Report of the ECCO workshop on anti-TNF therapy failures in inflammatory bowel diseases: Biological roles and effects of TNF and TNF antagonists

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Abstract

This second section of the first ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases addresses the biological roles of TNFα and the effects and mechanisms of action of TNFα antagonists. Mechanisms underlying their failure, including...
Understanding the biological activity of tumor-necrosis factor alpha (TNFα) and its available antagonists is essential in order to delineate mechanisms for anti-TNF failures. In this section, we review and discuss the current knowledge regarding possible mechanisms leading to failures of anti-TNF antibodies in the context of their effects at the cellular level, TNF-receptor mediated activities, transmembrane TNF-mediated activities, and the effect of TNFα and anti-TNF agents on different cell types and tissues.

1. Biology of TNFα in IBD (Table 1)

1.1. Biological activity of TNFα

TNFα is generated as a 26-kD transmembrane Type II polypeptide precursor (tmTNF) which is expressed on activated macrophages, T-lymphocytes, natural killer cells and to a lesser extent on non-immune cells such as endothelial cells, smooth muscle cells, keratinocytes and neurons.1-3 Cleavage of tmTNF by TNFα converting enzyme (TACE) releases soluble-TNFα (sTNF).4

TNFα exerts its effects both as a transmembrane protein (tmTNF) and as soluble homotrimeric cytokine (sTNF) through binding to its p55TNFR (TNFR1) and p75TNFR (TNFR2) receptors.5 The TNF receptors diverge in their cellular expression and despite the fact they share structural homology in their extracellular TNF-binding domains, they induce different cytoplasmic signaling pathways.6

The pleiotropic effects of TNFα may be explained by the distinct effects of sTNF and tmTNF and by signaling through the different receptors. This signaling is affected by the balance between tmTNF and sTNF, cell type, cellular activation status, the stimulus eliciting TNFα production, TACE activity, and expression of endogenous TACE inhibitors leading to divergent TNF-mediated effects on cellular viability.

Due to intracellular signaling motifs, tmTNF also serves as a cell surface receptor and allows for "reverse" signaling in TNFα producing cells,7 a fact highly relevant for understanding the effects of anti-TNF agents.

The complexity and plasticity of the TNFα mechanisms of action allows for a number of possibilities to explain primary and secondary anti-TNF therapy failure (primary non response (PNR) and loss of response (LOR)). Further insight into relevant physiological mechanisms that play a role in different disease states and the effect of the different anti-TNF agents on these pathways will improve our understanding of events that lead to loss of therapeutic response.

1.2. TNFR mediated activities at the cellular level

The two TNFRs are ubiquitously expressed and display structurally similar extracellular domains but signal through distinct intracellular regions. TNFR1 contains a death domain while TNFR2 does not.8

TNFRs are initially synthesized as membrane-anchored proteins which are cleaved by proteolysis, forming soluble
molecules capable of binding the TNFα ligand. Soluble TNFRs are constitutively released to the circulation and their levels increase in the course of various disease states and following TNFα stimulation. In cell culture systems, soluble receptors are rapidly produced in response to various stimuli such as TNFα, LPS, PMA, IL-10 and after T cell and neutrophil activation. Receptor shedding followed by decrease in their cell surface density may serve to desensitize cells to the TNFα stimulation. Additionally, the pool of soluble forms could function as physiological attenuators of the TNFα activity by competing for the ligand. On the other hand, it has been proposed that soluble receptors can stabilize and preserve circulating soluble TNFα and thus function as TNFα agonists.

It is generally admitted that TNFα mediates most of TNFα biological effects. Studies in TNFα-knockout mice suggest that it is the major mediator of TNFα toxicity and neutrophil and endothelial activation. Signaling by the TNFRs is mediated by binding of trimeric TNFα molecules to three monomeric subunits of the receptor. Receptor aggregation activates a downstream receptor-associated effect, or facilitates recruitment of downstream factors to the receptor complex, which then transduces signals through intracellular signaling molecules. The cytoplasmic domains of the TNFR are of modest length and function as docking sites for signaling molecules. The various TNFα activities and their coordinated induction are likely mediated by the heterogeneity of the intracellular functional domains of TNFR1 and adaptor proteins.

