



**HAL**  
open science

# Insights into the biology and therapeutic implications of TNF and regulatory T cells

Benoit L Salomon

► **To cite this version:**

Benoit L Salomon. Insights into the biology and therapeutic implications of TNF and regulatory T cells. Nature Reviews Rheumatology, 2021, 10.1038/s41584-021-00639-6 . hal-03287990

**HAL Id: hal-03287990**

**<https://hal.sorbonne-universite.fr/hal-03287990v1>**

Submitted on 16 Jul 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# 1 **Insights into the biology and therapeutic implications of TNF and regulatory** 2 **T cells**

3  
4 Benoit L. Salomon<sup>†</sup>

5  
6 Sorbonne Université, INSERM, CNRS, Centre d'Immunologie et des Maladies Infectieuses  
7 (CIMI-Paris), F-75013, Paris, France.

8  
9 <sup>†</sup>email: [benoit.salomon@inserm.fr](mailto:benoit.salomon@inserm.fr).

## 10 11 **Abstract**

12 Treatments that block tumour necrosis factor (TNF) have major beneficial effects in several  
13 autoimmune and rheumatic diseases, including rheumatoid arthritis. However, some patients do  
14 not respond to TNF inhibitor treatment and some rare occurrences of paradoxical disease  
15 exacerbation have been reported. These limitations on the clinical efficacy of TNF inhibitors can  
16 be explained by the differences between TNF receptor 1 (TNFR1) and TNFR2 signalling and by  
17 the diverse effects of TNF on multiple immune cells, including FOXP3<sup>+</sup> regulatory T (T<sub>reg</sub>) cells.  
18 This basic knowledge sheds light on the consequences of TNF inhibitor therapies on T<sub>reg</sub> cells in  
19 treated patients and on the limitations of such treatment in the control of diseases with an  
20 autoimmune component. Accordingly, the next generation of drugs targeting TNF is likely to be  
21 based on agents that selectively block the binding of TNF to TNFR1 and on TNFR2 agonists.  
22 These approaches could improve the treatment of rheumatic diseases in the future.

## 23 24 **[H1] Introduction**

25 Tumour necrosis factor (TNF) is an inflammatory cytokine that is detected in the blood within  
26 minutes after an injury and has a major protective role in infectious diseases. In the late 1980s,  
27 TNF was detected in the joints of patients with rheumatoid arthritis (RA)<sup>1,2</sup>. A few years later,  
28 overexpression of TNF in transgenic mice was shown to induce autoimmune arthritis<sup>3</sup>. Agents  
29 that block this cytokine, termed TNF inhibitors, include monoclonal antibodies (mAbs) and  
30 soluble TNF receptors. Anti-TNF therapy was first tested in patients with sepsis without clear  
31 success and then repurposed for the treatment of RA in the early 1990s<sup>1,2</sup>. TNF inhibitors are  
32 now widely used and have greatly improved the medical care of patients with RA, juvenile  
33 idiopathic arthritis, psoriasis, psoriatic arthritis and ankylosing spondylitis. Five original TNF

34 inhibitors and numerous biosimilars have been approved, mostly for the treatment of arthritis,  
35 psoriasis or ankylosing spondylitis (**Table 1**). However, not all patients respond to TNF inhibitor  
36 treatment. One-third of patients with RA have to stop taking these drugs within the first year  
37 because of insufficient efficacy or adverse events<sup>4</sup>. About 20% of patients with psoriasis do not  
38 respond to treatment with a TNF inhibitor and around one-third of initial responders lose  
39 response over time<sup>5</sup>. Similar efficacy profiles are observed for patients with inflammatory bowel  
40 disease (IBD)<sup>6</sup>. Although this Review mainly focuses on the effects of TNF inhibitors in  
41 rheumatic diseases, particularly RA, I also discuss their effects and use in the treatment of other  
42 autoimmune and inflammatory diseases to illustrate the role and mechanisms of these agents in  
43 general.

44 Treatment with TNF inhibitors is also associated with adverse effects, such as infections,  
45 which are explained by the intrinsic anti-inflammatory effects of these agents. More intriguing  
46 (and counterintuitive) is the paradoxical exacerbation of pre-existing autoimmune disease or the  
47 development of new-onset autoimmune disease following TNF inhibitor therapy. Rarely, treated  
48 patients can develop lupus-like syndrome, vasculitis, antiphospholipid syndrome or sarcoidosis.  
49 For example, the reported prevalence of systemic lupus erythematosus (SLE) among recipients  
50 of TNF inhibitor therapy is 0.1–0.2%<sup>7-9</sup>. A few patients develop organ-specific autoimmune  
51 conditions, such as interstitial lung disease, optical neuritis, demyelinating neuropathy, multiple  
52 sclerosis (MS), psoriasis or autoimmune hepatitis, with the highest prevalence (2.00–5.00%)  
53 reported for psoriasis and the lowest (0.05–0.20%) for demyelinating disease. Several reviews  
54 have discussed in depth the spectrum of autoimmune diseases occurring in TNF inhibitor-treated  
55 patients<sup>7-10</sup>, among which MS is of particular interest. In the late 1990s, before the increased risk  
56 of demyelinating neuropathy associated with TNF inhibitor treatment was known, two clinical  
57 trials investigated the efficacy of TNF inhibitors in MS. However, these drugs induced  
58 unexpected disease exacerbations that led to the worldwide contraindication of these drugs in  
59 these patients<sup>11,12</sup>. These observations sparked intense interest in elucidating why not all patients  
60 respond to TNF inhibitor therapy, developing biomarkers to predict response, and understanding  
61 why some treated patients develop paradoxical autoimmunity.

62 This Review focuses on the effects of TNF on inflammation and immunity. I describe the  
63 pro-inflammatory and regulatory roles of TNF, both of which are now well-established, and  
64 address the effects of this cytokine on diverse aspects of regulatory T ( $T_{reg}$ ) cell biology,  
65 including their expansion, differentiation and suppressive function. Finally, I describe the effect  
66 of TNF inhibitors on  $T_{reg}$  cells and explore potential candidates for the next generation of drugs  
67 that target TNF or its receptors. Although TNF also has important roles in organogenesis and the

68 development of lymphoid organs, protection of tissues in the nervous system, heart and joints<sup>13-15</sup>  
69 and inhibition of tumorigenesis<sup>16</sup>, these topics are outside the scope of the present Review and  
70 will not be considered.

71

## 72 [H1] The two Janus faces of TNF

73 TNF has complex regulatory and pro-inflammatory effects in diseases with an autoimmune  
74 component<sup>13-15</sup>, such as RA<sup>17</sup>. This cytokine is produced under various inflammatory conditions  
75 by multiple cell types and exists in two forms: a soluble form that acts as a ligand, and a  
76 membrane-bound form that can act as either a ligand or a receptor<sup>18-20</sup>. Furthermore, TNF can  
77 induce multiple downstream signalling pathways<sup>9</sup> as a result of binding to two different  
78 receptors, TNF receptor (TNFR) 1 and TNFR2, which are structurally related but have divergent  
79 biological properties. TNFR1 is broadly expressed whereas TNFR2 is expressed mostly by T  
80 cells, certain myeloid and endothelial cells and some cells of the central nervous system<sup>21,22</sup>.

81 The next sections describe the distinct functions of TNFR1 and TNFR2 and discuss the  
82 proinflammatory and anti-inflammatory effects of TNF on innate immune cells and  
83 lymphocytes, and present information on the cellular source of TNF.

84

## 85 [H2] TNFR1 and TNFR2

86 TNF is one of the most potent pro-inflammatory cytokines<sup>23</sup>, which explains the success of TNF  
87 inhibitor therapy in diseases with an inflammatory component. However, the paradoxical  
88 development or exacerbation of autoimmune disease in some patients treated with these drugs  
89 reveals the anti-inflammatory aspect of this cytokine, which is partly explained by effects  
90 downstream of TNFR2. Polymorphisms in *TNFRSF1B*, which encodes TNFR2, are frequently  
91 observed in patients with rheumatic diseases (RA, SLE, ankylosing spondylitis and systemic  
92 sclerosis) or IBD<sup>24</sup>. These mutations seem to alter the binding kinetics between TNF and TNFR2  
93 and lead to inhibition of downstream NF-κB signalling, which suggests that TNFR2 signalling  
94 has a protective role in these diseases<sup>25</sup>. A single nucleotide polymorphism in *TNFRSF1A*, which  
95 encodes TNFR1, is specifically associated with an increased risk of MS. This allele results in the  
96 expression of a novel soluble form of TNFR1 that binds to and blocks TNF, and therefore  
97 mimics the MS-exacerbating effect of TNF inhibitor therapy<sup>26</sup>. Other mutations in *TNFRSF1A*  
98 that cause TNFR1 misfolding and endoplasmic reticulum stress are found in patients with  
99 periodic fevers<sup>27</sup>.

100 The differential functions of TNFR1 and TNFR2 in rheumatic and autoimmune diseases  
101 have been defined in mouse models. Generally, TNFR1-knockout mice have reduced disease

102 severity whereas TNFR2-knockout mice develop exacerbated disease (**Table 2**). In addition,  
103 treatment with either TNFR1 antagonists or TNFR2 agonists suppresses disease symptoms in  
104 mouse models of arthritis and in mice with experimental autoimmune encephalomyelitis (EAE),  
105 a model of MS, further supporting a pathogenic role of TNFR1 and a protective role of TNFR2  
106 (**Table 2**). A pathogenic role of TNFR1 and a protective role of TNFR2 have also been observed  
107 in mouse models of IBD, at least during the chronic phase of the disease<sup>15,28</sup>. Thus, TNFR1 and  
108 TNFR2 seem to be pathogenic and protective, respectively, in some autoimmune and chronic  
109 inflammatory diseases.

## 110 **[H2] Effects of TNF on innate immunity**

### 111 ***[H3] Pro-inflammatory effects.***

112 The pro-inflammatory effects of TNF on innate immunity involve several distinct mechanisms  
113 (**Figure 1**). TNF is one of the main drivers of acute inflammation because it activates endothelial  
114 cells, induces chemokine release and promotes intense and early (within hours) recruitment of  
115 neutrophils and monocytes, via both TNFR1 and TNFR2<sup>29,30</sup>. Acute inflammation is also  
116 attributed to the TNF-mediated activation of canonical NF- $\kappa$ B signalling, which leads to the  
117 early induction of inflammatory cytokines, including TNF itself, IL-6, IL-8 and IL-1 $\beta$ <sup>14</sup>. TNF  
118 also sustains inflammation through the activation of receptor-interacting protein kinase (RIPK) 1  
119 and RIPK3, which promote necroptosis and the release of inflammatory compounds termed  
120 damage-associated molecular patterns (DAMPs)<sup>31</sup>. In addition, via TNFR1 signalling, TNF  
121 promotes innate immunity by favouring the maturation of dendritic cells<sup>32,33</sup>.

### 122 ***[H3] Regulatory effects.***

123 The immunoregulatory functions of TNF are likely to involve multiple mechanisms (**Figure 1**).  
124 TNF might promote the extra-adrenal production of immunoregulatory glucocorticoids<sup>34</sup> and  
125 inhibit haematopoiesis<sup>35</sup>. TNF also stimulates innate immunosuppressive cells (via TNFR2) and  
126 activates mesenchymal stem cells, which produce increased levels of immunosuppressive  
127 prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), as has been shown in synovial fluid from patients with RA<sup>36,37</sup>. TNF  
128 also promotes immunosuppression by favouring either the differentiation or the suppressive  
129 function of myeloid-derived suppressor cells via increasing their production of reactive oxygen  
130 species, arginase 1 and inducible nitric oxide synthase<sup>38-42</sup>.

### 131 ***[H3] Effects on dendritic cells, monocytes and macrophages.***

132 Although TNF seems to favour the production of T-bet and IL-12 by dendritic cells<sup>43</sup>, other  
133  
134  
135

136 studies suggest that the presence of TNF inhibits the production of p40 (the common chain of IL-  
137 12 and IL-23) by dendritic cells, macrophages and monocytes<sup>44-46</sup>. These divergent findings  
138 could be explained by differential actions of TNF depending on the maturation stage and type of  
139 both antigen presenting cells and dendritic cell subsets.

140 In addition, TNF can either promote or inhibit macrophage activation, effects that are  
141 both probably mediated by TNFR1 (reviewed elsewhere<sup>14</sup>). The early response of macrophages  
142 to incubation with TNF, observed after a few hours, is both NF- $\kappa$ B-dependent and MAPK-  
143 dependent and involves the expression of genes encoding various inflammatory molecules and  
144 cytokines. This initial response is followed (after 24 hours) by a state of desensitization, also  
145 called cross-tolerance or endotoxin tolerance. Desensitized (also termed tolerized) macrophages  
146 are unable to produce inflammatory factors when stimulated by potent activators such as Toll-  
147 like receptor ligands. The mechanism of desensitization involves NF- $\kappa$ B inhibition following  
148 activation of glycogen synthase kinase 3 (GSK3) and TNF-induced protein 3 (TNFAIP3)<sup>47</sup>.  
149 Tolerized macrophages have a transiently reduced capacity to produce IL-12 and IL-23, which  
150 are pro-inflammatory<sup>46,48</sup>. The physiological role of cross-tolerance is probably the prevention of  
151 life-threatening inflammation in the context of overwhelming macrophage activation by  
152 pathogens and Toll-like receptor ligands. Ultimately, after prolonged incubation with TNF,  
153 specifically in the presence of type 1 interferons, macrophages overcome this desensitized state  
154 and recover their inflammatory function by modifying their metabolism and epigenetic  
155 status<sup>49,50</sup>.

## 156 [H2] Effects of TNF on lymphocytes

### 157 [H3] Pro-inflammatory effects.

158 TNF can either promote or suppress immunity through its differential effects on lymphocytes  
159 (Figure 1). The pro-inflammatory effects of TNF result from co-stimulation of T cells, mainly  
160 via TNFR2. TNF activates NF- $\kappa$ B and AKT signalling pathways that lead to increased T cell  
161 proliferation and survival, which are associated with increased levels of BCL2, BCLXL, IL-2  
162 and survivin<sup>51-56</sup>. However, the co-stimulatory effect of TNF binding to TNFR2 on conventional  
163 T cells seems to be of marginal importance compared with its strong effect on T<sub>reg</sub> cells<sup>57</sup>, which  
164 is extensively discussed below.

### 165 [H3] Regulatory effects.

166 Although one report suggests that TNF promotes the expression of IL-10 by B regulatory (B<sub>reg</sub>)  
167 cells<sup>58</sup>, much more is known about the inhibitory effects of TNF on T cells. Prolonged exposure  
168  
169

170 to TNF attenuates T cell receptor signalling by impairing store-operated calcium influx<sup>59,60</sup> and  
171 also favours T cell exhaustion; in one report, TNF blockade during chronic infection with  
172 lymphocytic choriomeningitis virus abrogated the inhibitory gene expression signature<sup>61</sup>. TNF is  
173 able to induce activation-induced cell death via TNFR1 engagement<sup>62</sup>. Interestingly, TNFR2  
174 signalling also seems to increase T-cell apoptosis by interfering with signalling pathways  
175 downstream of TNFR1<sup>63</sup>. However, TNFR2-dependent cell death might specifically occur in  
176 autoreactive T cells, which have altered TNFR2 signalling<sup>25,62,64-68</sup>. Cross-talk between TNFR1  
177 and TNFR2 signalling is discussed in more detail in subsequent sections.

178 TNF also inhibits the differentiation of T<sub>H</sub>17 cells by increasing IL-2 production<sup>69</sup>, and  
179 decreases IL-17 production by conventional T cells and effector T<sub>reg</sub> cells via activation of  
180 TNFAIP3<sup>70,71</sup>. This mechanism might explain the increase in numbers of T<sub>H</sub>17 cells described in  
181 TNFR1-knockout mice or after treatment with TNF inhibitors in mouse models of RA and  
182 psoriasis<sup>44-46,72,73</sup>. A similar increase in T<sub>H</sub>17 cells has been reported specifically in non-  
183 responding patients with RA treated with TNF inhibitors<sup>44,74</sup>. Interestingly, these non-responding  
184 patients showed a T<sub>H</sub>1-mediated and T<sub>H</sub>17-mediated immune response against the TNF inhibitor,  
185 which might have precipitated their lack of clinical response<sup>75</sup>. Finally, in the late 2000s,  
186 regulatory properties of TNF were proposed to result from its effects on T<sub>reg</sub> cells. These  
187 mechanisms are extensively discussed below.

## 188 [H2] Cellular sources of TNF

189 Multiple cell types are able to produce TNF, but the immune cells that produce this cytokine in  
190 the highest amounts are myeloid cells and activated T cells<sup>76</sup>. The role of TNF produced by these  
191 two cell types in rheumatic and autoimmune diseases has been investigated using genetically  
192 modified mice with conditional knock-out of TNF only in myeloid cells or only in T cells. In  
193 mice with collagen-induced arthritis, conditional knockout of TNF in myeloid cells leads to  
194 reduced disease severity, showing that the TNF produced by these cells contributes to the  
195 pathology. By contrast, mice with TNF-deficient T cells have exacerbated arthritis, suggesting a  
196 protective role of the TNF produced by T cells<sup>77</sup>. Similarly, mice with EAE and TNF-deficient  
197 myeloid cells have attenuated disease, which is either delayed in onset or reduced in severity  
198 depending on the model<sup>76,78</sup>. Finally, the role of TNF produced by B cells has been analyzed in  
199 mice with TNF-deficient B cells. These mice have reduced arthritis and reduced levels of  
200 autoantibodies<sup>77</sup>.

