumab, reduced proliferation in the MLR. Since
the difference compared with our previous experiments was that the
MLR contained both T cells and monocytes, which can recognize the
Fc part of antibodies, we hypothesised that the effect may be Fc
receptor-dependent. Indeed, we were able to block the anti-prolifera-
tive effect of infliximab by saturating the Fc receptors with IgG,
and a Fab’2 fragment of infliximab did not reduce proliferation.
Furthermore the full anti-TNF that was originally made to gener-
ate certolizumab did inhibit proliferation. Thus in the MLR, a TNF-
blocking compound needs to both bind activated TNF-expressing
cells [as discussed above, etanercept seems to bind cells only when
a noncleavable form of mTNF is ectopically expressed] and have an
Fc part.

We observed that infliximab and adalimumab, but not certoli-
zumab, induce CD14+ CD206+ M2-type wound-healing macro-
phages which were mediating the anti-proliferative effects in
the MLR.45 Intriguingly, we subsequently found that azathioprine
potentiates anti-TNF induced M2 macrophage induction, and that
macrophages generated with combination therapy where more
immunosuppressive than macrophages generated with infliximab
alone.46 This provides a possible explanation for the fact that azas-
 thioprine potentiates the effect of infliximab,46 other than preventing
the development of anti-infliximab antibodies.

We next examined if M2 macrophages would be induced in patients
with infliximab in vivo. Indeed, we found that CD206+ macro-
phages were induced in endoscopic responders to infliximab but not
in the non-responders.45 Since this observation remains an associa-
tion and does not prove causation, we next performed an experi-
ment in the transfer model of colitis which is sensitive to treatment
with anti-TNF. We compared two different anti-TNFs with the same
TNF-binding variable region and the same affinity for TNF.47 One of
the antibodies, however, was mouse IgG2 and had high affinity for the
Fc, whereas the other was engineered to have mouse IgG1 which has
strongly diminished affinity for the Fc receptors. The IgG2 antibody
efficiently improved the histological severity of colitis, whereas IgG1
anti-TNF had no effect. In contrast, both anti-TNFs worked equally
well in the collagen-induced model of arthritis.48 These in vivo experi-
ments are consistent with the notion that the Fc receptor may play a
role in the efficacy of anti-TNF specifically in IBD.

The potential relevance of the Fc part of infliximab and adali-
numab is supported by two additional clinical observations. The
first is from studies that examined a polymorphism that encodes an
FcγRIIIa with higher affinity. Patients with the V/V high-affinity allo-
type did not have a different clinical response [CDAI] to infliximab
but did have a significantly higher decrease in CRP compared with
the low-affinity allotype [V/F or F/F].49-51 Unfortunately no studies
are available which have examined a potential correlation between
the FcγRIIIa allotype and endoscopic response, which would be the
most important outcome to examine in the light of the Fc receptor-
dependent induction of wound-healing macrophages. The second
important observation is that it has recently been shown that inflix-
imab and adalimumab can be degraded in inflamed intestinal tissues
by tissue matrix metalloproteinases [MMPs] which are activated
during inflammation, as IgG1s have a specific MMP cleavage site
in their hinge region.52 Cleavage results in the generation of Fab’2 frag-
ments which would abrogate Fc receptor binding. Patients who
did not respond to anti-TNF had higher serum levels of MMP3 and
MMP12 and cleaved IgG, suggesting that mucosal processing of full
antibody to Fab’2 fragments may contribute to non-response.

14. Conclusions

Anti-TNFs may have multiple modes of action in Crohn’s disease, which
contribute to the full clinical, biochemical, and endoscopic response.
Two major observations have been made in anti-TNF treated patients
with IBD in vivo, which may relate to these distinct mechanisms.

The first is the rapid induction of T cell apoptosis that seems to
occur already at 24 h after the administration of infliximab, as dem-
onstrated by both endoscopy plus biopsies and administration of
technetium-labelled annexin V.46,47 The second is that M2-type wound-
healing macrophages are specifically induced in responders to inflixi-
mas.48 Regarding the first mechanism, there is little evidence that T
 cell apoptosis is induced directly by anti-TNFs binding to mTNF on
activated T cells. Instead, it seems that anti-TNFs may interrupt anti-
apoptotic signalling by mTNF expressed on monocytes [and perhaps
other cells] through TNFR-II expressed on lamina propria T cells.49
This mechanism of action is shared by infliximab, adalimumab, and
certolizumab. It suggests that certolizumab would also rapidly induce
T cell apoptosis in the lamina propria, which has not been examined
to date. This would be consistent with the rapid reduction of CRP that
was observed in the PRECISE-1 study with certolizumab.

The second mechanism of action is specific for anti-TNFs that
contain an Fc region. Infliximab and adalimumab can induce M2-type
wound-healing macrophages in vitro and in vivo, which may make
an important contribution to complete mucosal healing.50,51 The
relevance of this Fc receptor-mediated mechanism of action is sup-
ported by: the poor rate of complete mucosal healing with certoli-
zumab;52 the specific induction of M2 macrophages in responders to
infliximab;53 experiments in the transfer model of colitis in which the
therapeutic effect of anti-TNF depends on Fc receptor binding54 and
clinical studies demonstrating a positive correlation between FcγRIIIa
allotype with higher affinity and biochemical response. Furthermore,
the observation that MMP-mediated cleavage of infliximab and
adalimumab to generate Fab’2 fragments may contribute to non-
response,55 further supports this mechanism of action.

The above not only provides a potential explanation for the dif-
ferent clinical activity of anti-TNFs in Crohn’s disease but may also
point the way for the development of novel anti-TNFs with increased
rates of mucosal healing. Novel anti-TNFs with enhanced affinity for
the Fc receptor may be developed similar to the third-generation anti-
CD20s. As suggested by Biancheri et al.,56 perhaps these antibodies
can also be made resistant to MMP-mediated cleavage in order to
prevent loss of Fc receptor binding in the inflamed tissue.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

ADL performed a review of the literature and wrote the manuscript, MEW
critically reviewed the manuscript, and GRB supervised the review of the lit-
erature and wrote the manuscript.

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