Three intracellular TNFR1 functional domains including the C-terminal death domain, neutral sphingomyelase and acidic sphingomyelase activating domains mediate downstream signaling events of the activated TNFR1 complex. Signaling occurs through two principal classes of cytoplasmic adaptor proteins: TNF-associated factor (TRAF) and TNFR-associated death domain (TRADD). How the receptor ligand complex activates downstream signaling is not completely understood. The precise pathways for activation of downstream caspases, AP-1, NFκB, and other intracellular responses involve a variety of kinases, including IκB, P38MAPK, JNK, sphingosine kinase, sphingolipase, phospholipase, and other specialized signaling proteins. A number of drug design studies are in progress based on the fact that TNFα exerts its effects through TNFα/TNF complex formation.

Almost all of the signaling mentioned above has been described and focused on TNFα, which was extensively investigated in an attempt to understand the control of cell death mechanisms as a possible therapeutic intervention. The association of TNFα with TRADD/FADD occurs through the death domain which exists on TNFR1, but not on TNFR2, and recruits a series of downstream signaling events associated with cell death. TNFR2 activation by TNFα differs from TNFα activation. Once activated, TNFR2 is readily cleaved by metalloproteases into a soluble shed form which is still capable of TNFα binding. The role of TNFR2 in cellular responses is not fully understood. Activation of TNFR2 in some cells (mainly T-cells and thymocytes) has been shown to induce proliferation, but it was also shown to regulate TNFα induced apoptosis. TNFR2 does not contain a death domain motif but still recruits adaptor proteins. It is thought to directly signal for apoptosis. It has also been proposed that TNFα signal through a so-called ‘ligand-passing’ mechanism. Indeed, TNFα binding to TNFα may increase its concentration in the vicinity of TNFR1 receptors. More precisely, TNFα may accept the TNF ligand from TNFR2, then inducing an apoptotic signal. Others have additionally shown that TNFR2 signals for cell death through its cytoplasmic domain to induce endogenous mTNF expression, which then signals for apoptosis on itself through its own expressed TNFR1.

### 1.3. Transmembrane tumor-necrosis factor alpha (tmTNF)

Binding of sTNF to TNFRI was long-time considered as the principal mechanism of TNFα induced pro-inflammatory action. However, increasing evidence underlines a sTNF-independent and biological relevant role of tmTNF and TNF-RII mediated signaling in chronic inflammation. TmTNF was shown to induce colitis and arthritis in the absence of sTNF. Besides tmTNF “forward signaling” via binding of TNF-receptors on effector cells, tmTNF also functions as a cellular receptor on TNFα producing cells by “reverse signaling”. Reverse signaling through tmTNF in monocytes leads to subsequent phosphorylation of the cytoplasmic tail, binding of casein kinase I, elevation of intracellular calcium and
signaling through p38 — a mitogen-activated protein (MAP-) kinase extracellular signal-regulated kinase pathway. \(^7,35,36\) The phosphorylation of human tmTNF by antibody-treatment seems restricted to serine residues of its cytoplasmic domain. \(^52,53\) A 10 kDa proteolytic fragment of the intracellular portion of tmTNF was observed as a putative nuclear localization signal. \(^38\) Recent evidence in dendritic cells suggests that signal peptide peptidase-like proteases (SPPLa and SPPLb) cleave the intramembrane site of tmTNF, thereby initializing reverse signaling. \(^39\)

2. Mechanism of action of anti-TNF mAbs (Table 2)

Binding of anti-TNF antibodies to tmTNF can block tmTNF interaction with TNF-receptors and neutralize forward signaling via TNF-receptors and/or activate the reverse signaling pathway. Infliximab (IFX), adalimumab (ADA), etanercept and certolizumab pegol (CZP) were shown to bind to tmTNF in transfected human cell lines and induce reverse signaling. \(^40,45\) IFX, ADA and — with conflicting results — etanercept were shown to induce apoptosis in peripheral blood monocytes, leukemic TPH-1 cells and lamina propria T-cells by reverse signaling, whereas CZP did not. \(^42,43,46-49\)

IFX, ADA and etanercept were shown to equally neutralize sTNF, \(^41\) but surprisingly, etanercept failed to show clinical effectiveness in granulomatous diseases such as Wegener’s granulomatosis, \(^50\) pulmonary sarcoidosis \(^51\) and Crohn’s disease, \(^52\) indicating that there is an additional anti-inflammatory action of anti-TNF-antagonists besides neutralization of sTNF. Although differences in ligand binding, crosslinking and induction of complement dependent and antibody dependent cytotoxicity \(^41,53\) may explain these biological differences, the exact reason why etanercept treatment fails in granulomatous disease remains unclear.