## 201 [H2] TNF structure and signalling

204 **[H3] Soluble and transmembrane TNF.**

205 Crystallographic studies show that trimers of TNF interact with trimers of either TNFR1 or  
206 TNFR2<sup>79,80</sup>. This trimeric association of the cytokine with its receptor is characteristic of the  
207 TNF superfamily and is critical for downstream signalling<sup>63,81</sup>.

208 TNF is initially produced as a transmembrane molecule that can be processed by  
209 disintegrin and metalloproteinase domain-containing protein 17 (also known as TNF converting  
210 enzyme (TACE)) encoded by the *ADAM17* gene<sup>82</sup>. Thus, activated myeloid and T cells produce  
211 transmembrane TNF and secreted soluble TNF, which are both biologically active<sup>77,83,84</sup>. The  
212 role of soluble TNF in the pathophysiology of rheumatic and autoimmune diseases has been  
213 assessed in mice engineered to express a TNF protein that cannot be cleaved by TACE. Thus,  
214 these mice produce normal levels of transmembrane TNF but no soluble TNF<sup>83</sup>. Importantly,  
215 such mice do not develop EAE or arthritis, showing that soluble TNF but not transmembrane  
216 TNF contributes to these diseases<sup>77,83,84</sup>. By contrast, mice with global deletion of TNF (full  
217 knock-out) still develop EAE, which suggests that transmembrane TNF has protective effects in  
218 the disease<sup>83</sup>.

219 Despite a similarly high binding affinity for its two receptors, trimeric soluble TNF  
220 triggers TNFR1 signalling much more efficiently than it does TNFR2 signalling<sup>85</sup>. Although this  
221 observation requires further confirmation, TNFR1 signalling is usually considered to be  
222 triggered by both soluble and transmembrane TNF whereas TNFR2 signalling is preferentially  
223 triggered by transmembrane TNF<sup>86</sup>. These observations suggest that soluble TNF (notably that  
224 produced by myeloid cells at the onset of a rheumatic or autoimmune disease) binds to TNFR1  
225 to promote inflammation and precipitate the disease, whereas transmembrane TNF (probably  
226 that expressed by both myeloid and T cells) has regulatory effects mostly derived from triggering  
227 TNFR2. These observations might have implications for the use of TNF inhibitors. For example,  
228 etanercept (a TNFR2–Fc fusion protein) can efficiently block soluble TNF (as well as  $\alpha 3$  and  
229  $\alpha 2\beta 1$  lymphotoxins) but not transmembrane TNF, whereas anti-TNF monoclonal antibodies  
230 block both soluble and transmembrane TNF<sup>82</sup>. This concept also has implications for the design  
231 of next-generation TNF inhibitors, as discussed below.

232  
233 **[H3] TNFR1 and TNFR2 signalling pathways.**

234 TNFR1 and TNFR2 signalling pathways are complex and have been extensively reviewed  
235 elsewhere<sup>14,15,63,86</sup>. Accordingly, only the pathways most relevant to this review are outlined  
236 here. Most of the available knowledge has been obtained in cell lines and non-immune cells and  
237 deserves further investigation to confirm its relevance in immune cells.

238 Upon binding of trimeric TNF to TNFR1, the cytoplasmic tail of the receptor recruits the  
239 adaptor protein TNFR1-associated death domain (TRADD) via its death domain. TRADD can  
240 then interact with other adaptor proteins, such as TNF receptor associated factor 2 (TRAF2), and  
241 kinases, such as receptor-interacting serine/threonine-protein kinase 1 (RIPK1) or cellular  
242 inhibitor of apoptosis (cIAP) 1 and cIAP2. The resulting molecular complex, named complex 1,  
243 is able to phosphorylate and ubiquitinate several other molecules, ultimately leading to potent  
244 activation of canonical NF- $\kappa$ B and MAPK pathways. Members of these pathways, such as c-Jun  
245 N-terminal kinase (JNK) and p38, in turn, activate AP1 complex<sup>14,15,63,86</sup>. This complex-1-  
246 dependent signalling pathway favours cell proliferation and survival. Alternatively, TNFR1 and  
247 TRADD interact with the Fas associated death domain (FADD) adaptors RIPK1 and RIPK3,  
248 forming the complex 2 interactome, which is able to induce cell death: either apoptosis (via  
249 caspase 8 activation) or necroptosis (via mixed lineage kinase domain-like (MLKL) protein  
250 activation)<sup>14,15,63,86</sup>.

251 Complex 1 and complex 2 are downstream effectors of TNFR1 signalling. Complex 1 is  
252 probably involved in most of the effects of TNF on dendritic cells and macrophages, including  
253 activation of inflammatory target genes and production of inflammatory cytokines. Complex 2 is  
254 involved in TNF-dependent, activation-induced cell death and the formation of inflammation-  
255 dependent DAMPs.

256 The signal transduction pathway downstream of TNFR2 lacks a death domain and  
257 involves different adaptors. Binding of transmembrane TNF to TNFR2 recruits TRAF1 or  
258 TRAF2 adaptors to this receptor, leading to activation of cIAP1 or cIAP2 kinases and activation  
259 of canonical and non-canonical NF- $\kappa$ B, JNK and AKT pathways that promote cell proliferation  
260 and survival<sup>14,15,63,86-88</sup>. These pathways are likely to be involved in the TNF-dependent  
261 activation of mesenchymal stem cells and myeloid-derived stem cells as well as T cell co-  
262 stimulation. TRAF2 recruitment to TNFR2 also decreases the amount of cytoplasmic TRAF2,  
263 which interferes with TNFR1 signalling by favouring the formation of (cell death-promoting)  
264 complex 2 to the detriment of (survival-promoting) complex 1<sup>63</sup>. This cross-talk between TNFR1  
265 and TNFR2 signalling pathways seems to be responsible for TNFR2-dependent T cell death<sup>89</sup>.

### 267 **[H3] TNF reverse signalling.**

268 Reverse (extracellular to intracellular) signalling induced by transmembrane TNF has been  
269 described but remains poorly documented. This phenomenon is only outlined here as it has been  
270 reviewed elsewhere<sup>18-20</sup>. In this context, TNFR1 or TNFR2 can act as ligands for transmembrane  
271 TNF, which can function as a cell receptor transducing a signal in several different situations.

272 For example, TNFR2-expressing T cells promote the increased expression of TNF in monocytes  
273 and/or macrophages via transmembrane TNF, a phenomenon that has been observed in the joints  
274 of patients with RA. Also, TNFR1-expressing endothelial cells induce cross-tolerance in  
275 monocytes and/or macrophages via transmembrane TNF. Finally, TNF inhibitors are also able to  
276 bind to transmembrane TNF and thereby induce the apoptosis of transmembrane TNF-expressing  
277 cells; this phenomenon has been observed for instance in T cells and synovial macrophages from  
278 patients with RA<sup>90,91</sup>. The mechanism of TNF reverse signalling involves increased intracellular  
279 levels of calcium and TGF- $\beta$  and activation of the MAPK-ERK pathway. However, the in vivo  
280 relevance of reverse signalling is difficult to assess because this phenomenon has been poorly  
281 described. I consider that reverse signalling might contribute to the spectrum of effects of TNF  
282 and might have an important role in inducing cross-tolerance of macrophages and in the death of  
283 transmembrane TNF-expressing cells induced by administration of TNF inhibitors.

284

## 285 [H2] Integrative view of TNF functions

286 Here, I present a simplified and integrated view of what I believe is the major role of TNF in  
287 immunity (**Figure 1**). TNF is one of the most potent inflammatory cytokines owing to its  
288 capacity to activate endothelial cells, neutrophils, macrophages and dendritic cells, leading to  
289 leukocyte recruitment and massive release of inflammatory cytokines at sites of inflammation.  
290 Most of these pro-inflammatory phenomena are mediated by TNFR1 signalling. Besides its pro-  
291 inflammatory functions, TNF also has anti-inflammatory (regulatory) functions, although their  
292 role and mechanisms in immunity are yet to be clarified. The regulatory functions involve TNF-  
293 dependent activation of suppressive cells, such as mesenchymal stem cells, myeloid-derived  
294 stem cells and of course T<sub>reg</sub> cells (which are extensively discussed below). TNF might also  
295 promote death or exhaustion of T cells and inhibit pathogenic T<sub>H</sub>17 cells. Most of these  
296 regulatory phenomena are mediated by TNFR2 signalling.

297 The end result of TNF blockade depends on the type of autoimmune disease present and  
298 the timing of treatment. Blocking the interaction between TNF and TNFR1 led to increased  
299 numbers of pathogenic T<sub>H</sub>1 and T<sub>H</sub>17 cells in mouse models of arthritis and psoriasis<sup>44-46,72,73</sup>.  
300 This increase was associated with exacerbation of psoriasis (as might logically be expected) but  
301 surprisingly with attenuation of arthritis because this treatment also blocked the migration of  
302 pathogenic T cells to the joints<sup>46</sup>. Similarly, patients with RA treated with TNF inhibitors have  
303 increased levels of circulating T<sub>H</sub>1 and T<sub>H</sub>17 cells<sup>44,74,75</sup>, which could explain some of the  
304 paradoxical inflammation observed in a subset of these patients. The effects of TNF blockade  
305 could also depend on the timing of treatment in relation to the course of disease. To investigate

306 the role of TNF signalling via TNFR2 in a model of collagen-induced arthritis, TNFR1-knockout  
307 mice were treated with TNF on either days 2–20 or days 22–40 after disease induction<sup>92</sup>.  
308 Interestingly, early TNF treatment led to disease exacerbation whereas late TNF treatment led to  
309 attenuation of arthritis. An opposite effect of TNF that is similarly dependent on the stage of  
310 disease progression has been documented in non-obese diabetic mice; TNF seems to exacerbate  
311 diabetes in young mice by activating dendritic cells and to attenuate it in adult mice by inhibiting  
312 conventional T cells and promoting T<sub>reg</sub> cell activation<sup>59,93-95</sup>.

### 314 [H1] Effects of TNF on T<sub>reg</sub> cells

315 T<sub>reg</sub> cells are master regulators of autoimmune diseases. Mice and humans that are genetically  
316 deficient in T<sub>reg</sub> cells die soon after birth from a massive and systemic autoimmune syndrome,  
317 which reveals the critical role of these cells in the suppression of autoimmunity<sup>96</sup>. Functional or  
318 quantitative defects of T<sub>reg</sub> cells have been reported in many human autoimmune diseases<sup>97</sup>.  
319 Other indirect evidence supports the concept that T<sub>reg</sub> cells contribute to human autoimmune  
320 diseases. For instance, some biomarkers of disease activity, such as C reactive protein levels, are  
321 inversely correlated with T<sub>reg</sub> cell proportion in patients with RA<sup>98</sup> or IBD<sup>99,100</sup>. Moreover, T<sub>reg</sub>  
322 cell transfer seems to have beneficial effects in patients with various autoimmune diseases<sup>101</sup>.

323 Transcriptomic analyses that compared T<sub>reg</sub> with conventional T cells in lymphoid tissues  
324 showed that several members of the TNFR superfamily, including TNFR2, TNFR superfamily  
325 member 4 (OX40), TNFR superfamily member 9 (41BB) and TNFR superfamily member 18  
326 (GITR) are included in the T<sub>reg</sub> cell signature<sup>102</sup>. These molecules are further upregulated upon  
327 stimulation of either the T cell receptor (TCR) or T cell-specific surface glycoprotein CD28 and  
328 are therefore preferentially expressed by effector T<sub>reg</sub> cells rather than resting T<sub>reg</sub> cells<sup>103,104</sup>. At  
329 steady state, 30% of T<sub>reg</sub> cells express TNFR2 and most of this subset are effector T<sub>reg</sub> cells that  
330 have a stronger suppressive function in vitro than do TNFR2<sup>-</sup> resting T<sub>reg</sub> cells<sup>105,106</sup>. Thus,  
331 TNFR2 belongs to the T<sub>reg</sub> cell signature and is a marker of highly suppressive T<sub>reg</sub> cells.

### 333 [H2] Effects on T<sub>reg</sub> cell expansion

334 Expansion is defined as an increase in cell numbers, and results from a combination of increased  
335 proliferation, prolonged survival and phenotypic stability. TNFR2 signalling seems to expand  
336 T<sub>reg</sub> cells by increasing all three of these factors.

337 Initially, TNF and/or TNFR2 co-stimulation were shown to increase T<sub>reg</sub> cell proliferation  
338 in mice<sup>22,107</sup>. Our group and others showed that effector T cells, in particular T<sub>H</sub>17 cells, are a  
339 major source of the TNF that induces this increase in the T<sub>reg</sub> cell population in vivo<sup>108-110</sup>.

340 Similar findings were obtained for human T<sub>reg</sub> cells<sup>84,111,112</sup>. TNF can also substantially prolong  
341 T<sub>reg</sub> cell survival<sup>103</sup>. Indirect evidence indicates that TNFR2 signalling also maintains *FOXP3*  
342 expression, which increases T<sub>reg</sub> cell phenotypic stability and therefore their long-term  
343 expansion<sup>112-115</sup>.

344 In many of these in vitro studies, soluble TNF was capable of boosting T<sub>reg</sub> cell  
345 expansion. Although transmembrane TNF has a stronger effect than soluble TNF on induction of  
346 TNFR2 signalling<sup>85</sup>, strong evidence indicates that soluble TNF can indeed stimulate the  
347 expansion of T<sub>reg</sub> cells by binding to TNFR2. Furthermore, TNFR1 expression has not been  
348 detected on T<sub>reg</sub> cells (unlike TNFR2 expression)<sup>22</sup>. The expansion-promoting effect of soluble  
349 TNF on T<sub>reg</sub> cells was lost in TNFR2-deficient T<sub>reg</sub> cells and when TNFR2, but not TNFR1, was  
350 blocked<sup>113</sup>. Finally, treatment with TNF or TNFR2 agonists induced similar co-stimulation of  
351 T<sub>reg</sub> cells<sup>111</sup>. The capacity of soluble TNF to efficiently induce TNFR2 signalling could be  
352 explained by the use of high concentrations of this cytokine or the presence of TNF aggregates  
353 with crosslinking properties in the preparations. TNFR2 agonists, which are either multimers of  
354 mutated TNF or mAbs that bind only to TNFR2 (discussed in more detail below), strongly co-  
355 stimulate T<sub>reg</sub> cells in both mice and humans<sup>57,103,111,116-118</sup>. In a study of pre-activated T cells,  
356 TNFR2 co-stimulation strongly increased the proliferation of T<sub>reg</sub> but had no effect on  
357 conventional T cells<sup>57</sup>. The capacity of TNFR2 co-stimulation to promote T<sub>reg</sub> cell expansion was  
358 confirmed in vivo in animals treated with TNFR2 agonists<sup>86,117,119-121</sup>.

359 Although very little is known about TNFR2 signal transduction in T<sub>reg</sub> cells,  
360 transcriptomic analyses showed that binding of TNF to TNFR2 on purified mouse or human T<sub>reg</sub>  
361 cells induced a gene expression signature indicative of NF-κB pathway activation<sup>103,122</sup>. More  
362 precisely, TNFR2 signalling induced nuclear translocation and binding of RelA to its DNA  
363 target sequence, which suggests that the canonical NF-κB pathway is activated by TNFR2  
364 signalling in T<sub>reg</sub> cells. Importantly, the increased proliferation and prolonged survival of T<sub>reg</sub>  
365 cells induced by TNFR2 triggering was severely attenuated in RelA-deficient T<sub>reg</sub> cells<sup>103,104</sup>.  
366 Some evidence also suggests that the non-canonical NF-κB pathway is also activated by TNFR2  
367 signalling in T<sub>reg</sub> cells but this observation has to be treated with caution because these assays  
368 were conducted on a cell population with low T<sub>reg</sub> cell purity<sup>123</sup>. Other data suggest that TNFR2  
369 signalling induces activation of the MAPK pathway, notably via p38<sup>124,125</sup>. TNFR2-mediated co-  
370 stimulation of T<sub>reg</sub> cells also induced a glycolytic switch associated with activation of  
371 mammalian target of rapamycin complex 1 (mTORC1) signalling via phosphoinositide-3 kinase  
372 (PI3K), although the signalling pathway connecting TNFR2 to PI3K was not identified<sup>57</sup>.

373 Overall, strong evidence indicates that the boost in T<sub>reg</sub> cell numbers induced by TNFR2  
374 signalling involves activation of the canonical NF-κB pathway. The role of the other signalling  
375 pathways mentioned here requires further documentation.

## 376 [H2] Effects on T<sub>reg</sub> suppressive function

377 The effects of TNF on the suppressive function of mouse and human T<sub>reg</sub> cells have been  
378 assessed in vitro (**Table 3**). The first of these studies showed no effect of low-dose (≤5 ng/ml)  
379 TNF in human cells<sup>98</sup>. Five subsequent reports showed that treatment with TNF, usually at a high  
380 dose (50 ng/ml), reduced the suppression of conventional T cell activation by human T<sub>reg</sub>  
381 cells<sup>122,126-129</sup>. By contrast, in vitro studies performed in mouse cells showed that the presence of  
382 high amounts of TNF either had no effect or even increased T<sub>reg</sub> cell-mediated suppression of  
383 conventional T cell activation<sup>22,107</sup>. Moreover, other evidence also suggests that TNF does not  
384 inhibit T<sub>reg</sub> cell-mediated suppression of conventional T cells, and might even increase it. For  
385 instance, administration of a TNFR2 agonist to mice with graft versus host disease (GvHD) or  
386 collagen-induced arthritis promoted T<sub>reg</sub> cell expansion and had a therapeutic effect<sup>117,119,121</sup>.  
387 Also, treatment of cultured T<sub>reg</sub> cells with TNF increased their capacity to suppress colitis or  
388 GvHD after transfer<sup>103,130</sup>, whereas TNFR2-deficient T<sub>reg</sub> cells had a reduced capacity to  
389 suppress colitis or GvHD<sup>114,131</sup>. However, these observations provide only indirect evidence that  
390 TNF either had no effect on or increased T<sub>reg</sub> cell suppressive function in mice. Indeed, this  
391 cytokine might influence other parameters of T<sub>reg</sub> cell biology (such as proliferation, survival,  
392 functional stability or migration). Interestingly, EAE was exacerbated in genetically modified  
393 mice in which TNFR2 was ablated only in T<sub>reg</sub> cells. Ablation of TNFR2 in T<sub>reg</sub> cells seems to  
394 decrease their suppressive function specifically in the inflamed central nervous system<sup>132</sup>. In this  
395 context, the expression of TNFR2 by T<sub>reg</sub> cells might be essential for their suppressive function  
396 and their capacity to control EAE.

397  
398 Our group also performed an analysis of the suppressive capacity of T<sub>reg</sub> cells from  
399 numerous different human donors under three different T cell activation conditions. We  
400 consistently found that TNF (added either before or during the suppression assay) either had no  
401 effect on or even slightly increased the suppressive activity of human T<sub>reg</sub> cells<sup>133</sup>. The  
402 preservation of T<sub>reg</sub> cell suppressive activity after TNFR2 co-stimulation (achieved using a  
403 TNFR2 agonist) in humans has also been confirmed<sup>57</sup>.

404 Several factors might account for the contrasting findings in mouse and human cells.  
405 First, as none of the available markers can exclusively characterize the population of human T<sub>reg</sub>  
406 cells, the purified T<sub>reg</sub> cell populations used in some of these studies might still have some level

407 of contamination by activated conventional T cells, especially when only CD4 and CD25  
408 expression was used to sort the cells<sup>134</sup>. Second, given the high inter-individual variability in T<sub>reg</sub>  
409 cell phenotypes, responses to TNF and suppressive activity, it is important to collect data from a  
410 sufficiently large sample of individuals. Finally, a T<sub>reg</sub> cell functional defect identified in a  
411 suppression assay could be due either to intrinsic T<sub>reg</sub> cell dysfunction or to the presence of  
412 contaminating conventional T cells that are resistant to T<sub>reg</sub> cell suppression. This last point is  
413 critical with regard to the effects of TNF. Indeed, in addition to its proliferation-promoting effect  
414 on T<sub>reg</sub> cells, TNF not only increases the proliferation of conventional T cells<sup>51,53,133</sup> but also  
415 increases their resistance to T<sub>reg</sub> cell-mediated suppression<sup>135</sup>. In several studies performed in  
416 human cells, TNF was present during the suppression assays and might act on any contaminating  
417 conventional T cells, which would impair the evaluation of T<sub>reg</sub> cell suppressive function (**Table**  
418 **3**). Accordingly, pre-incubation of the T<sub>reg</sub> cells with TNF is appropriate before testing their  
419 capacity to suppress conventional T cells.

420 Another critical point is the choice of parameter used to assess the activation of  
421 conventional T cells. As TNF strongly increases T<sub>reg</sub> cell proliferation (and possibly also  
422 cytokine production), measuring the activation of only the conventional T cells within the  
423 population is critical. This measurement can be done by analyzing fluorescent marker dilution or  
424 assessing intracellular cytokine production using flow cytometry techniques such as  
425 fluorescence-activated cell sorting (FACS). Researchers should not use thymidine incorporation  
426 or enzyme-linked immunosorbent assays (ELISA) to measure the proliferation or cytokine  
427 production of the whole cell population, which includes both conventional T cells and T<sub>reg</sub> cells.  
428 For this reason, to accurately determine whether TNF alters the suppressive function of T<sub>reg</sub> cells,  
429 we recommend that TNF is added only during the pre-incubation phase (that is, before the  
430 suppressive assay), and that activation of only the conventional T cells is measured by FACS.  
431 The absence of these two precautionary measures in some of the reports claiming that TNF  
432 inhibits T<sub>reg</sub> cell suppressive activity in humans undermines their conclusions (**Table 3**).

433 To conclude, weak evidence indicates that TNF is able to either inhibit or increase the  
434 suppressive activity of T<sub>reg</sub> cells. After careful analyses of the data from in vitro assays, I would  
435 say that TNF has no or only a minor effect on T<sub>reg</sub> cell suppressive function in this context.  
436 However, this cytokine seems to have an essential role in stimulation of T<sub>reg</sub> cell function in  
437 some conditions associated with inflammation.

438 The data derived from in vitro studies of mechanisms underlying the suppressive activity  
439 of T<sub>reg</sub> cells reflect only the tip of the iceberg, as only two or three suppressive mechanisms have  
440 been analyzed in these studies to date. However, it is now well established that T<sub>reg</sub> cells in vivo

441 are able to use a wide range of suppressive mechanisms depending on their tissue localization  
442 and the type of inflammation present<sup>136,137</sup>. The suppressive activity of T<sub>reg</sub> cells also involves  
443 many different effector molecules. Some have been thoroughly studied and shown to be essential  
444 for aspects of T<sub>reg</sub> cell suppression, such as cytotoxic T-lymphocyte protein 4 (CTLA4) and IL-  
445 10<sup>138</sup>. FOXP3 expression is also critical because its loss leads to loss of T<sub>reg</sub> cell function<sup>138</sup>, but  
446 no single marker has been shown to easily quantify the level of T<sub>reg</sub> cell suppression.

447 Several mechanisms have been suggested to explain how TNF might increase the  
448 suppressive function of T<sub>reg</sub> cells in mice. TNF promotes full differentiation of effector T<sub>reg</sub> cells  
449 by stimulating NF-κB, which might increase some of these cells' suppressive functions<sup>103,104</sup>.  
450 TNF also synergizes with IL-2 to increase the expression of CD25 (the IL-2 receptor α-chain)  
451 and FOXP3<sup>22,133</sup>. Moreover, TNF increases the IL-2-induced phosphorylation of STAT5<sup>22</sup> and  
452 limits the loss of FOXP3 expression in cultured cells by preventing re-methylation of the *Foxp3*  
453 promoter<sup>113,115</sup>. Thus, TNF might increase T<sub>reg</sub> cell suppression and stability by favouring both  
454 phosphorylation of STAT5 and FOXP3 expression, which are key determinants of these T<sub>reg</sub> cell  
455 features<sup>139,140</sup>. Finally, TNF limits IL-17 production by T<sub>reg</sub> cells by activating TNFAIP3<sup>71</sup>.

456 Other mechanisms have been proposed to explain how TNF might decrease T<sub>reg</sub> cell  
457 function. TNF decreases FOXP3 expression by increasing the expression of deleted in breast  
458 cancer 1 (DBC1) and miR-34a, which respectively promote FOXP3 degradation and reduce  
459 *FOXP3* transcription and translation<sup>128,141,142</sup>. Alternatively, TNF might increase the expression  
460 of serine/threonine-protein phosphatase PP1, which dephosphorylates FOXP3, thereby  
461 decreasing its effect on T<sub>reg</sub> cell suppressive function<sup>126</sup>.

462

## 463 [H2] Effects on T<sub>reg</sub> cell differentiation

464 The population of FOXP3<sup>+</sup> T<sub>reg</sub> cells is composed of thymic T<sub>reg</sub> cells, which acquire their T<sub>reg</sub>  
465 cell state during their development in the thymus, and peripheral T<sub>reg</sub> cells, which acquire their  
466 T<sub>reg</sub> cell state during peripheral differentiation of mature naive conventional T cells. Finally,  
467 induced T<sub>reg</sub> cells can be differentiated in vitro from naive conventional T cells by TCR  
468 stimulation in the presence of IL-2 and TGF-β. Thus, induced T<sub>reg</sub> cells are the in vitro  
469 counterpart of peripheral T<sub>reg</sub> cells.

470 However, whereas TNF alone has no effect on thymic T<sub>reg</sub> cell differentiation,  
471 experiments in mice show that TNF inhibits the differentiation of induced T<sub>reg</sub> cells, whereas  
472 treatment with TNF inhibitors increased the differentiation of induced T<sub>reg</sub> cells<sup>143,144</sup>. This  
473 inhibitory effect of TNF was also observed on peripheral T<sub>reg</sub> cells in vivo. In mice with EAE,  
474 injection of anti-TNF or anti-TNFR2 mAbs at the time of disease induction led to reduced

475 disease severity, which was associated with an increased proportion of T<sub>reg</sub> cells and evidence of  
476 increased peripheral T<sub>reg</sub> cell differentiation<sup>144</sup>. Two other papers do not support this observation  
477 and even suggest that the TNF–TNFR2 axis promotes the differentiation of induced T<sub>reg</sub> and  
478 peripheral T<sub>reg</sub> cells<sup>28,73</sup>. However, the design of these two studies meant that contaminating  
479 natural T<sub>reg</sub> cells were present in the starting inoculum, and thus treatment with TNF might boost  
480 the expansion of these contaminating cells rather than increase the differentiation of induced T<sub>reg</sub>  
481 cells<sup>28,73</sup>. TNF does not seem to affect thymic T<sub>reg</sub> cell differentiation at steady state, because  
482 mice lacking TNFR2 have normal thymic T<sub>reg</sub> cell numbers. However, ablation or neutralization  
483 of TNFR2 combined with ablation or neutralization of two other members of the TNFR  
484 superfamily, namely OX40 and GITR, led to reduced differentiation of thymic T<sub>reg</sub> cells<sup>145</sup>.  
485 Overall, whereas the effect of TNF on T<sub>reg</sub> cell differentiation is still open to discussion, an  
486 excess of TNF seems to impair the differentiation of induced T<sub>reg</sub> cells and peripheral T<sub>reg</sub> cells in  
487 mice.

488 In humans, the inhibition of T<sub>reg</sub> cell differentiation by TNF was first observed in patients  
489 with RA. TNF inhibitor treatment increased the in vitro differentiation of induced T<sub>reg</sub> cells  
490 derived from patients with RA but not those from healthy controls<sup>146</sup>. This observation explained  
491 why blood samples from patients with RA treated with infliximab had an increased proportion of  
492 T<sub>reg</sub> cells, which might result from increased differentiation of peripheral T<sub>reg</sub> cells<sup>98,146</sup>. Other  
493 members of the TNF family, such as 41BB, OX40 or TNFR superfamily member 25 (also  
494 known as death receptor 3 (DR3)), can also inhibit the differentiation of induced T<sub>reg</sub> cells<sup>147-149</sup>.  
495 These observations suggest that a shared mechanism is involved, perhaps implicating NF-κB,  
496 PI3K or MAPK pathways. IFN $\gamma$  produced by T cells following TNFR co-stimulation has also  
497 been proposed to inhibit the differentiation of induced T<sub>reg</sub> cells. Alternatively, the increased  
498 activation of the PI3K–AKT pathway resulting from TNFR signalling could lead to reduced  
499 activation of phosphorylated SMAD3, which transactivates *Foxp3* expression in mouse induced  
500 T<sub>reg</sub> cells<sup>144</sup>.

501

## 502 [H2] Overall effects of TNF on T<sub>reg</sub> cells

503 In summary, TNF has multiple negative and positive effects on T<sub>reg</sub> cell biology, most probably  
504 resulting from TNFR2 rather than TNFR1 signalling (**Figure 2**). The best-characterized of the  
505 positive effects of TNF are increased T<sub>reg</sub> cell proliferation and expansion. TNF also seems to  
506 promote T<sub>reg</sub> cell survival in vitro, although the relevance of this effect in vivo is difficult to  
507 evaluate. The TNF-dependent increases in T<sub>reg</sub> cell proliferation and survival are at least partially  
508 dependent on RelA and activation of the canonical NF-κB pathway. Involvement of p38 and

509 PI3K–AKT pathway activation has also been suggested but requires further investigation.  
510 Finally, weak evidence indicates that TNF increases the stability and suppressive function of T<sub>reg</sub>  
511 cells, a phenomenon that might be partially due to TNF signalling synergizing with IL-2  
512 signalling and with phosphorylation of STAT5. Other reports suggest a negative effect of TNF  
513 on T<sub>reg</sub> cell biology in vitro. Whether this cytokine truly has a negative effect on T<sub>reg</sub> cell  
514 function is questionable. By contrast, TNF seems to increase T<sub>reg</sub> cell suppressive function in  
515 vivo, at least in some inflammatory contexts. However, the evidence of an inhibitory effect of  
516 TNF on differentiation of induced T<sub>reg</sub> cells is fairly solid and might involve the PI3K–AKT  
517 pathway (**Figure 2**).

518

### 519 **[H1] T<sub>reg</sub> cells in RA**

520 As T<sub>reg</sub> cells have an important role in the suppression of autoimmunity, numerous studies have  
521 attempted to identify whether these cells have a quantitative or functional defect in patients with  
522 autoimmune diseases. Major drawbacks of these studies include the use of sample sizes too small  
523 to account for interindividual variability and the absence of a specific marker for human T<sub>reg</sub>  
524 cells, which has led to the utilization of different combinations of markers. As a result, the  
525 literature is full of conflicting data.

526 I present here the main findings on T<sub>reg</sub> cell proportion and function in patients with RA.  
527 This disease is particularly interesting as T<sub>reg</sub> cells can be obtained from both the blood and joints  
528 (the target tissue of the disease), which are easily accessible for analysis. Data obtained in other  
529 autoimmune diseases are also included where relevant.

530

### 531 **[H2] T<sub>reg</sub> cell proportion**

532 Contrasting findings have been reported in studies of the proportion of T<sub>reg</sub> cells in the blood of  
533 patients with RA receiving conventional immunosuppressive treatment (the effects of TNF  
534 inhibitors are discussed below). Among studies that compared patients with RA with healthy  
535 control individuals, four described a decreased T<sub>reg</sub> cell proportion<sup>75,150-152</sup>, five found no  
536 difference<sup>98,126,146,153,154</sup> and one found an increased T<sub>reg</sub> cell proportion in the patients with  
537 RA<sup>155</sup>.

538 Most studies that have analyzed both blood and synovial fluid of patients with RA  
539 concluded that the proportion of T<sub>reg</sub> cells was higher in synovial fluid than in blood, and  
540 remained stable over time in individual patients<sup>152,155-157</sup>. The T<sub>reg</sub> cells isolated from synovial  
541 fluid seem to be bona fide T<sub>reg</sub> cells because they exhibit *FOXP3* promoter demethylation. Also,  
542 the phenotype of these T<sub>reg</sub> cells indicates that they have an activated status<sup>157</sup>. The synovial fluid

543 of patients with RA contains high amounts of IL-6, TNF and IFN $\gamma$ , low levels of IL-17A, IL-10  
544 or IL-13 and does not contain IL-1<sup>126,157</sup>. Which of these factors is responsible for the increased  
545 proportion and activation of synovial T<sub>reg</sub> cells remains unclear. However, IL-6 is not likely to be  
546 involved, because this cytokine (which is produced by joint fibroblasts) induces  
547 transdifferentiation of T<sub>reg</sub> cells into highly pathogenic T<sub>H</sub>17 cells in a mouse model of  
548 autoimmune arthritis, a phenomenon that might also take place in patients with RA<sup>158</sup>. IL-6 also  
549 induced proteasomal degradation of FOXP3 and loss of the suppressive activity of T<sub>reg</sub>  
550 cells<sup>159,160</sup>. We do not know much about the effect of IFN $\gamma$  on T<sub>reg</sub> cells. Therefore, the activation  
551 and/or expansion of T<sub>reg</sub> cells in the synovial fluid of patients with RA is likely to be caused by  
552 high local levels of TNF.

553

## 554 [H2] T<sub>reg</sub> cell function

555 Compared with T<sub>reg</sub> cells obtained from the blood of healthy control individuals, T<sub>reg</sub> cells  
556 isolated from the blood of patients with RA were shown to have similar suppressive activity in  
557 one study<sup>155</sup> and decreased suppressive activity in another<sup>128</sup>. In a third study, the capacity of  
558 these cells to suppress conventional T cell proliferation was maintained but their cytokine  
559 production was reduced<sup>98</sup>. Contrasting findings have also been reported for the suppressive  
560 activity of T<sub>reg</sub> cells isolated from the synovial fluid of patients with RA. Several studies showed  
561 that synovial fluid T<sub>reg</sub> cells from patients with RA were as active, or were more active, than  
562 blood T<sub>reg</sub> cells from either patients with RA or healthy control individuals in terms of  
563 suppression of proliferation or IFN $\gamma$  production<sup>152,155-157</sup>. Another publication reported that  
564 synovial fluid T<sub>reg</sub> cells from patients with RA had decreased suppressive activity<sup>126</sup>.  
565 Importantly, these studies noted considerable variation between patients, with T<sub>reg</sub> cells from  
566 some individuals but not others showing a high level of suppression<sup>157</sup>. This observation might  
567 explain the contrasting results and further emphasizes the importance of generating data from at  
568 least 7–10 different patients, which was not the case for most of these studies.