Crosslinking seems to be dispensable for reverse signaling, but may explain the different biological potency of the drugs depending on the amount tmTNF expression on cell surfaces and micro environmental conditions at site of inflammation. \(^41\) IFX and ADA, both binding two tmTNF trimers simultaneously, have the potential to crosslink neighboring tmTNF molecules. \(^40\)

Binding of anti-TNF antibodies to tmTNF was shown to suppress pro-inflammatory cytokines, and cell activation as well as downregulate adhesion molecules in different tmTNF transfected cell lines. \(^54\) In line with this hypothesis, resistance to endotoxin mediated cytokine production by reverse signaling has been observed in monocytic cells following treatment with anti-TNF antibodies. \(^7,41\)

Complement dependent cytotoxicity and antibody dependent cytotoxicity of tmTNF bearing cells after stimulation with anti-TNF antibodies was demonstrated. \(^49,53\) IFX and ADA exerted almost equal complement dependent activities in tmTNF transfected cells, while Etanercept was considerably less effective in this regard. \(^51,44,53\) Nevertheless, these observations were not confirmed in normal activated human peripheral blood mononuclear cells. \(^45\)

It should be noted that most of the experimental data on tmTNF signaling were obtained using transfected in vitro systems with cells expressing abnormally high amounts of tmTNF in the absence of TNFRI/II and sTNF. In vivo tmTNF may act simultaneously in concert with other agonistic and antagonistic signaling pathways, turning the TNF\(\alpha\) signaling pathways into a highly complex system. Further insight into the different binding properties of TNF\(\alpha\) antagonists, the intra- and extracellular signaling mechanisms and adaptations are needed in order to better understand the reasons of loss of response.

3. Hypotheses regarding anti-TNF failures (Table 3)

3.1. Cellular mechanisms for loss of response to anti-TNF

TNF\(\alpha\) is central to cellular inflammatory responses. As such, it is not surprising that there are several ways that cells tightly regulate TNF\(\alpha\) and TNF\(\alpha\) receptor activity. When considering how this occurs, it is important to discuss three different events. First, signaling pathways are complex and incompletely understood. For example, sTNF preferentially binds TNFR1 and tmTNF preferentially binds TNFR2, \(^55\) but in reality the precise roles of each agonist and receptor are uncertain. Second, responses will vary depending on cellular activation and cell type. Third, our understanding is largely derived from experiments in single cell systems, which may not represent the more complex in vivo state.
3.2. Other inflammatory pathways

Lack of response to anti-TNF agents could be due to the importance of other inflammatory pathways which are potentially TNFα-independent. Another possibility is that anti-TNF agents may promote some alternative inflammatory pathways. This could explain the observation of paradoxical inflammatory events in immune-mediated inflammatory disorders (IMID) patients treated with anti-TNF agents. This section is divided in 3 parts: first, we review data on cases of paradoxical inflammation, second, we propose some potential explanations for the induction of inflammation through TNFα blockade, and third, we review other inflammatory pathways which are potentially TNF-independent.

3.3. Paradoxical inflammation

Paradoxical inflammatory events have been described in patients with diverse IMID treated with anti-TNF agents. It can be defined by the development of inflammatory lesions in IMID patients after initiation of treatment with anti-TNF agents, normally used to treat them. IBD patients treated with anti-TNF agents can paradoxically experience the onset of other IMID, particularly skin inflammatory lesions resembling psoriasis. These anti-TNF-induced paradoxical inflammatory lesions observed in IMID suggest that anti-TNF could promote inflammation in some patients. We first review clinical data regarding these paradoxical inflammatory events, and then discuss the potential role of anti-TNF in other inflammatory pathways.

Inflammatory skin disorders occurring under anti-TNF therapy are an emerging problem. A recent study has shown that the incidence rate of new onset psoriasis was high in rheumatoid arthritis patients treated with anti-TNF therapy, while no cases of new onset psoriasis were identified in a large cohort of patients treated with traditional therapy. Interestingly, the risk of developing psoriasis varied with the anti-TNF agent. In a recent case series describing the experience with use of a third anti-TNF agent in CD, psoriasis-like lesions occurred under the first anti-TNF treatment in 3 patients, relapsing with the second and third line. One patient developed de novo psoriasis with the third anti-TNF. In all cases, improvement was observed when anti-TNF was stopped; but psoriasis relapsed in all cases with use of the next anti-TNF agent. In most cases, anti-TNF had to be stopped, but one patient used topical treatment and continued the anti-TNF.