569 Firm conclusions are difficult to draw because the available evidence does not provide a  
570 clear picture of whether T<sub>reg</sub> cells in the blood of patients with RA have similar proportions and  
571 functions to those of healthy control individuals. The situation is a little bit clearer for synovial  
572 fluid T<sub>reg</sub> cells, which seem to be present at an increased proportion in patients with RA.

573

## 574 [H2] Effects of TNF inhibitors

### 575 [H3] T<sub>reg</sub> cell proportion

576 The proportion of T<sub>reg</sub> cells in the blood has been analyzed in many studies of patients with RA  
577 3–6 months (typically 3 months) after initiation of TNF inhibitor treatment. In studies of  
578 infliximab-treated patients with RA, the T<sub>reg</sub> cell proportion increased<sup>75,98,115,146,151</sup> after  
579 treatment (**Table 4**). In studies of patients with RA treated with either adalimumab or etanercept,  
580 the T<sub>reg</sub> cell proportion was either increased<sup>150,154,161</sup> or unchanged<sup>153,154,162</sup> (**Table 4**). This T<sub>reg</sub>  
581 cell increase was more often observed in responding than in non-responding patients.

582 Moreover, in studies of infliximab-treated patients with Crohn disease or IBD  
583 (**Supplementary Table 1**), the T<sub>reg</sub> cell proportion was also either unchanged<sup>99,163</sup> or  
584 increased<sup>100,115,163-168</sup>. Some of the studies in patients with IBD or Crohn disease also analyzed  
585 the kinetics of this treatment-related increase in T<sub>reg</sub> cell proportion. In a study of patients with  
586 Crohn disease, the increase was transient and only occurred after the first injection<sup>165</sup>. In two  
587 studies of patients with IBD, the increase occurred 2 weeks after the first injection and was  
588 maintained for  $\geq 22$  weeks<sup>100,166</sup>, whereas in another study in patients with Crohn disease no  
589 increase was detected after 1 week but an increase was detected at week 24 in patients who had  
590 low T<sub>reg</sub> cell proportions before treatment<sup>99</sup> (**Supplementary Table 1**).

591 Two studies in patients with uveitis<sup>169,170</sup> and one in patients with ankylosing  
592 spondylitis<sup>171</sup> showed an increase in the T<sub>reg</sub> cell proportion after TNF inhibitor therapy.  
593 However, one study in patients with juvenile idiopathic arthritis observed no difference<sup>172</sup> and  
594 one in patients with sarcoidosis observed a decrease in the T<sub>reg</sub> cell proportion<sup>173</sup> following TNF  
595 inhibitor therapy (**Table 4**).

596 Some general conclusions can be drawn from these data. Most publications described an  
597 increase in the proportion of T<sub>reg</sub> cells in blood after TNF inhibitor therapy. Discrepancies  
598 between some studies could be due to the following factors: first, infliximab seems to induce an  
599 increase in the T<sub>reg</sub> cell proportion more consistently than either adalimumab or etanercept.  
600 Second, a T<sub>reg</sub> cell increase seems to be more consistent among patients who responded to TNF  
601 inhibitor treatment. The type of concomitant medications might also matter. For instance,  
602 although methotrexate monotherapy induces an increase in T<sub>reg</sub> cell proportion<sup>150</sup>, combination  
603 therapy with methotrexate and a TNF inhibitor provided an optimal increase in T<sub>reg</sub> cells in  
604 vitro<sup>174</sup>. Also, steroid treatment might increase T<sub>reg</sub> cell proportion and function<sup>175,176</sup>. Finally, as  
605 discussed above, technical factors related to the way that T<sub>reg</sub> cells were purified might influence  
606 the conclusions of these studies. Some activated conventional T cells (which also express CD25)  
607 are likely to contaminate the population identified as T<sub>reg</sub> cells. Thus, the findings of these  
608 studies have to be considered carefully because the level of conventional T cell contamination  
609 could differ between healthy control individuals and patients with rheumatic or autoimmune

610 disease, or before and after TNF inhibitor treatment. Use of the CD45RA (naive T cell) or  
611 CD45RO (memory T cell) markers, in addition to CD25 or CD127, would help to limit the risk  
612 of such contamination<sup>134</sup>.

613 Several mechanisms by which T<sub>reg</sub> cells might increase after TNF inhibitor treatment are  
614 supported by experimental evidence. First, T<sub>reg</sub> cells might increase because treatment with TNF  
615 blockers such as infliximab favour the differentiation of peripheral T<sub>reg</sub> cells<sup>146</sup>. Second, T<sub>reg</sub> cells  
616 might increase because treatment with anti-TNF mAbs such as adalimumab augments the  
617 expression of transmembrane TNF on monocytes, which then triggers T<sub>reg</sub> cell expansion via  
618 TNFR2 signalling. Thus, anti-TNF mAbs that are intended to inhibit TNF might paradoxically  
619 increase its activity<sup>84</sup>. The preferential expansion of activated T<sub>reg</sub> cells rather than resting T<sub>reg</sub>  
620 cells in patients receiving anti-TNF mAbs supports this hypothesis<sup>100</sup>. In patients with RA<sup>151</sup> or  
621 IBD<sup>168</sup>, T<sub>reg</sub> cells that are more sensitive to spontaneous apoptosis than are those of healthy  
622 control individuals, might be present at an increased proportion in patients treated with TNF  
623 inhibitors because they are protected from cell death by this therapy. In patients with IBD, TNF  
624 inhibitor therapy blocks T<sub>reg</sub> cell migration to inflamed tissues, which results in increased T<sub>reg</sub>  
625 cell levels in blood and decreased levels in the intestinal mucosa<sup>166</sup>. Finally, TNF inhibitor  
626 therapy leads to a decrease in inflammatory cytokine levels and pathogenic T cells while sparing  
627 T<sub>reg</sub> cells in patients with Crohn disease<sup>161</sup> or ankylosing spondylitis<sup>165,171</sup>. Therefore, this  
628 treatment might target conventional T cells in preference to T<sub>reg</sub> cells, thereby explaining the  
629 relative increase in the T<sub>reg</sub> cell proportion within the population of CD4<sup>+</sup> T cells. As the  
630 increased proportion of blood T<sub>reg</sub> cells following TNF inhibitor treatment is an in vivo  
631 phenomenon that occurs over a long time period, determining which of the above-described  
632 mechanisms is most relevant remains a challenge.

633

### 634 ***[H3] Suppressive function.***

635 Treatment with mAb TNF inhibitors affects not only the T<sub>reg</sub> cell proportion but also their  
636 suppressive function. Early work showed that T<sub>reg</sub> cells from patients with RA obtained before  
637 the initiation of TNF inhibitor therapy had a poor capacity to suppress cytokine production by  
638 conventional T cells, and that the suppressive activity of these T<sub>reg</sub> cells was restored following  
639 anti-TNF treatment<sup>98</sup>. These functional T<sub>reg</sub> cells resulted from either the generation of new  
640 peripheral T<sub>reg</sub> cells following infliximab treatment<sup>146</sup> or from the expansion of differentiated T<sub>reg</sub>  
641 cells following adalimumab treatment<sup>84</sup>. These restored T<sub>reg</sub> cells were even able to suppress  
642 pathogenic T<sub>H</sub>17 cells, unlike the T<sub>reg</sub> cells of healthy control individuals<sup>154</sup>. Dysfunction of T<sub>reg</sub>  
643 cells obtained from the blood or synovial fluid of patients with RA and restoration of their

644 suppressive function after TNF inhibitor treatment (infliximab) were also confirmed in two other  
645 studies<sup>126,128</sup>. Restoration of functional blood T<sub>reg</sub> cells after TNF inhibitor treatment has also  
646 been described in patients with IBD<sup>164</sup>.

647 TNF inhibitors based on mAbs seem to act, at least in part, by restoring the functional  
648 T<sub>reg</sub> cell compartment. By contrast, etanercept is likely to act by suppressing conventional T cells  
649 and/or rendering them sensitive to the suppressive effects of T<sub>reg</sub> cells<sup>172,177</sup>. In another study, T<sub>reg</sub>  
650 cells obtained from patients with Crohn disease were shown to be functional even before  
651 initiation of infliximab treatment<sup>178</sup>. However, the T<sub>reg</sub> cell purification strategy used in this  
652 paper meant that activated conventional T cells might have contaminated the population of T<sub>reg</sub>  
653 cells, thereby resulting in an inaccurate measurement of the suppressive activity of genuine T<sub>reg</sub>  
654 cells. Therefore, the conclusions of this report have to be interpreted with caution.

655 In summary, the beneficial effects of TNF inhibitor therapies could be due to either  
656 restoration of fully functional T<sub>reg</sub> cells or to an increased susceptibility of conventional T cells  
657 to the suppressive effects of T<sub>reg</sub> cells.

658

### 659 *[H3] T<sub>reg</sub> cell biomarkers of response.*

660 The development of biomarkers to identify the 20–30% of patients with RA or IBD who will not  
661 respond to TNF inhibitor therapy is highly desirable, and some T<sub>reg</sub> cell-related biomarkers are  
662 potential candidates. In some studies, an increase in the T<sub>reg</sub> cell proportion after TNF inhibitor  
663 treatment was observed only in patients who responded to this therapy (**Table 4**). Thus, the T<sub>reg</sub>  
664 cell proportion before TNF inhibitor treatment has been proposed as a predictive biomarker to  
665 predict treatment response. However, patients with IBD who respond to TNF inhibitors could  
666 have T<sub>reg</sub> cell proportions before therapy that are either higher<sup>99,100</sup> or lower<sup>178</sup> than those of non-  
667 responding patients. Moreover, in patients with ankylosing spondylitis<sup>171,174</sup> or RA<sup>171,174</sup>, the T<sub>reg</sub>  
668 cell proportion before TNF inhibitor therapy was not predictive of treatment efficacy.

669 As discussed above, a possible mechanism for the observed increase of T<sub>reg</sub> cells upon  
670 mAb TNF inhibitor treatment is binding of the mAb to transmembrane TNF on myeloid cells,  
671 leading first to its increased expression and then to a boost in T<sub>reg</sub> cell numbers mediated by  
672 TNFR2 signalling<sup>84</sup>. As the expression of transmembrane TNF on monocytes can be readily  
673 assessed by flow cytometry, the capacity of adalimumab to provoke an increase in T<sub>reg</sub> cell  
674 numbers in a 3-day culture has been used to identify which patients with RA would respond to  
675 this treatment<sup>174</sup>.

676 In summary, pretreatment T<sub>reg</sub> cell proportion does not seem to be a reliable biomarker of  
677 response to anti-TNF therapies. The expression of transmembrane TNF on myeloid cells as a  
678 biomarker of treatment response deserves to be confirmed in other studies.

### 680 [H1] Next-generation drugs targeting TNF

681 The putative mechanisms underlying non-response and paradoxical autoimmunity to TNF  
682 inhibitor treatment could be explained by the regulatory aspect of TNF. Blocking TNF is  
683 associated with an increased risk of impairing the activity of some suppressor cells, including  
684 T<sub>reg</sub> cells, or increasing the activation of autoreactive T cells. The overall effect of these  
685 treatments is likely to depend on the specific autoimmune disease present, its stage and severity,  
686 and on genetic and environmental factors unique to each patient. At the time of treatment, if TNF  
687 has a dominant inflammatory and pathogenic role, TNF inhibitors will be beneficial. By contrast,  
688 if TNF has a dominant regulatory and protective role, TNF inhibitors will be detrimental.

689 Given that most of the pro-inflammatory properties of TNF are due to TNFR1 signalling  
690 induced by soluble TNF and most of the regulatory properties of are due to TNFR2 signalling  
691 induced by transmembrane TNF, the next generation of TNF inhibitors might preferentially  
692 target TNFR1 or TNFR2<sup>14,25,82,86,88,179,180</sup>. Two types of TNFR-specific agents have been  
693 proposed: mAbs and so-called TNF muteins, which are forms of this cytokine harbouring  
694 mutations in the receptor-interacting domains<sup>181-183</sup>.

### 696 [H2] Selective TNFR1 antagonists

697 The capacity of TNFR1 antagonists to block the pro-inflammatory interaction of TNF with  
698 TNFR1 has been investigated in mouse models of autoimmune diseases.

699 Both mAbs and TNF muteins have been developed that have potent TNFR1 antagonist  
700 activity and a strong therapeutic effect in mouse models of autoimmune diseases (**Table 5**). For  
701 example, the mAb DMS5540 was as effective as etanercept in the treatment of collagen-induced  
702 arthritis. In addition, the effects of DMS5540 on immune cells were superior to those of  
703 etanercept, as DMS5540 induced T<sub>reg</sub> cell activation and reduced the activation of conventional  
704 T cells, a phenomenon not observed with etanercept<sup>184</sup>. Several anti-TNFR1 mAbs (namely  
705 atosab, trivalent nanobody TNFR1 silencer (TROS) and HM1097) were able to suppress  
706 EAE<sup>185-187</sup>. Finally, the muteins XPro1595 and R1antTNF had therapeutic effects in arthritis or  
707 EAE; when these agents were compared with etanercept, they sometimes demonstrated  
708 improved efficacy<sup>188-192</sup>.

710 **[H2] TNFR2 agonists**

711 As TNFR2 signalling stimulates the expansion of T<sub>reg</sub> cells, TNFR2 agonists such as the mAbs  
712 MR2-1 and another unnamed version are interesting candidates for improving T<sub>reg</sub> cell therapy in  
713 autoimmune diseases (**Table 5**). When added to human T<sub>reg</sub> cell cultures, these mAbs promote  
714 the expansion and improve the stability and purity of T<sub>reg</sub> cells over time<sup>111,118</sup>.

715 Two additional TNF muteins with human TNFR2 agonist activity (TNF07 and TNC-  
716 scTNFR<sub>2</sub>) have been generated<sup>116,193</sup> and TNF07 has been shown to promote T<sub>reg</sub> cell activation  
717 in vitro. In the future, mAbs or TNF muteins with TNFR2 agonist activity might be used to  
718 improve cell culture methods used to generate T<sub>reg</sub> cell preparations for use in cell therapy. This  
719 notion is supported by mouse studies showing that adding TNF or a TNFR2 agonist to T<sub>reg</sub> cell  
720 cultures increased the capacity of these cells to suppress colitis<sup>103,130</sup> or GvHD<sup>103,130</sup> after their  
721 reintroduction in vivo<sup>103,130</sup>.

722 The capacity of TNFR2 agonists to stimulate T<sub>reg</sub> cells in vivo has been tested in mouse  
723 models. Treatment with either of two TNF muteins with TNFR2 agonist activity (STAR2 and  
724 EHD2-sc-mTNFR<sub>2</sub>)<sup>194,195</sup> induced in vivo T<sub>reg</sub> cell activation and expansion<sup>117,120</sup> that was  
725 associated with prevention or amelioration of arthritis<sup>119,121</sup>, EAE<sup>132</sup> or GvHD<sup>117</sup>. These agents  
726 also protected the central nervous system of treated animals from inflammation and neuronal  
727 injury induced by chronic nerve constriction or drug treatment, respectively<sup>195,196</sup>.

728 Whereas classical immunosuppressive drugs aim to suppress autoimmunity by  
729 neutralizing pathogenic cells, an alternative approach is based on increasing the expansion or  
730 suppressive capacity of T<sub>reg</sub> cells. A prototype of this new class of drugs is IL-2<sup>197</sup>. Our group  
731 showed that administration of low-dose IL-2 boosts the proliferation of T<sub>reg</sub> cells and induces  
732 remission of type 1 diabetes mellitus in non-obese diabetic mice<sup>198,199</sup>. Low-dose IL-2 is now  
733 being investigated as a treatment for other autoimmune diseases in multiple clinical trials. One  
734 study has investigated this treatment in 14 different autoimmune diseases, including RA and  
735 ankylosing spondylitis (NCT01988506). TNFR2 agonists are another type of drug that are able  
736 to boost the number or function of T<sub>reg</sub> cells. No clinical trial has so far investigated the use of  
737 TNFR2 agonists to treat an autoimmune disease. However, bacillus Calmette–Guérin (BCG)  
738 vaccine can induce TNF release without secondary effects, thereby providing an indirect way to  
739 trigger TNFR2 signalling. BCG vaccine has been tested for efficacy in type 1 diabetes  
740 (NCT00607230 and NCT02081326).

741

742 **[H1] Conclusions**

743 TNF has a long and fascinating yet chaotic history. This cytokine was discovered in the mid-

744 1970s and named for its effect as a tumour cell killer. Major milestones in its history include its  
745 cloning in the mid-1980s, the discovery that TNF binds to two receptors, that its signalling  
746 transduction is highly complex (and still remains to be fully explored), and that it has multiple  
747 effects at steady state.

748 TNF is now known to be one of the most important inflammatory cytokines. Although  
749 TNF is critical for beneficial immune responses, the realization that TNF is also harmful in many  
750 autoimmune diseases led to the great success of TNF inhibitors and ultimately to the flowering  
751 of research into other biological therapies. The regulatory role of this cytokine is also important  
752 to consider. Here again, the mechanisms underlying the immunosuppressive activity of TNF are  
753 complex. However, one of its main features seems to involve the expression of transmembrane  
754 TNF on myeloid or T cells, which interacts with TNFR2 on T<sub>reg</sub> cells to boost their proliferation  
755 and maybe also their stability and suppressive function.

756 The inflammatory and regulatory roles of TNF are both essential to take into account in  
757 the design of future generations of TNF inhibitors. Preclinical studies have shown that selective  
758 antagonists of TNFR1 inhibit the inflammatory action of TNF whereas selective agonists of  
759 TNFR2 boost T<sub>reg</sub> cell numbers and potentially also improve their function. Therefore, TNFR1  
760 antagonists and TNFR2 agonists could be beneficial in future treatments of several diseases with  
761 an autoimmune component. In the future, biotechnology and pharmaceutical companies are  
762 expected to work hand in hand with academic laboratories towards the successful translation of  
763 these fascinating observations into the clinic.

764

#### 765 **Acknowledgements**

766 The author thanks current and past members of his laboratory for their hard work and fruitful and  
767 passionate exchanges on the effects of TNF on T<sub>reg</sub> cells. His research work is supported by  
768 Agence Nationale de la Recherche (grants ANR-15-CE15-0015-03 and ANR-17-CE15-0030-01)  
769 and Fondation pour la Recherche Médicale (équipe FRM).

770

#### 771 **Competing interests**

772 B.S. declares that he received consultancy fees from HiFiBio Therapeutics regarding the  
773 applications of TNFR2 agonists and antagonists in cancer and autoimmunity.

774

#### 775 **Peer review information**

776 *Nature Reviews Rheumatology* thanks [Referee#1 name], [Referee#2 name] and the other,  
777 anonymous, reviewer(s) for their contribution to the peer review of this work.

778

779 **Supplementary information**

780 Supplementary information is available for this paper at <https://doi.org/10.1038/s415XX-XXX-XXXX-X>

782

783 **Key points**

784

- Tumour necrosis factor (TNF) is a major inflammatory cytokine that has deleterious effects in several rheumatic and autoimmune diseases, as attested by the success of TNF inhibitor therapy.

785

786

787

- Some patients do not respond to TNF inhibitors and others develop paradoxical autoimmune exacerbations that can be explained by the immunoregulatory role of TNF.

788

789

- The pro-inflammatory and anti-inflammatory properties of TNF are largely segregated by the capacity of this cytokine to bind to TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2), respectively.

790

791

792

- The anti-inflammatory effects of TNF are explained by its capacity to increase the proliferation, stability and suppressive function of FOXP3<sup>+</sup> regulatory T cells via TNFR2 signalling.

793

794

795

- Antagonists of TNFR1 and agonists of TNFR2 constitute a new generation of drugs that might be more effective and have fewer adverse effects than classical TNF inhibitors.

796

797

798

799

**Table 1. Clinically approved TNF inhibitors in the USA and Europe**

Drug	Molecule	Biosimilars	Approved rheumatic disease indications <sup>a</sup>
Etanercept	Human TNFR2—IgG1–Fc fusion protein	Benpali, Erelzi, Nepexto	RA, JIA, psoriatic arthritis, plaque psoriasis, AS
Infliximab	Humanized chimeric anti-TNF IgG1/κ mAb	Remsima, Inflectra, Flixabi, Ixifi, Zessly, Avsola	RA, psoriatic arthritis, plaque psoriasis, AS
Adalimumab	Fully human anti-TNF IgG1/κ mAb	Exemptia, Adftrar, Amjevita, Cyltezo, Amgevita, Solymbic, Imraldi, Cyltezo, Halimatoz, Hefiya, Hyrimoz, Hulio, Idacio, Kromeza, Hadlima, Abrilada, Amsparity	RA, JIA, psoriatic arthritis, plaque psoriasis, AS, hidradenitis suppurativa, non-infectious uveitis
Certolizumab pegol	PEGylated human Fab fragment of anti-TNF mAb	NA	RA (Europe only), psoriatic arthritis
Golimumab	Fully human anti-TNF IgG1/κ mAb	NA	RA, psoriatic arthritis, AS

800 <sup>a</sup>Disease indications for biosimilars can differ from those of the original drug and depend on the  
 801 countries where they are registered. AS, ankylosing spondylitis; JIA, juvenile idiopathic arthritis;  
 802 mAb, monoclonal antibody; NA, not applicable; RA, rheumatoid arthritis.

803

804

**Commenté [JM1]:** Caroline: Check against table 1 in the published paper below for overlap:

<https://www.mdpi.com/2073-4468/4/1/48/html?>

This table might need to be adjusted to avoid overlap (or else might need permissions).

(see handover sheet for one idea of alt layout)

805 Table 2. Pathogenic and protective roles of TNFR1 and TNFR2 in models of rheumatic and  
806 autoimmune diseases

Mouse model	<i>TNFR1</i> knockout	<i>TNFR2</i> knockout	TNFR1 antagonist	TNFR2 agonist	Refs
Collagen-induced arthritis	Attenuated	Exacerbated	Attenuated	Attenuated	200,201,184,119,121
Antigen-induced arthritis	ND	Exacerbated	ND	ND	201
DTHA	ND	Exacerbated	ND	ND	115
Arthritis in TNF-transgenic mice	Attenuated	Exacerbated	ND	ND	202
EAE	Attenuated	Exacerbated	Attenuated	Attenuated	28,187,203-205,28,187,191,203,204,206,207,208,132

807 DTHA, delayed-type hypersensitivity arthritis; EAE, experimental autoimmune  
808 encephalomyelitis; ND, not determined.

809

810

811 Table 3. Effect of TNF on T<sub>reg</sub> cell function in vitro.

T <sub>reg</sub> cell population	Culture condition(s)	<i>n</i>	TNF added	Assay type	Effect of TNF on T <sub>reg</sub> cell suppression	Refs
Human CD4 <sup>+</sup> CD25 <sup>hi</sup>	Soluble anti-CD3 and anti-CD28 mAbs	6	Before or during	Cytokine FACS	No change	98
Human CD4 <sup>+</sup> CD25 <sup>hi</sup>	Coated anti-CD3 mAbs	6	Before	Proliferation <sup>3</sup> H	Decreased	128
Human CD4 <sup>+</sup> CD25 <sup>+</sup>	HBV e-antigen	7	None	Proliferation <sup>3</sup> H	Decreased	127
Human CD4 <sup>+</sup> CD25 <sup>+</sup>	Coated anti-CD3 mAbs	NR	During	Proliferation FACS, cytokine ELISA	Decreased	129
Human CD4 <sup>+</sup> CD25 <sup>hi</sup>	Coated anti-CD3 mAbs	3	Before or during	Proliferation FACS	Decreased	122
Human CD4 <sup>+</sup> CD25 <sup>hi</sup> CD127 <sup>low</sup>	Coated anti-CD3 and anti-CD28 mAbs	5?	Before	Proliferation FACS	Decreased	126
Human CD4 <sup>+</sup> CD25 <sup>hi</sup> CD127 <sup>low</sup> CD45RA <sup>+</sup>	Coated anti-CD3 and anti-CD28 mAbs; APC and soluble anti-CD3 mAbs; APC and coated anti-CD3 mAbs	28	Before or during	Proliferation FACS	No change or increased	133
Mouse CD4 <sup>+</sup> CD25 <sup>+</sup>	APC and soluble anti-CD3 mAbs	6	Before or during	Proliferation FACS	No change or increased	22
Mouse CD4 <sup>+</sup> CD25 <sup>+</sup>	APC and soluble anti-CD3 mAbs	3	Before	Proliferation FACS	Increased	107

812 APC, antigen presenting cells; ELISA, enzyme-linked immunosorbent assay; FACS,  
813 fluorescence-activated cell sorting; *n*, number of healthy individuals; NR, not reported; TNF,  
814 tumour necrosis factor.

815

816

817 Table 4. T<sub>reg</sub> cell proportions in blood before and after TNF inhibitor therapy

Study population	TNF inhibitor (concomitant medications)	Sampling time points <sup>a</sup>	T <sub>reg</sub> cells			Refs
			Cell population	Pre-treatment (proportion) <sup>b</sup>	Post-treatment (proportion)	
27 patients with RA; 8 healthy controls	Infliximab (NSAIDs, methotrexate)	Baseline, 1.5 and 3.0 months	CD4 <sup>+</sup> CD25 <sup>hi</sup>	Same	Increased from baseline; increased in responders vs nonresponders	98
17 patients with RA; 15 healthy controls	Infliximab (NSAIDs, methotrexate)	Baseline and 3.0 months	CD4 <sup>+</sup> CD25 <sup>hi</sup>	Decreased <sup>c</sup>	Increased <sup>c</sup> from baseline	151
31 patients with RA; 20 healthy controls	Infliximab (NSAIDs, methotrexate)	Baseline and 4.0–6.0 months	CD4 <sup>+</sup> FOXP3 <sup>+</sup>	Same	Increased from baseline	146
40 patients with RA; 10 healthy controls	Infliximab (methotrexate, salazopyrin, hydroxychloroquine, steroids)	NR	CD4 <sup>+</sup> CD25 <sup>+</sup> FOXP3 <sup>+</sup>	Decreased	Increased from baseline and in responders vs nonresponders	75
10 patients with RA; 10 healthy controls	Adalimumab (NSAIDs, methotrexate, steroids)	Baseline and 3.0 months	CD4 <sup>+</sup> CD25 <sup>hi</sup>	Same	No change from baseline	153
50 patients with RA; 15 healthy controls	Adalimumab or etanercept (NR)	NR	CD4 <sup>+</sup> FOXP3 <sup>+</sup>	Same	Increased from baseline; increased in responders vs nonresponders to adalimumab; no change from baseline with etanercept	154
48 patients with RA	Adalimumab or etanercept (methotrexate, leflunomide)	Baseline, 1.5 and 3.0 months	CD4 <sup>+</sup> FOXP3 <sup>+</sup> , CD25 <sup>hi</sup> 127 <sup>low</sup>	ND	No change from baseline, no difference between responders and nonresponders	162
20 patients with RA; 10 healthy controls	Etanercept (methotrexate)	Baseline and 3.0 months	CD4 <sup>+</sup> CD25 <sup>hi</sup> FOXP3 <sup>+</sup>	Decreased	Increased from baseline	150
33 patients with RA	Etanercept (methotrexate)	Baseline, 3.0 and 6.0 months	CD4 <sup>+</sup> CD25 <sup>+</sup> FOXP3 <sup>+</sup>	ND	Increased from baseline	161
16 patients with RA	Infliximab or etanercept <sup>d</sup> (NR)	Baseline and 3.0 months	CD4 <sup>+</sup> CD25 <sup>+</sup> 127 <sup>lo</sup> w FOXP3 <sup>+</sup>	ND	Increased from baseline	115
7 patients with JIA	Etanercept (NSAIDs, methotrexate)	Baseline and 1.0–5.0 months	CD4 <sup>+</sup> FOXP3 <sup>+</sup>	ND	No change from baseline	172
222 patients with AS; 68 healthy controls	Infliximab or etanercept (NSAIDs)	Baseline and 6.0 months	CD4 <sup>+</sup> CD25 <sup>hi</sup> FOXP3 <sup>+</sup>	Decreased	Increased from baseline; increased in responders versus nonresponders	171
46 patients with sarcoidosis; 26 healthy controls	Infliximab (NR)	Baseline, 3.5 and 6.0 months	CD4 <sup>+</sup> CD25 <sup>hi</sup>	Increased	Decreased from baseline	173
12 patients with uveitis	Adalimumab (NR)	Baseline, 1.0 and 6.0 months	CD4 <sup>+</sup> CD25 <sup>hi</sup> 127 <sup>lo</sup> w FOXP3 <sup>+</sup>	ND	Increased from baseline	169
16 patients with uveitis; 15 healthy controls	Infliximab (NR)	Baseline, 4.0–27.0 months	CD4 <sup>+</sup> FOXP3 <sup>+</sup>	Same	Increased from baseline <sup>e</sup>	170

818 <sup>a</sup>Baseline (before initiation of TNF inhibitor treatment). <sup>b</sup>In patients versus controls. <sup>c</sup>Absolute819 number. <sup>d</sup>Three patients also received golimumab, adalimumab or certolizumab. <sup>e</sup>Versus patients

820 treated only with ciclosporine or colchicine. AS, ankylosing spondylitis; JIA, juvenile idiopathic  
821 arthritis; ND, not determined; NR, not reported; NSAID, nonsteroidal anti-inflammatory drug.

822

823 **Table 5. Therapeutic effects of drugs targeting TNFRs in autoimmune disease models**

Agent	Structure	Therapeutic efficacy	Refs
<i>Antagonists of TNFR1</i>			
DMS5540	Bispecific anti-TNFR1 and anti-albumin mAb	Arthritis (CIA)	184
ASTROSAB	Humanized anti-TNFR1 IgG1 mAb, mutated in the Fc fragment to abrogate complement and immune complex activation	EAE	185
TROS	Trivalent nanobody comprising two mAb domains binding to TNFR1 and one mAb domain binding to albumin	EAE	186
HM1097	Hamster IgG	EAE	187
XPro1595	Dominant-negative PEGylated TNF muteins that interact with soluble TNF to form inactive heterotrimers, which have low binding and signalling activity	Arthritis (CIA) and EAE	188,190,192
R1antTNF	PEGylated TNF mutein that binds specifically to TNFR1 without signalling activity	Arthritis (CIA) and EAE	189,191
<i>Agonists of TNFR2</i>			
MR2-1	Mouse mAb against human TNFR2	Increased expansion and stability of T <sub>reg</sub> cells; not tested in vivo	57, 118
Unnamed	Mouse mAb against human TNFR2	Increased expansion and stability of T <sub>reg</sub> cells; not tested in vivo	111
TNF07	Human TNF mutein trimer	Increased expansion of T <sub>reg</sub> cells; not tested in vivo	116
TNC-scTNFR <sub>2</sub>	Human TNF mutein trimer	Not tested in vitro or vivo	193
STAR2	Mouse TNF mutein nanomer	Increased expansion, survival and function of T <sub>reg</sub> cells; effective in CIA, EAE and GvHD	121,103,117,120,132
EHD2-sc-mTNFR <sub>2</sub>	Mouse TNF mutein hexamer	Increased expansion of T <sub>reg</sub> cells; effective in EAE and CIA	119,208

824 CIA, collagen-induced arthritis; EAE, experimental autoimmune encephalomyelitis; GvHD,  
825 graft versus host disease; mAb, monoclonal antibody; TNF, tumour necrosis factor; TNFR, TNF  
826 receptor.

827

828 **Figure 1. The proinflammatory and anti-inflammatory activities of TNF are driven by**  
829 **effects on innate and adaptive immunity.** Tumour necrosis factor (TNF) is a major  
830 proinflammatory cytokine (top panel) that activates both innate (left side) and adaptive (right  
831 side) immunity. TNF promotes recruitment of leukocytes, favours the production of other  
832 proinflammatory cytokines, activates neutrophils and participates in co-stimulation of  
833 conventional T cells. TNF also has regulatory activities (bottom panel) such as inhibition of  
834 haematopoiesis, increased glucocorticoid production, activation of suppressive cells (such as  
835 mesenchymal stem cells (MSC) and myeloid-derived suppressor cells (MDSC)) or altering the  
836 function of dendritic cells (DCs) and macrophages. TNF also regulates immunity by promoting  
837 IL-10-producing B cells, inducing T cell apoptosis, altering T cell receptor (TCR) signalling,  
838 inhibiting  $T_H17$  cell differentiation and boosting numbers and function of regulatory T ( $T_{reg}$ )  
839 cells. APC, antigen-presenting cell; FLS, fibroblast-like synoviocyte; HSC, haematopoietic stem  
840 cell.

841  
842 **Figure 2. The overall effects of TNF on regulatory T cells.** Most of the effects of tumour  
843 necrosis factor (TNF) on regulatory T ( $T_{reg}$ ) cells are due to induction of TNF receptor 1  
844 (TNFR2) signalling, which is probably preferentially mediated by transmembrane TNF rather  
845 than soluble TNF. Signal transduction downstream of TNFR2 that does not involve kinase  
846 activity involves TNF receptor-associated factor (TRAF) adaptor proteins. Multiple downstream  
847 signalling pathways lead to positive (left) and negative (right) effects on  $T_{reg}$  cell biology.  
848 TNFR2 signalling strongly induces  $T_{reg}$  cell proliferation and has a moderate survival-promoting  
849 effect on  $T_{reg}$  cells; both of these effects depend on RelA and probably also on the activation of  
850 p38, AKT and mTORC1 by phosphorylation (p). Weak evidence indicates that TNF also  
851 promotes the stability and suppressive function of  $T_{reg}$  cells, perhaps via TNF-induced protein 3  
852 (TNFAIP3) and signal transducer and activator of transcription 5 (STAT5) signalling pathways.  
853 In addition to these positive effects of TNF, the negative effects of this cytokine are clear in  
854 relation to the inhibition of induced  $T_{reg}$  cell differentiation (which involves phosphoinositide 3-  
855 kinase (PI3K) and/or phosphorylated RAC $\alpha$  serine/threonine-protein kinase (AKT) pathway  
856 activation). Weak evidence suggests that TNF induces  $T_{reg}$  cell dysfunction, perhaps via a  
857 mechanism involving deleted in breast cancer 1 (DBC1), microRNA 34a (miR-34a) and  
858 serine/threonine-protein phosphatase PP1. Arrow thickness and box shading intensity is  
859 proportional to the importance of the effect or the likelihood that a given molecule is involved in  
860 the pathway.