New onset of IBD in ankylosing spondylitis patients treated with anti-TNF has been described, but is infrequent and may be only due to the association between both diseases. New onset of CD has been reported in a series of patients with juvenile idiopathic arthritis treated with anti-TNF agents. In a retrospective study on paradoxical adverse events in 296 patients with spondylarthropathies treated with anti-TNF agents, 4 cases with de novo intestinal manifestations under etanercept or IFX were reported, corresponding to respective rates of 1 and 0.3 per 100 patient-years. In the same series, there were 5 cases of de novo psoriasis and 3 cases of acute anterior uveitis. None of these events appeared to be specific of the different anti-TNF agents.

<table>
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<th>Table 3</th>
<th>Hypotheses regarding anti-TNF failures.</th>
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<td><strong>Key messages</strong></td>
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<td>1) Paradoxical inflammatory events can occur in IMID patients treated with anti-TNF agents.</td>
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<td>2) Paradoxical inflammation can occur in the intestine and thus contribute to loss of response.</td>
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<td>3) Little is known about the effect of anti-TNF agents on other inflammatory pathways.</td>
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<td><strong>Questions to address in the future</strong></td>
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<tr>
<td>1) What are the effects of anti-TNF agents on “other” inflammatory pathways in IBD?</td>
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<td>2) Are there inflammatory pathways in IBD which are TNFα independent?</td>
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<td>3) Can loss of response to anti-TNF agents be explained by the induction/promotion of other inflammatory pathways which are normally downregulated by TNFα?</td>
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<td>4) Could the identification of these pathways in anti-TNF failures help us to perform a rational drug switch or select the best drug combination?</td>
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These events of paradoxical inflammation in IMID patients treated with anti-TNF agents clearly suggest that there are TNFα-independent inflammatory pathways relevant to human disease, and even suggest that these pathways could be induced or promoted by the anti-TNF agents themselves.

3.4. Induction of other pro-inflammatory pathways through TNF blockade

The mechanisms responsible for the appearance of “paradoxical inflammation” in some IBD patients treated with anti-TNF are mostly unknown. Some important inflammatory pathways described in CD may be independent of TNFα, and some pro-inflammatory pathways could even be upregulated by TNFα blockade.

3.4.1. IL17/IL23

In a recently published paper, Notley and co-workers analyzed the effect of anti-TNF therapy on Th1 and Th17 cells. TNF blockade in mice with collagen-induced arthritis, using either TNFR-Fc fusion protein or an anti-TNF monoclonal antibody, not only resulted in the expected improvement in joint inflammation, but also in the presence of expanded populations of Th1 and Th17 cells in the periphery. The pathogenic capacity of these cells was further confirmed using adoptive transfer models. The authors provided a plausible explanation to reconcile the anti-inflammatory effect observed in the mice joints and the finding of expanded Th1 and Th17 cells through additional experiments in which they demonstrated that while TNFα blockade increased the numbers of Th1 and Th17 cells in lymph nodes, it simultaneously inhibited their accumulation in the joints. Another study used the CD45RB high T-cell transfer model of colitis to demonstrate that in addition to inducing a marked improvement in colonic inflammation, anti-TNF therapy, downregulated IL2, IFNγ gamma and TNFα.
secretion by lamina propria CD4+ T cells and also resulted in a significantly decreased expression of IL23p19 and IL17 in the inflamed colon.63

Probably more relevant for human IBD are the studies conducted in rheumatoid arthritis (RA) patients. In one of these studies, Kageyama et al. found that treatment of RA with IFX, but not with methotrexate, resulted in a significant reduction in serum IL-23 levels, with no changes in serum IL17 levels.64 The same group previously reported that infliximab therapy in RA patients caused a marked reduction in serum IL-15 levels, whereas it has no effect on serum IL16, IL17 or GM-CSF levels.65

The notion that anti-TNF therapy is able to down-regulate other well-known pro-inflammatory pathways was further supported by Popa and co-workers, using a different experimental approach. These investigators performed cultures of whole blood obtained from RA patients under ADA or etanercept therapy and from healthy volunteers and demonstrated that upon exposure to a range of bacterial stimuli, including heat-killed Salmonella typhimurium, or Staphylococcus aureus, as well as with Salmonella typhimurium LPS, lower production of IL1β and TNFα and a trend towards lower IL6 and IFNγ production is found in RA patients, compared to healthy subjects.66

Taken together it appears that a growing body of evidence suggests that anti-TNF therapy has a broad impact on the immune system which exceeds the blockade of TNFα itself. Some studies suggest that the decrease in Th1 and Th17 cells downregulates several pro-inflammatory cytokines that ultimately contribute to the beneficial effect of this therapy.