861

862 **References**

- 863 1. Feldmann, M. Translating molecular insights in autoimmunity into effective therapy.  
864 *Annu Rev Immunol* **27**, 1-27 (2009).
- 865 2. Monaco, C., Nanchahal, J., Taylor, P. & Feldmann, M. Anti-TNF therapy: past, present  
866 and future. *Int Immunol* **27**, 55-62 (2015).
- 867 3. Keffer, J. et al. Transgenic mice expressing human tumour necrosis factor: a predictive  
868 genetic model of arthritis. *EMBO J* **10**, 4025-31 (1991).
- 869 4. Silva-Fernandez, L. & Hyrich, K. Rheumatoid arthritis: When TNF inhibitors fail in RA-  
870 -weighing up the options. *Nat Rev Rheumatol* **10**, 262-4 (2014).
- 871 5. Roda, G., Jharap, B., Neeraj, N. & Colombel, J.F. Loss of Response to Anti-TNFs:  
872 Definition, Epidemiology, and Management. *Clin Transl Gastroenterol* **7**, e135 (2016).
- 873 6. Esposito, M. et al. Survival rate of antitumour necrosis factor-alpha treatments for  
874 psoriasis in routine dermatological practice: a multicentre observational study. *Br J*  
875 *Dermatol* **169**, 666-72 (2013).
- 876 7. Ramos-Casals, M., Brito-Zeron, P., Soto, M.J., Cuadrado, M.J. & Khamashta, M.A.  
877 Autoimmune diseases induced by TNF-targeted therapies. *Best Pract Res Clin Rheumatol*  
878 **22**, 847-61 (2008).
- 879 8. Ramos-Casals, M. et al. Autoimmune diseases induced by biological agents: a double-  
880 edged sword? *Autoimmun Rev* **9**, 188-93 (2010).
- 881 9. Brenner, D., Blaser, H. & Mak, T.W. Regulation of tumour necrosis factor signalling:  
882 live or let die. *Nat Rev Immunol* **15**, 362-74 (2015).
- 883 10. Conrad, C. et al. TNF blockade induces a dysregulated type 1 interferon response without  
884 autoimmunity in paradoxical psoriasis. *Nat Commun* **9**, 25 (2018).
- 885 11. van Oosten, B.W. et al. Increased MRI activity and immune activation in two multiple  
886 sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2.  
887 *Neurology* **47**, 1531-4 (1996).
- 888 12. Study-group. TNF neutralization in MS: results of a randomized, placebo-controlled  
889 multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of  
890 British Columbia MS/MRI Analysis Group. *Neurology* **53**, 457-65 (1999).
- 891 13. Aggarwal, B.B., Gupta, S.C. & Kim, J.H. Historical perspectives on tumor necrosis  
892 factor and its superfamily: 25 years later, a golden journey. *Blood* **119**, 651-65 (2012).
- 893 14. Kalliolias, G.D. & Ivashkiv, L.B. TNF biology, pathogenic mechanisms and emerging  
894 therapeutic strategies. *Nat Rev Rheumatol* **12**, 49-62 (2016).
- 895 15. Atrekhany, K.N., Gogoleva, V.S., Drutskaya, M.S. & Nedospasov, S.A. Distinct modes  
896 of TNF signaling through its two receptors in health and disease. *J Leukoc Biol* (2020).
- 897 16. Salomon, B.L. et al. Tumor Necrosis Factor alpha and Regulatory T Cells in  
898 Oncoimmunology. *Front Immunol* **9**, 444 (2018).
- 899 17. Davignon, J.L. et al. Modulation of T-cell responses by anti-tumor necrosis factor  
900 treatments in rheumatoid arthritis: a review. *Arthritis Res Ther* **20**, 229 (2018).
- 901 18. Horiuchi, T., Mitoma, H., Harashima, S., Tsukamoto, H. & Shimoda, T. Transmembrane  
902 TNF-a: structure, function and interaction with anti-TNF agents. *Rheumatology (Oxford)*  
903 **49**, 1215-1228 (2010).
- 904 19. Lee, W.H., Seo, D., Lim, S.G. & Suk, K. Reverse Signaling of Tumor Necrosis Factor  
905 Superfamily Proteins in Macrophages and Microglia: Superfamily Portrait in the  
906 Neuroimmune Interface. *Front Immunol* **10**, 262 (2019).
- 907 20. Qu, Y., Zhao, G. & H., L. Forward and Reverse Signaling Mediated by Transmembrane  
908 Tumor Necrosis Factor-Alpha and TNF Receptor 2: Potential Roles in an  
909 immunosuppressive Tumor Microenvironment. *Front Immunol* **8**, 1675 (2017).
- 910 21. Aggarwal, B.B. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat*  
911 *Rev Immunol* **3**, 745-56 (2003).

- 912 22. Chen, X., Baumel, M., Mannel, D.N., Howard, O.M. & Oppenheim, J.J. Interaction of  
913 TNF with TNF receptor type 2 promotes expansion and function of mouse CD4+CD25+  
914 T regulatory cells. *J Immunol* **179**, 154-61 (2007).
- 915 23. Ware, C.F. Network communications: lymphotoxins, LIGHT, and TNF. *Annu Rev*  
916 *Immunol* **23**, 787-819 (2005).
- 917 24. Yang, S., Wang, J., Brand, D.D. & Zheng, S.G. Role of TNF-TNF Receptor 2 Signal in  
918 Regulatory T Cells and Its Therapeutic Implications. *Front Immunol* **9**, 784 (2018).
- 919 25. Faustman, D. & Davis, M. TNF receptor 2 pathway: drug target for autoimmune  
920 diseases. *Nat Rev Drug Discov* **9**, 482-93 (2010).
- 921 26. Gregory, A.P. et al. TNF receptor 1 genetic risk mirrors outcome of anti-TNF therapy in  
922 multiple sclerosis. *Nature* **488**, 508-511 (2012).
- 923 27. Park, H., Bourla, A.B., Kastner, D.L., Colbert, R.A. & Siegel, R.M. Lighting the fires  
924 within: the cell biology of autoinflammatory diseases. *Nat Rev Immunol* **12**, 570-80  
925 (2012).
- 926 28. Yang, S. et al. Differential roles of TNFalpha-TNFR1 and TNFalpha-TNFR2 in the  
927 differentiation and function of CD4(+)Foxp3(+) induced Treg cells in vitro and in vivo  
928 periphery in autoimmune diseases. *Cell Death Dis* **10**, 27 (2019).
- 929 29. Rampart, M., De Smet, W., Fiers, W. & Herman, A.G. Inflammatory properties of  
930 recombinant tumor necrosis factor in rabbit skin in vivo. *J Exp Med* **169**, 2227-32 (1989).
- 931 30. Venkatesh, D. et al. Endothelial TNF receptor 2 induces IRF1 transcription factor-  
932 dependent interferon-beta autocrine signaling to promote monocyte recruitment.  
933 *Immunity* **38**, 1025-37 (2013).
- 934 31. Duprez, L. et al. RIP kinase-dependent necrosis drives lethal systemic inflammatory  
935 response syndrome. *Immunity* **35**, 908-18 (2011).
- 936 32. Ding, X. et al. TNF receptor 1 mediates dendritic cell maturation and CD8 T cell  
937 response through two distinct mechanisms. *J Immunol* **187**, 1184-91 (2011).
- 938 33. Menges, M. et al. Repetitive injections of dendritic cells matured with tumor necrosis  
939 factor alpha induce antigen-specific protection of mice from autoimmunity. *J Exp Med*  
940 **195**, 15-21 (2002).
- 941 34. Noti, M., Corazza, N., Mueller, C., Berger, B. & Brunner, T. TNF suppresses acute  
942 intestinal inflammation by inducing local glucocorticoid synthesis. *J Exp Med* **207**, 1057-  
943 66 (2010).
- 944 35. Wang, W. et al. Enhanced human hematopoietic stem and progenitor cell engraftment by  
945 blocking donor T cell-mediated TNFalpha signaling. *Sci Transl Med* **9** (2017).
- 946 36. Ghannam, S., Pene, J., Moquet-Torcy, G., Jorgensen, C. & Yssel, H. Mesenchymal stem  
947 cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell  
948 phenotype. *J Immunol* **185**, 302-12 (2010).
- 949 37. Sayegh, S. et al. Rheumatoid Synovial Fluids Regulate the Immunomodulatory Potential  
950 of Adipose-Derived Mesenchymal Stem Cells Through a TNF/NF-kappaB-Dependent  
951 Mechanism. *Front Immunol* **10**, 1482 (2019).
- 952 38. Raveney, B.J., Copland, D.A., Dick, A.D. & Nicholson, L.B. TNFR1-dependent  
953 regulation of myeloid cell function in experimental autoimmune uveoretinitis. *J Immunol*  
954 **183**, 2321-9 (2009).
- 955 39. Zhao, X. et al. TNF signaling drives myeloid-derived suppressor cell accumulation. *J*  
956 *Clin Invest* **122**, 4094-104 (2012).
- 957 40. Chavez-Galan, L. et al. Transmembrane Tumor Necrosis Factor Controls Myeloid-  
958 Derived Suppressor Cell Activity via TNF Receptor 2 and Protects from Excessive  
959 Inflammation during BCG-Induced Pleurisy. *Front Immunol* **8**, 999 (2017).
- 960 41. Hu, X. et al. Transmembrane TNF-alpha promotes suppressive activities of myeloid-  
961 derived suppressor cells via TNFR2. *J Immunol* **192**, 1320-31 (2014).

- 962 42. Sade-Feldman, M. et al. Tumor necrosis factor-alpha blocks differentiation and enhances  
963 suppressive activity of immature myeloid cells during chronic inflammation. *Immunity*  
964 **38**, 541-54 (2013).
- 965 43. Bachus, H. et al. Impaired Tumor-Necrosis-Factor-alpha-driven Dendritic Cell Activation  
966 Limits Lipopolysaccharide-Induced Protection from Allergic Inflammation in Infants.  
967 *Immunity* **50**, 225-240 e4 (2019).
- 968 44. Alzabin, S. et al. Incomplete response of inflammatory arthritis to TNF $\alpha$  blockade is  
969 associated with the Th17 pathway. *Ann Rheum Dis* **71**, 1741-1748 (2012).
- 970 45. Mayordomo, A.C. et al. IL-12/23p40 overproduction by dendritic cells leads to an  
971 increased Th1 and Th17 polarization in a model of Yersinia enterocolitica-induced  
972 reactive arthritis in TNFRp55 $^{-/-}$  mice. *PLoS One* **13**, e0193573 (2018).
- 973 46. Notley, C.A. et al. Blockade of tumor necrosis factor in collagen-induced arthritis reveals  
974 a novel immunoregulatory pathway for Th1 and Th17 cells. *J Exp Med* **205**, 2491-7  
975 (2008).
- 976 47. Park, S.H., Park-Min, K.H., Chen, J., Hu, X. & Ivashkiv, L.B. Tumor necrosis factor  
977 induces GSK3 kinase-mediated cross-tolerance to endotoxin in macrophages. *Nat*  
978 *Immunol* **12**, 607-15 (2011).
- 979 48. Zakharova, M. & Ziegler, H.K. Paradoxical anti-inflammatory actions of TNF-alpha:  
980 inhibition of IL-12 and IL-23 via TNF receptor 1 in macrophages and dendritic cells. *J*  
981 *Immunol* **175**, 5024-33 (2005).
- 982 49. Kusunadi, A. et al. The Cytokine TNF Promotes Transcription Factor SREBP Activity and  
983 Binding to Inflammatory Genes to Activate Macrophages and Limit Tissue Repair.  
984 *Immunity* **51**, 241-257 e9 (2019).
- 985 50. Park, S.H. et al. Type I interferons and the cytokine TNF cooperatively reprogram the  
986 macrophage epigenome to promote inflammatory activation. *Nat Immunol* **18**, 1104-1116  
987 (2017).
- 988 51. Tartaglia, L.A. et al. Stimulation of human T-cell proliferation by specific activation of  
989 the 75-kDa tumor necrosis factor receptor. *J Immunol* **151**, 4637-41 (1993).
- 990 52. Kim, E.Y. & Teh, H.S. Critical role of TNF receptor type-2 (p75) as a costimulator for  
991 IL-2 induction and T cell survival: a functional link to CD28. *J Immunol* **173**, 4500-9  
992 (2004).
- 993 53. Kim, E.Y., Priatel, J.J., Teh, S.J. & Teh, H.S. TNF receptor type 2 (p75) functions as a  
994 costimulator for antigen-driven T cell responses in vivo. *J Immunol* **176**, 1026-35 (2006).
- 995 54. Calzascia, T. et al. TNF-alpha is critical for antitumor but not antiviral T cell immunity in  
996 mice. *J Clin Invest* **117**, 3833-45 (2007).
- 997 55. Chen, X. et al. TNFR2 expression by CD4 effector T cells is required to induce full-  
998 fledged experimental colitis. *Sci Rep* **6**, 32834 (2016).
- 999 56. Soloviova, K., Puliaiev, M., Haas, M. & Via, C.S. In vivo maturation of allo-specific  
1000 CD8 CTL and prevention of lupus-like graft-versus-host disease is critically dependent  
1001 on T cell signaling through the TNF p75 receptor but not the TNF p55 receptor. *J*  
1002 *Immunol* **190**, 4562-72 (2013).
- 1003 57. de Kivit, S. et al. Stable human regulatory T cells switch to glycolysis following TNF  
1004 receptor 2 costimulation. *Nat Metab* **2**, 1046-1061 (2020).
- 1005 58. Schioppa, T. et al. B regulatory cells and the tumor-promoting actions of TNF-alpha  
1006 during squamous carcinogenesis. *Proc Natl Acad Sci U S A* **108**, 10662-7 (2011).
- 1007 59. Cope, A.P. et al. Chronic tumor necrosis factor alters T cell responses by attenuating T  
1008 cell receptor signaling. *J Exp Med* **185**, 1573-84 (1997).
- 1009 60. Aspalter, R.M., Wolf, H.M. & Eibl, M.M. Chronic TNF-alpha exposure impairs TCR-  
1010 signaling via TNF-RII but not TNF-RI. *Cell Immunol* **237**, 55-67 (2005).