Even if this has not been specifically addressed, all the cited evidence supports the hypothesis that the appearance of anti-TNF induced paradoxical inflammation is mediated by consistent upregulation of other pro-inflammatory pathways. Paradoxical inflammation, which occurs in a minority of IBD patients treated with TNFα antagonists, could be associated with the up regulation of some inflammatory cytokines. The observation that Th1 and Th17 cells may increase in organs other than those that are inflamed and target of anti-inflammatory therapy supports this hypothesis. Studies aimed at thoroughly characterizing cell population and cytokine expression in mucosal, circulating inflammatory cells and cells in organs affected with IMID in these patients should provide a final answer to this question.

### 3.4.2. IFN α

TNFα downregulates the production of IFNα by plasmacytoid dendritic cells, and vice versa.67 Anti-TNF agents increase the production of IFNα.68 Consequently, a prolonged blockade of TNFα using anti-TNF agents could by itself promote IFNα production. Such situation mimics infections where there is increased production of IFNα. IFNα has a potential role in the pathogenesis of psoriasis, where it is involved in induction of the chemokine receptor CXCR3 on T cells, maturation and stimulation of myeloid dendritic cells, and expression of pathogenic T cells via IL-15 production in the skin. Furthermore, an increased expression of IFNα has been demonstrated in psoriatic lesions of patients treated with anti-TNF agents. Thus, a side effect of TNFα blockade may be an over expression of IFNα resulting in IFNα-driven inflammation.

### 3.5. Other pathways involved in intestinal inflammation

A number of experimental systems show that altered regulation of intestinal T cell function can result in chronic intestinal inflammation which may be due either to impaired regulatory T cell activity or excessive effector T cell function.69 Several receptors expressed on effector T cells have been recently described in IBD. Some of these inflammatory pathways could be TNF-independent.

#### 3.5.1. NKG2D pathway

NKG2D is an activating receptor preferentially expressed on CD8+ T cells, γδ T cells and NK cells. NKG2D ligands are expressed on the intestinal epithelium, and most of them appear up regulated in IBD. A subset of mucosal CD4+ T cells characterized by NKG2D expression on their surface and mediating cytotoxic and inflammatory response has been described in CD.70 These CD4+ effector T cells expand in the mucosa and the periphery of CD patients but not in UC patients. CD4+ NKG2D+ T cells have been recently described in the transfer-induced colitis in SCID mice.71,72 NKG2D may play a role in the early stage of colitis in this model, since early administration of anti-NKG2D mAb attenuated the development of colitis. The effect of anti-TNF therapies on the NKG2D pathway has not been studied yet.

#### 3.5.2. TNF/TNFR superfamily members

Several co-stimulatory molecules, belonging to the TNF superfamily, amplify the immune response and can promote inflammation.73 These pathways play a critical role in mucosal inflammation and experimental colitis that could be TNF-independent. Members of the TNF superfamily that may be involved in these inflammatory processes include CD40 (TNFRSF5)/CD40L (CD154), TL1A (TNFSF15)/DR3 (TNFRSF25), OX40 ligand (CD252 and TNFSF4) and OX40 (CD134 and TNFRSF4), 4-1BB ligand (TNFSF9) and 4-1BB (TNFRSF25), OX40 ligand (CD252 and TNFSF4) and OX40 ligand (CD137 and TNFRSF9), ICOS (CD278)/B7RP-1 (CD275), CD70 (TNFSF7) and CD27 (TNFSF7), BTNL2.