- 1011 61. Beyer, M. et al. Tumor-necrosis factor impairs CD4(+) T cell-mediated immunological  
1012 control in chronic viral infection. *Nat Immunol* **17**, 593-603 (2016).
- 1013 62. Qin, H.Y., Chaturvedi, P. & Singh, B. In vivo apoptosis of diabetogenic T cells in NOD  
1014 mice by IFN-gamma/TNF-alpha. *Int Immunol* **16**, 1723-32 (2004).
- 1015 63. Naude, P.J., den Boer, J.A., Luiten, P.G. & Eisel, U.L. Tumor necrosis factor receptor  
1016 cross-talk. *FEBS J* **278**, 888-98 (2011).
- 1017 64. Lin, R.H., Hwang, Y.W., Yang, B.C. & Lin, C.S. TNF receptor-2-triggered apoptosis is  
1018 associated with the down-regulation of Bcl-xL on activated T cells and can be prevented  
1019 by CD28 costimulation. *J Immunol* **158**, 598-603 (1997).
- 1020 65. Ban, L. et al. Selective death of autoreactive T cells in human diabetes by TNF or TNF  
1021 receptor 2 agonism. *Proc Natl Acad Sci U S A* **105**, 13644-9 (2008).
- 1022 66. Bhattacharyya, S. et al. Tumor-induced oxidative stress perturbs nuclear factor-kappaB  
1023 activity-augmenting tumor necrosis factor-alpha-mediated T-cell death: protection by  
1024 curcumin. *Cancer Res* **67**, 362-70 (2007).
- 1025 67. Kim, E.Y., Teh, S.J., Yang, J., Chow, M.T. & Teh, H.S. TNFR2-deficient memory CD8  
1026 T cells provide superior protection against tumor cell growth. *J Immunol* **183**, 6051-7  
1027 (2009).
- 1028 68. Luckey, U. et al. T cell killing by tolerogenic dendritic cells protects mice from allergy. *J*  
1029 *Clin Invest* **121**, 3860-71 (2011).
- 1030 69. Miller, P.G., Bonn, M.B. & McKarns, S.C. Transmembrane TNF-TNFR2 Impairs Th17  
1031 Differentiation by Promoting IL2 Expression. *J Immunol* **195**, 2633-47 (2015).
- 1032 70. Urbano, P.C.M. et al. TNF-alpha-induced protein 3 (TNFAIP3)/A20 acts as a master  
1033 switch in TNF-alpha blockade-driven IL-17A expression. *J Allergy Clin Immunol* **142**,  
1034 517-529 (2018).
- 1035 71. Urbano, P.C.M. et al. TNFalpha-Signaling Modulates the Kinase Activity of Human  
1036 Effector Treg and Regulates IL-17A Expression. *Front Immunol* **10**, 3047 (2019).
- 1037 72. Elicabe, R.J. et al. Lack of TNFR p55 results in heightened expression of IFN-gamma  
1038 and IL-17 during the development of reactive arthritis. *J Immunol* **185**, 4485-95 (2010).
- 1039 73. Ma, H.L. et al. Tumor necrosis factor alpha blockade exacerbates murine psoriasis-like  
1040 disease by enhancing Th17 function and decreasing expansion of Treg cells. *Arthritis*  
1041 *Rheum* **62**, 430-40 (2010).
- 1042 74. Hull, D.N. et al. Anti-tumour necrosis factor treatment increases circulating T helper type  
1043 17 cells similarly in different types of inflammatory arthritis. *Clin Exp Immunol* **181**,  
1044 401-6 (2015).
- 1045 75. Talotta, R. et al. Paradoxical Expansion of Th1 and Th17 Lymphocytes in Rheumatoid  
1046 Arthritis Following Infliximab Treatment: a Possible Explanation for a Lack of Clinical  
1047 Response. *J Clin Immunol* **35**, 550-7 (2015).
- 1048 76. Kruglov, A.A., Lampropoulou, V., Fillatreau, S. & Nedospasov, S.A. Pathogenic and  
1049 protective functions of TNF in neuroinflammation are defined by its expression in T  
1050 lymphocytes and myeloid cells. *J Immunol* **187**, 5660-5670 (2011).
- 1051 77. Kruglov, A. et al. Contrasting contributions of TNF from distinct cellular sources in  
1052 arthritis. *Ann Rheum Dis* **79**, 1453-1459 (2020).
- 1053 78. Wolf, Y. et al. Autonomous TNF is critical for in vivo monocyte survival in steady state  
1054 and inflammation. *J Exp Med* **214**, 905-917 (2017).
- 1055 79. Mukai, Y. et al. Solution of the structure of the TNF-TNFR2 complex. *Sci Signal* **3**, ra83  
1056 (2010).
- 1057 80. Chan, F.K. The pre-ligand binding assembly domain: a potential target of inhibition of  
1058 tumour necrosis factor receptor function *Ann Rheum Dis* **59 Suppl 1**, i50-53 (2000).
- 1059 81. Croft, M. & Siegel, R.M. Beyond TNF: TNF superfamily cytokines as targets for the  
1060 treatment of rheumatic diseases. *Nat Rev Rheumatol* **13**, 217-233 (2017).

- 1061 82. Van Hauwermeiren, F., Vandenbroucke, R.E. & Libert, C. Treatment of TNF mediated  
1062 diseases by selective inhibition of soluble TNF or TNFR1. *Cytokine Growth Factor Rev*  
1063 **22**, 311-9 (2011).
- 1064 83. Alexopoulou, L. et al. Transmembrane TNF protects mutant mice against intracellular  
1065 bacterial infections, chronic inflammation and autoimmunity. *Eur J Immunol* **36**, 2768-  
1066 2780 (2006).
- 1067 84. Nguyen, D.X. & Ehrenstein, M.R. Anti-TNF drives regulatory T cell expansion by  
1068 paradoxically promoting membrane TNF-TNF-RII binding in rheumatoid arthritis. *J Exp*  
1069 *Med* **213**, 1241-53 (2016).
- 1070 85. Grell, M. et al. The transmembrane form of tumor necrosis factor is the prime activating  
1071 ligand of the 80 kDa tumor necrosis factor receptor *Cell* **83**, 793-802 (1995).
- 1072 86. Medler, J. & Wajant, H. Tumor necrosis factor receptor-2 (TNFR2): an overview of an  
1073 emerging drug target. *Expert Opin Ther Targets* **23**, 295-307 (2019).
- 1074 87. So, T. & Croft, M. Regulation of PI-3-Kinase and Akt Signaling in T Lymphocytes and  
1075 Other Cells by TNFR Family Molecules. *Front Immunol* **4**, 139 (2013).
- 1076 88. Wajant, H. & Beilhack, A. Targeting Regulatory T Cells by Addressing Tumor Necrosis  
1077 Factor and Its Receptors in Allogeneic Hematopoietic Cell Transplantation and Cancer.  
1078 *Front Immunol* **10**, 2040 (2019).
- 1079 89. Twu, Y.C., Gold, M.R. & Teh, H.S. TNFR1 delivers pro-survival signals that are  
1080 required for limiting TNFR2-dependent activation-induced cell death (AICD) in CD8+ T  
1081 cells. *Eur J Immunol* **41**, 335-44 (2011).
- 1082 90. Catrina, A.C. et al. Evidence that anti-tumor necrosis factor therapy with both etanercept  
1083 and infliximab induces apoptosis in macrophages, but not lymphocytes, in rheumatoid  
1084 arthritis joints: extended report. *Arthritis Rheum* **52**, 61-72 (2005).
- 1085 91. Mitoma, H. et al. Mechanisms for cytotoxic effects of anti-tumor necrosis factors agents  
1086 on transmembrane tumor necrosis factor a-expressing cells. *Arthritis Rheum* **58**, 1248-  
1087 1252 (2008).
- 1088 92. Tada, Y. et al. Collagen-induced arthritis in TNF receptor-1-deficient mice: TNF  
1089 receptor-2 can modulate arthritis in the absence of TNF receptor-1. *Clinical Immunol* **99**,  
1090 325-333 (2001).
- 1091 93. Lee, L.F. et al. The role of TNF-alpha in the pathogenesis of type 1 diabetes in the  
1092 nonobese diabetic mouse: analysis of dendritic cell maturation. *Proc Natl Acad Sci U S A*  
1093 **102**, 15995-6000 (2005).
- 1094 94. McDevitt, H., Munson, S., Ettinger, R. & Wu, A. Multiple roles for tumor necrosis  
1095 factor-alpha and lymphotoxin alpha/beta in immunity and autoimmunity. *Arthritis Res* **4**  
1096 **Suppl 3**, S141-52 (2002).
- 1097 95. Green, E.A. & Flavell, R.A. The temporal importance of TNFalpha expression in the  
1098 development of diabetes. *Immunity* **12**, 459-69 (2000).
- 1099 96. Sakaguchi, S., Yamaguchi, T., Nomura, T. & Ono, M. Regulatory T cells and immune  
1100 tolerance. *Cell* **133**, 775-87 (2008).
- 1101 97. Buckner, J.H. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. *Nat Rev Immunol* **10**, 849-59 (2010).
- 1102 98. Ehrenstein, M.R. et al. Compromised function of regulatory T cells in rheumatoid  
1103 arthritis and reversal by anti-TNFalpha therapy. *J Exp Med* **200**, 277-85 (2004).
- 1104 99. Dige, A. et al. Adalimumab treatment in Crohn's disease does not induce early changes in  
1105 regulatory T cells. *Scand J Gastroenterol* **46**, 1206-14 (2011).
- 1106 100. Li, Z. et al. Restoration of Foxp3+ Regulatory T-cell Subsets and Foxp3- Type 1  
1107 Regulatory-like T Cells in Inflammatory Bowel Diseases During Anti-tumor Necrosis  
1108 Factor Therapy. *Inflamm Bowel Dis* **21**, 2418-28 (2015).
- 1109

- 1110 101. Bluestone, J.A. & Tang, Q. Treg cells-the next frontier of cell therapy. *Science* **362**, 154-  
1111 155 (2018).
- 1112 102. Zemmour, D. et al. Single-cell gene expression reveals a landscape of regulatory T cell  
1113 phenotypes shaped by the TCR. *Nat Immunol* **19**, 291-301 (2018).
- 1114 103. Lubrano di Ricco, M. et al. Tumor necrosis factor receptor family costimulation increases  
1115 regulatory T-cell activation and function via NF-kappaB. *Eur J Immunol* (2020).
- 1116 104. Vasanthakumar, A. et al. The TNF Receptor Superfamily-NF-kappaB Axis Is Critical to  
1117 Maintain Effector Regulatory T Cells in Lymphoid and Non-lymphoid Tissues. *Cell Rep*  
1118 **20**, 2906-2920 (2017).
- 1119 105. Chen, X. et al. Co-expression of TNFR2 and CD25 identifies more of the functional  
1120 CD4(+)FoxP3(+) regulatory T cells in human peripheral blood. *Eur J Immunol* **40**, 1099-  
1121 1106 (2010).
- 1122 106. Chen, X. et al. Cutting edge: expression of TNFR2 defines a maximally suppressive  
1123 subset of mouse CD4+CD25+FoxP3+ T regulatory cells: applicability to tumor-  
1124 infiltrating T regulatory cells. *J Immunol* **180**, 6467-71 (2008).
- 1125 107. Hamano, R., Huang, J., Yoshimura, T., Oppenheim, J.J. & Chen, X. TNF optimally  
1126 activates regulatory T cells by inducing TNF receptor superfamily members TNFR2, 4-  
1127 1BB and OX40. *Eur J Immunol* **41**, 2010-20 (2011).
- 1128 108. Grinberg-Bleyer, Y. et al. Pathogenic T cells have a paradoxical protective effect in  
1129 murine autoimmune diabetes by boosting Tregs. *J Clin Invest* **120**, 4558-68 (2010).
- 1130 109. Baeyens, A. et al. Effector T Cells Boost Regulatory T Cell Expansion by IL-2, TNF,  
1131 OX40, and Plasmacytoid Dendritic Cells Depending on the Immune Context. *J Immunol*  
1132 **194**, 999-1010 (2015).
- 1133 110. Zhou, Q., Hu, Y., Howard, O.M., Oppenheim, J.J. & Chen, X. In vitro generated Th17  
1134 cells support the expansion and phenotypic stability of CD4(+)Foxp3(+) regulatory T  
1135 cells in vivo. *Cytokine* **65**, 56-64 (2014).
- 1136 111. Okubo, Y., Mera, T., Wang, L. & Faustman, D.L. Homogeneous expansion of human T-  
1137 regulatory cells via tumor necrosis factor receptor 2. *Sci Rep* **3**, 3153 (2013).
- 1138 112. Urbano, P.C.M., Koenen, H., Joosten, I. & He, X. An Autocrine TNFalpha-Tumor  
1139 Necrosis Factor Receptor 2 Loop Promotes Epigenetic Effects Inducing Human Treg  
1140 Stability In Vitro. *Front Immunol* **9**, 573 (2018).
- 1141 113. Chen, X. et al. TNFR2 is critical for the stabilization of the CD4+Foxp3+ regulatory T.  
1142 cell phenotype in the inflammatory environment. *J Immunol* **190**, 1076-84 (2013).
- 1143 114. Housley, W.J. et al. Natural but not inducible regulatory T cells require TNF-alpha  
1144 signaling for in vivo function. *J Immunol* **186**, 6779-87 (2011).
- 1145 115. Santinon, F. et al. Involvement of Tumor Necrosis Factor Receptor Type II in FoxP3  
1146 Stability and as a Marker of Treg Cells Specifically Expanded by Anti-Tumor Necrosis  
1147 Factor Treatments in Rheumatoid Arthritis. *Arthritis Rheumatol* **72**, 576-587 (2020).
- 1148 116. Ban, L. et al. Strategic internal covalent cross-linking of TNF produces a stable TNF  
1149 trimer with improved TNFR2 signaling. *Mol Cell Ther* **3**, 7 (2015).
- 1150 117. Chopra, M. et al. Exogenous TNFR2 activation protects from acute GvHD via host T reg  
1151 cell expansion. *J Exp Med* **213**, 1881-900 (2016).
- 1152 118. He, X. et al. A TNFR2-Agonist Facilitates High Purity Expansion of Human Low Purity  
1153 Treg Cells. *PLoS One* **11**, e0156311 (2016).
- 1154 119. Fischer, R. et al. Selective Activation of Tumor Necrosis Factor Receptor II Induces  
1155 Antiinflammatory Responses and Alleviates Experimental Arthritis. *Arthritis Rheumatol*  
1156 **70**, 722-735 (2018).
- 1157 120. Joedicke, J.J. et al. Activated CD8+ T cells induce expansion of Vbeta5+ regulatory T  
1158 cells via TNFR2 signaling. *J Immunol* **193**, 2952-60 (2014).

- 1159 121. Lamontain, V. et al. Stimulation of TNF receptor type 2 expands regulatory T cells and  
1160 ameliorates established collagen-induced arthritis in mice. *Cell Mol Immunol* **16**, 65-74  
1161 (2019).
- 1162 122. Nagar, M. et al. TNF activates a NF-kappaB-regulated cellular program in human  
1163 CD45RA- regulatory T cells that modulates their suppressive function. *J Immunol* **184**,  
1164 3570-81 (2010).
- 1165 123. Wang, J. et al. TNFR2 ligation in human T regulatory cells enhances IL2-induced cell  
1166 proliferation through the non-canonical NF-kappaB pathway. *Sci Rep* **8**, 12079 (2018).
- 1167 124. Bittner, S. & Ehrenschrwender, M. Multifaceted death receptor 3 signaling-promoting  
1168 survival and triggering death. *FEBS Lett* **591**, 2543-2555 (2017).
- 1169 125. He, T. et al. The p38 MAPK Inhibitor SB203580 Abrogates Tumor Necrosis Factor-  
1170 Induced Proliferative Expansion of Mouse CD4(+)Foxp3(+) Regulatory T Cells. *Front*  
1171 *Immunol* **9**, 1556 (2018).
- 1172 126. Nie, H. et al. Phosphorylation of FOXP3 controls regulatory T cell function and is  
1173 inhibited by TNF-alpha in rheumatoid arthritis. *Nat Med* **19**, 322-8 (2013).
- 1174 127. Stoop, J.N. et al. Tumor necrosis factor alpha inhibits the suppressive effect of regulatory  
1175 T cells on the hepatitis B virus-specific immune response. *Hepatology* **46**, 699-705  
1176 (2007).
- 1177 128. Valencia, X. et al. TNF downmodulates the function of human CD4+CD25hi T-  
1178 regulatory cells. *Blood* **108**, 253-61 (2006).
- 1179 129. Zanin-Zhorov, A. et al. Protein kinase C-theta mediates negative feedback on regulatory  
1180 T cell function. *Science* **328**, 372-6 (2010).
- 1181 130. Pierini, A. et al. TNF-alpha priming enhances CD4+FoxP3+ regulatory T-cell  
1182 suppressive function in murine GVHD prevention and treatment. *Blood* **128**, 866-71  
1183 (2016).
- 1184 131. Leclerc, M. et al. Control of GVHD by regulatory T cells depends on TNF produced by T  
1185 cells and TNFR2 expressed by regulatory T cells. *Blood* **128**, 1651-9 (2016).
- 1186 132. Ronin, E. et al. Tissue-restricted control of established central nervous system  
1187 autoimmunity by TNF receptor 2-expressing  
1188 Treg cells. *Proc Natl Acad Sci U S A* **118**, e2014043118 (2021).
- 1189 133. Zaragoza, B. et al. Suppressive activity of human regulatory T cells is maintained in the  
1190 presence of TNF. *Nat Med* **22**, 16-7 (2016).
- 1191 134. Miyara, M. et al. Functional delineation and differentiation dynamics of human CD4+ T  
1192 cells expressing the FoxP3 transcription factor. *Immunity* **30**, 899-911 (2009).
- 1193 135. Chen, X. et al. Expression of costimulatory TNFR2 induces resistance of CD4+FoxP3-  
1194 conventional T cells to suppression by CD4+FoxP3+ regulatory T cells. *J Immunol* **185**,  
1195 174-82 (2010).
- 1196 136. Yamaguchi, T., Wing, J.B. & Sakaguchi, S. Two modes of immune suppression by  
1197 Foxp3(+) regulatory T cells under inflammatory or non-inflammatory conditions. *Semin*  
1198 *Immunol* **23**, 424-30 (2011).
- 1199 137. Chaudhry, A. & Rudensky, A.Y. Control of inflammation by integration of  
1200 environmental cues by regulatory T cells. *J Clin Invest* **123**, 939-44 (2013).
- 1201 138. Williams, L.M. & Rudensky, A.Y. Maintenance of the Foxp3-dependent developmental  
1202 program in mature regulatory T cells requires continued expression of Foxp3. *Nat*  
1203 *Immunol* **8**, 277-84 (2007).
- 1204 139. Chinen, T. et al. An essential role for the IL-2 receptor in T reg cell function *Nat*  
1205 *Immunol* **17**, 1322-1333 (2016).
- 1206 140. Feng, Y. et al. Control of the inheritance of regulatory T cell identity by a cis element in  
1207 the Foxp3 locus *Cell* **158**, 749-763 (2014).

- 1208 141. Gao, Y. et al. Inflammation negatively regulates FOXP3 and regulatory T-cell function  
1209 via DBC1. *Proc Natl Acad Sci U S A* **112**, E3246-54 (2015).
- 1210 142. Xie, M. et al. NF-kappaB-driven miR-34a impairs Treg/Th17 balance via targeting  
1211 Foxp3. *J Autoimmun* **102**, 96-113 (2019).
- 1212 143. Molinero, L.L., Miller, M.L., Evaristo, C. & Alegre, M.L. High TCR stimuli prevent  
1213 induced regulatory T cell differentiation in a NF-kappaB-dependent manner. *J Immunol*  
1214 **186**, 4609-17 (2011).
- 1215 144. Zhang, Q. et al. TNF-alpha impairs differentiation and function of TGF-beta-induced  
1216 Treg cells in autoimmune diseases through Akt and Smad3 signaling pathway. *J Mol Cell*  
1217 *Biol* **5**, 85-98 (2013).
- 1218 145. Mahmud, S.A. et al. Costimulation via the tumor-necrosis factor receptor superfamily  
1219 couples TCR signal strength to the thymic differentiation of regulatory T cells. *Nat*  
1220 *Immunol* **15**, 473-81 (2014).
- 1221 146. Nadkarni, S., Mauri, C. & Ehrenstein, M.R. Anti-TNF-alpha therapy induces a distinct  
1222 regulatory T cell population in patients with rheumatoid arthritis via TGF-beta. *J Exp*  
1223 *Med* **204**, 33-9 (2007).
- 1224 147. So, T. & Croft, M. Cutting edge: OX40 inhibits TGF-beta- and antigen-driven conversion  
1225 of naive CD4 T cells into CD25+Foxp3+ T cells. *J Immunol* **179**, 1427-30 (2007).
- 1226 148. Madireddi, S. et al. SA-4-1BBL costimulation inhibits conversion of conventional CD4+  
1227 T cells into CD4+ FoxP3+ T regulatory cells by production of IFN-gamma. *PLoS One* **7**,  
1228 e42459 (2012).
- 1229 149. Khan, S.Q. et al. Cloning, expression, and functional characterization of TL1A-Ig. *J*  
1230 *Immunol* **190**, 1540-1550 (2013).
- 1231 150. Lina, C., Conghua, W., Nan, L. & Ping, Z. Combined treatment of etanercept and MTX  
1232 reverses Th1/Th2, Th17/Treg imbalance in patients with rheumatoid arthritis. *J Clin*  
1233 *Immunol* **31**, 596-605 (2011).
- 1234 151. Toubi, E. et al. Increased spontaneous apoptosis of CD4+CD25+ T cells in patients with  
1235 active rheumatoid arthritis is reduced by infliximab. *Ann N Y Acad Sci* **1051**, 506-514  
1236 (2005).
- 1237 152. Cao, D., van Vollenhoven, R., Klareskog, L., Trollmo, C. & Malmstrom, V.  
1238 CD25brightCD4+ regulatory T cells are enriched in inflamed joints of patients with  
1239 chronic rheumatic disease. *Arthritis Res Ther* **6**, R335-46 (2004).
- 1240 153. Dombrecht, E.J. et al. Influence of anti-tumor necrosis factor therapy (Adalimumab) on  
1241 regulatory T cells and dendritic cells in rheumatoid arthritis. *Clin Exp Rheumatol* **24**, 31-  
1242 7 (2006).
- 1243 154. McGovern, J.L. et al. Th17 cells are restrained by Treg cells via the inhibition of  
1244 interleukin-6 in patients with rheumatoid arthritis responding to anti-tumor necrosis  
1245 factor antibody therapy. *Arthritis Rheum* **64**, 3129-38 (2012).
- 1246 155. van Amelsfort, J.M., Jacobs, K.M., Bijlsma, J.W., Lafeber, F.P. & Taams, L.S.  
1247 CD4(+)CD25(+) regulatory T cells in rheumatoid arthritis: differences in the presence,  
1248 phenotype, and function between peripheral blood and synovial fluid. *Arthritis Rheum*  
1249 **50**, 2775-85 (2004).
- 1250 156. Cao, D. et al. Isolation and functional characterization of regulatory CD25brightCD4+ T  
1251 cells from the target organ of patients with rheumatoid arthritis. *Eur J Immunol* **33**, 215-  
1252 23 (2003).
- 1253 157. Herrath, J. et al. The inflammatory milieu in the rheumatic joint reduces regulatory T-cell  
1254 function. *Eur J Immunol* **41**, 2279-90 (2011).
- 1255 158. Komatsu, N. et al. Pathogenic conversion of Foxp3+ T cells into TH17 cells in  
1256 autoimmune arthritis. *Nat Med* **20**, 62-8 (2014).

- 1257 159. Chen, Z. et al. The ubiquitin ligase Stub1 negatively modulates regulatory T cell  
1258 suppressive activity by promoting degradation of the transcription factor Foxp3.  
1259 *Immunity* **39**, 272-85 (2013).
- 1260 160. van Loosdregt, J. et al. Stabilization of the transcription factor Foxp3 by the  
1261 deubiquitinase USP7 increases Treg-cell-suppressive capacity. *Immunity* **39**, 259-71  
1262 (2013).
- 1263 161. Huang, Z. et al. Anti-TNF-alpha therapy improves Treg and suppresses Teff in patients  
1264 with rheumatoid arthritis. *Cell Immunol* **279**, 25-9 (2012).
- 1265 162. Blache, C. et al. Number and phenotype of rheumatoid arthritis patients' CD4+CD25hi  
1266 regulatory T cells are not affected by adalimumab or etanercept. *Rheumatology (Oxford)*  
1267 **50**, 1814-22 (2011).
- 1268 163. Hvas, C.L. et al. Discrete changes in circulating regulatory T cells during infliximab  
1269 treatment of Crohn's disease. *Autoimmunity* **43**, 325-33 (2010).
- 1270 164. Boschetti, G. et al. Therapy with anti-TNFalpha antibody enhances number and function  
1271 of Foxp3(+) regulatory T cells in inflammatory bowel diseases. *Inflamm Bowel Dis* **17**,  
1272 160-70 (2011).
- 1273 165. Kato, K. et al. Infliximab therapy impacts the peripheral immune system of  
1274 immunomodulator and corticosteroid naive patients with Crohn's disease. *Gut Liver* **5**,  
1275 37-45 (2011).
- 1276 166. Li, Z. et al. Reciprocal changes of Foxp3 expression in blood and intestinal mucosa in  
1277 IBD patients responding to infliximab. *Inflamm Bowel Dis* **16**, 1299-310 (2010).
- 1278 167. Ricciardelli, I., Lindley, K.J., Londei, M. & Quarantino, S. Anti tumour necrosis-alpha  
1279 therapy increases the number of FOXP3 regulatory T cells in children affected by Crohn's  
1280 disease. *Immunology* **125**, 178-83 (2008).
- 1281 168. Veltkamp, C. et al. Apoptosis of regulatory T lymphocytes is increased in chronic  
1282 inflammatory bowel disease and reversed by anti-TNFalpha treatment. *Gut* **60**, 1345-53  
1283 (2011).
- 1284 169. Calleja, S. et al. Adalimumab specifically induces CD3(+) CD4(+) CD25(high) Foxp3(+)  
1285 CD127(-) T-regulatory cells and decreases vascular endothelial growth factor plasma  
1286 levels in refractory immuno-mediated uveitis: a non-randomized pilot intervention study.  
1287 *Eye (Lond)* **26**, 468-77 (2012).
- 1288 170. Sugita, S., Yamada, Y., Kaneko, S., Horie, S. & Mochizuki, M. Induction of regulatory T  
1289 cells by infliximab in Behcet's disease. *Invest Ophthalmol Vis Sci* **52**, 476-84 (2011).
- 1290 171. Xueyi, L. et al. Levels of circulating Th17 cells and regulatory T cells in ankylosing  
1291 spondylitis patients with an inadequate response to anti-TNF-alpha therapy. *J Clin*  
1292 *Immunol* **33**, 151-61 (2013).
- 1293 172. Wehrens, E.J. et al. Anti-tumor necrosis factor alpha targets protein kinase B/c-Akt-  
1294 induced resistance of effector cells to suppression in juvenile idiopathic arthritis. *Arthritis*  
1295 *Rheum* **65**, 3279-3284 (2013).
- 1296 173. Verwoerd, A. et al. Infliximab therapy balances regulatory T cells, tumour necrosis factor  
1297 receptor 2 (TNFR2) expression and soluble TNFR2 in sarcoidosis. *Clin Exp Immunol*  
1298 **185**, 263-70 (2016).
- 1299 174. Nguyen, D.X. et al. Regulatory T cells as a biomarker for response to adalimumab in  
1300 rheumatoid arthritis. *J Allergy Clin Immunol* **142**, 978-980 e9 (2018).
- 1301 175. Chen, X., Oppenheim, J.J., Winkler-Pickett, R.T., Ortaldo, J.R. & Howard, O.M.  
1302 Glucocorticoid amplifies IL-2-dependent expansion of functional  
1303 FoxP3(+)/CD4(+)/CD25(+) T regulatory cells in vivo and enhances their capacity to  
1304 suppress EAE. *Eur J Immunol* **36**, 2139-49 (2006).
- 1305 176. Kim, D. et al. Anti-inflammatory Roles of Glucocorticoids Are Mediated by Foxp3 +  
1306 Regulatory T Cells via a miR-342-Dependent Mechanism. *Immunity* **53**, 581-596 (2020).

- 1307 177. Byng-Maddick, R. & Ehrenstein, M.R. The impact of biological therapy on regulatory T  
1308 cells in rheumatoid arthritis. *Rheumatology (Oxford)* **54**, 768-75 (2015).
- 1309 178. Di Sabatino, A. et al. Peripheral regulatory T cells and serum transforming growth factor-  
1310 beta: relationship with clinical response to infliximab in Crohn's disease. *Inflamm Bowel*  
1311 *Dis* **16**, 1891-7 (2010).
- 1312 179. Zou, H., Li, R., Hu, H., Hu, Y. & Chen, X. Modulation of Regulatory T Cell Activity by  
1313 TNF Receptor Type II-Targeting Pharmacological Agents. *Front Immunol* **9**, 594 (2018).
- 1314 180. Fischer, R., Kontermann, R.E. & Pfizenmaier, K. Selective Targeting of TNF Receptors  
1315 as a Novel Therapeutic Approach. *Front Cell Dev Biol* **8**, 401 (2020).
- 1316 181. Mukai, Y. et al. Structure-function relationship of tumor necrosis factor (TNF) and its  
1317 receptor interaction based on 3D structural analysis of a fully active TNFR1-selective  
1318 TNF mutant. *J Mol Biol* **385**, 1221-9 (2009).
- 1319 182. Ando, D. et al. Creation of mouse TNFR2-selective agonistic TNF mutants using a phage  
1320 display technique. *Biochem Biophys Res Commun* **440**, 309-315 (2013).
- 1321 183. Van Ostade, X., Vandenabeele, P., Tavernier, J. & Fiers, W. Human tumor necrosis  
1322 factor mutants with preferential binding to and activity on either the R55 or R75 receptor.  
1323 *Eur J Biochem* **220**, 771-9 (1994).
- 1324 184. McCann, F.E. et al. Selective tumor necrosis factor receptor I blockade is  
1325 antiinflammatory and reveals immunoregulatory role of tumor necrosis factor receptor II  
1326 in collagen-induced arthritis. *Arthritis Rheumatol* **66**, 2728-38 (2014).
- 1327 185. Williams, S.K. et al. Anti-TNFR1 targeting in humanized mice ameliorates disease in a  
1328 model of multiple sclerosis. *Sci Rep* **8**, 13628 (2018).
- 1329 186. Steeland, S. et al. TNFR1 inhibition with a Nanobody protects against EAE development  
1330 in mice. *Sci Rep* **7**, 13646 (2017).
- 1331 187. Williams, S.K. et al. Antibody-mediated inhibition of TNFR1 attenuates disease in a  
1332 mouse model of multiple sclerosis. *PLoS One* **9**, e90117 (2014).
- 1333 188. Zalevsky, J. et al. Dominant-negative inhibitors of soluble TNF attenuate experimental  
1334 arthritis without suppressing innate immunity to infection. *J Immunol* **179**, 1872-83  
1335 (2007).
- 1336 189. Shibata, H. et al. The treatment of established murine collagen-induced arthritis with a  
1337 TNFR1-selective antagonistic mutant TNF. *Biomaterials* **30**, 6638-47 (2009).
- 1338 190. Brambilla, R. et al. Inhibition of soluble tumour necrosis factor is therapeutic in  
1339 experimental autoimmune encephalomyelitis and promotes axon preservation and  
1340 remyelination. *Brain* **134**, 2736-54 (2011).
- 1341 191. Nomura, T. et al. Therapeutic effect of PEGylated TNFR1-selective antagonistic mutant  
1342 TNF in experimental autoimmune encephalomyelitis mice. *J Control Release* **149**, 8-14  
1343 (2011).
- 1344 192. Taoufik, E. et al. Transmembrane tumour necrosis factor is neuroprotective and regulates  
1345 experimental autoimmune encephalomyelitis via neuronal nuclear factor-kappaB *Brain*  
1346 **134**, 2722-2735 (2011).
- 1347 193. Maier, O., Fischer, R., Agresti, C. & Pfizenmaier, K. TNF receptor 2 protects  
1348 oligodendrocyte progenitor cells against oxidative stress. *Biochem Biophys Res Commun*  
1349 **440**, 336-41 (2013).
- 1350 194. Rauert, H. et al. Membrane tumor necrosis factor (TNF) induces p100 processing via  
1351 TNF receptor-2 (TNFR2). *J Biol Chem* **285**, 7394-7404 (2009).
- 1352 195. Dong, Y. et al. Essential protective role of tumor necrosis factor receptor 2 in  
1353 neurodegeneration. *Proc Natl Acad Sci U S A* **113**, 12304-12309 (2016).
- 1354 196. Fischer, R. et al. TNFR2 promotes Treg-mediated recovery from neuropathic pain across  
1355 sexes. *Proc Natl Acad Sci U S A* **116**, 17045-17050 (2019).

- 1356 197. Klatzmann, D. & Abbas, A.K. The promise of low-dose interleukin-2 therapy for  
1357 autoimmune and inflammatory diseases. *Nat Rev Immunol* **15**, 283-94 (2015).
- 1358 198. Grinberg-Bleyer, Y. et al. IL-2 reverses established type 1 diabetes in NOD mice by a  
1359 local effect on pancreatic regulatory T cells. *J Exp Med* **207**, 1871-8 (2010).
- 1360 199. Tang, Q. et al. Central role of defective interleukin-2 production in the triggering of islet  
1361 autoimmune destruction. *Immunity* **28**, 687-97 (2008).
- 1362 200. Mori, L., Iselin, S., De Libero, G. & Lesslauer, W. Attenuation of collagen-induced  
1363 arthritis in 55-kDa TNF receptor type 1 (TNFR1)-IgG1-treated and TNFR1-deficient  
1364 mice. *J Immunol* **157**, 3178-82 (1996).
- 1365 201. Tseng, W.Y. et al. TNF receptor 2 signaling prevents DNA methylation at the Foxp3  
1366 promoter and prevents pathogenic conversion of regulatory T cells. *Proc Natl Acad Sci U*  
1367 *SA* **116**, 21666-21672 (2019).
- 1368 202. Bluml, S. et al. Antiinflammatory effects of tumor necrosis factor on hematopoietic cells  
1369 in a murine model of erosive arthritis. *Arthritis Rheum* **62**, 1608-19 (2010).
- 1370 203. Eugster, H.P. et al. Severity of symptoms and demyelination in MOG-induced EAE  
1371 depends on TNFR1. *Eur J Immunol* **29**, 626-32 (1999).
- 1372 204. Suvannavejh, G.C. et al. Divergent roles for p55 and p75 tumor necrosis factor receptors  
1373 in the pathogenesis of MOG(35-55)-induced experimental autoimmune  
1374 encephalomyelitis. *Cell Immunol* **205**, 24-33 (2000).
- 1375 205. Kassiotis, G. & Kollias, G. Uncoupling the proinflammatory from the  
1376 immunosuppressive properties of tumor necrosis factor (TNF) at the p55 TNF receptor  
1377 level: implications for pathogenesis and therapy of autoimmune demyelination. *J Exp*  
1378 *Med* **193**, 427-34 (2001).
- 1379 206. Miller, P.G., Bonn, M.B., Franklin, C.L., Ericsson, A.C. & McKarns, S.C. TNFR2  
1380 Deficiency Acts in Concert with Gut Microbiota To Precipitate Spontaneous Sex-Biased  
1381 Central Nervous System Demyelinating Autoimmune Disease. *J Immunol* **195**, 4668-84  
1382 (2015).
- 1383 207. Wang, Y.L. et al. Targeting pre-ligand assembly domain of TNFR1 ameliorates  
1384 autoimmune diseases - an unrevealed role in downregulation of Th17 cells. *J Autoimmun*  
1385 **37**, 160-70 (2011).
- 1386 208. Fischer, R. et al. Exogenous activation of tumor necrosis factor receptor 2 promotes  
1387 recovery from sensory and motor disease in a model of multiple sclerosis. *Brain Behav*  
1388 *Immun* **81**, 247-259 (2019).
- 1389  
1390