CD40, expressed by lamina propria B lymphocytes and non-immune cells, such as human intestinal fibroblasts (HIFs) and human intestinal microvascular endothelial cells (HIMECs), interacts with the CD40 ligand on T cells and activated platelets (Danese 2004). These interactions are implicated in the pathogenesis of IBD through multiple effects on humoral, cell-mediated immunity and inflammation.74 The expression of both CD40 and CD40L is increased in the inflamed human intestine, whereas blockade of the CD40–CD40L pathway can prevent or improve colitis in several animal models of IBD.75,76 Uhlig et al. have shown that injection of an agonistic CD40 mAb to T and B cell-deficient mice was sufficient to induce a pathogenic systemic and intestinal innate inflammatory response that was functionally dependent on TNFα and IFNγ as well as IL-12 p40 and IL-23 p40 secretion. This CD40-induced colitis depended on IL-23 p19 secretion, whereas IL-12 p35 secretion controlled wasting disease and serum cytokine production but not mucosal immunopathology. Intestinal inflammation was associated with IL-23 (p19) mRNA-producing intestinal dendritic cells and IL-17A mRNA within the intestine.77
the presence of IL-12. Although ICOS/B7RP-1 interactions are from CD secreted significantly increased amounts of IFN-

amounts of IL-5. In contrast, anti-CD3/ICOS-stimulated-LPMC

stimulated-LPMC from UC secreted significantly increased

OX40-IgG fusion protein to mice with ongoing colitis (but not

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ICOS alone had little to no effect on disease induction. Recent data suggest that anti-CTLA4 administration in mice

induced amelioration of colitis which correlated with Indolea-

min-2,3-dioxygenase (IDO) expression and infiltration of

macrophages, and epithelial cells are significantly increased in

the inflamed mucosa of IBD patients. Anti-CD3/ICOS-stimulated-LPMC from UC secreted significantly increased

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suppresses immune responses. Recent data, however,

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tration prevents and ameliorates experimental colitis. The

role of TL1A–DR3 pathway in infectious and inflam-

matory diseases is beginning to be elucidated. Both TL1A and

DR3 expressions is increased in human and murine IBD. TL1A

enhances cytokine production (Th1 and Th17) by

cytokine-stimulated T cells, whereas anti-TL1A Ab adminis-

tration prevents and ameliorates experimental colitis. The

4-1BBL and 4-1BB as well as the CD70/CD27 pathways

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(PD-1), interacts with B7-H1 (PD-L1) and B7-dendritic cells

(B7-DC; PD-L2) and provides a negative signal that is

essential for immune homeostasis. Expression of PD-1 on T

cells and of B7-H1 on T, B, and macrophage/DCs is increased

in inflamed colon from both IBD patients and colitic mice.

Furthermore, administration of anti-B7-H1, but not anti-B7-

DC, mAb after transfer of CD4+CD45RBhigh T cells suppressed

wasting disease with colitis. The interaction between the TNF family member LIGHT and the TNF family receptor herpes virus entry mediator (HVEM or TNFRSF14) co-stimulates T cells and promotes inflammation. LIGHT also binds the lymphotoxin beta receptor. These molecules play an important role in regulating immunity, particularly in the intestinal mucosa. LIGHT-transgenic mice exhibit abnormalities in both lymphoid tissue architecture and the distribution of lymphocyte subsets and develop intestinal inflammation. HVEM can also act as a ligand for immunoglobulin family molecules, including B- and T-lymphocyte attenuator (BTLA), which suppresses immune responses. Recent data, however, suggest an anti-inflammatory role for HVEM and the importance of HVEM expression by innate immune cells in preventing intestinal inflammation. Butyrophilin-like 2 (BTNL2) is a butyrophilin family member with homology to the B7 co-stimulatory molecules. BTNL2 is over expressed during both the asymptomatic and symptomatic phase of the MDR1a knockout model of spontaneous colitis. BTNL2-Fc reduces proliferation and cytokine production from T cells activated by anti-CD3 and B7-related protein 1, suggesting a role for BTNL2 as a negative co-stimulatory molecule.

4. Conclusion

TNFα blockade can lead to shifts of inflammatory and regulatory cell populations and differentially regulate cells secreting specific cytokines. Under anti-TNFα therapies most pro-inflammatory cytokines are downregulated although some pro-inflammatory cytokines pathways may be upregulated. Additional inflammatory pathways than TNFα have indeed been shown to play a pivotal role in intestinal inflammation and future studies should address the role of these non-TNFα mediated pathways in loss of response to TNFα blockade and development of paradoxical inflammations in other organs.

Conflict of interest

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MA and YC organised the workshop, on behalf of the ECCO scientific committee, drafted and edited the manuscript. All authors wrote specific parts of the manuscript, and critically reviewed the manuscript. All authors approved the final manuscript.

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