Targeting TNF receptors in rheumatoid arthritis

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Abstract

Tumour necrosis factor (TNF) is a pro-inflammatory cytokine that signals through two distinct receptors, TNFR1 and TNFR2. TNF is essentially involved in the pathogenesis of various inflammatory and autoimmune diseases. Blocking TNF, in turn, has been proven to be highly effective in treating a variety of diseases. However, the role of its two receptors in these conditions is not very well understood. It is established that TNFR1 is mainly responsible for the detrimental effects of TNF. However, accumulating evidence suggests differential or even opposing effects of TNFR2 in the pathogenesis of a number of inflammatory and autoimmune conditions. In this review, we summarize the available data concerning biological and functional properties of the two TNF receptors and potential therapeutic consequences of these insights.

Keywords: anti-TNF, autoimmunity, infection, rheumatoid arthritis, TNF receptor function

Introduction

TNF and its receptors form a prototypic pro-inflammatory cytokine system that has well-recognized and important functions in various biological processes (1). It was the first and name-giving member of the TNF–TNF-receptor superfamily, which currently includes >40 members that share structural similarities. All of these ligands and receptors are self-assembling trimeric transmembrane proteins and are mainly expressed on cells of the immune system. Major biological functions of the TNF–TNF-receptor superfamily include a prominent role in host defence, organogenesis, apoptosis, inflammation, septic shock and autoimmunity (2).

TNF (also known as TNFSF2) is known to signal via two distinct receptors, namely TNFR1 (also designated as p55 or TNFRSF1A) and TNFR2 (also known as p75 or TNFRSF1B). In analogy to the cytokine, both of its receptors can also be cleaved off the cell surface and circulate in soluble forms, where they can act as ‘decoy’ (i.e. non-signalling) receptors for TNF (3, 4). Complexity is added by the fact that TNF receptors, like most superfamily receptors, interact with more than one ligand of the corresponding superfamily. In this regard, both TNF receptors have also been shown to be activated by lymphotoxin α (LTα, also known as TNFSF1) (5).

Signalling

General remarks

TNF triggers a variety of intracellular signalling events; these mainly result in transcription of pro-inflammatory-mediator genes on one hand and of genes related to apoptosis on the other hand (6). The major parts of the signalling cascade of TNFR1 and TNFR2 are depicted in Fig. 1 and detailed below.

Tumour necrosis factor receptor 1

TNFR1 is expressed on almost all nucleated cells. It is activated by binding of the soluble, or the membrane-bound, cytokine although soluble TNF preferentially binds TNFR1 (7). Both TNFR1 and TNFR2 have been shown to possess a pre-ligand-binding assembly domain, which does not interact with the ligand but is required for initiation of signalling (8). Therefore, the receptor already exists in its trimeric form, even before ligating with TNF. Binding of TNF to TNFR1 is considered to be an irreversible event, and this ligand–receptor interaction then triggers intracellular signalling. Many components of these pathways have been characterized in great detail.

The inflammatory pathway. The pro-inflammatory cascade starts by binding of the TNF trimer to the extracellular
domain of TNFR1. This binding releases the protein silencer of death domains (SODD), which inhibits TNF signalling, from the intracellular domain of TNFR1. The release of SODD then allows for binding of TNFR-associated death domain (TRADD), receptor-interacting protein-1 (RIP-1), TNFR-associated factor 2 (TRAF2), TNFR-associated factor 1 (TRAF1), Fas-associated protein with death domain (FADD), NF-κB essential modulator (NEMO), Inhibitor of NF-κB kinase subunit α (IKKα), Inhibitor of NF-κB kinase subunit β (IKKβ), cellular inhibitor of apoptosis (c-IAP), mitogen-activated protein kinase (MAP-kinase), MAP kinase kinase (MAP3K), mitogen-activated protein (MAP) kinase and NF-κB (nuclear factor kappa light chain enhancer of activated B-cells).

These adaptor proteins are then involved in activating key downstream-signalling pathways. RIP-1 recruitment of mitogen-activated protein kinase kinase kinase 3 (MAP3K3; also known as MEK kinase 3 [MEKK-3]) and transforming growth factor β-activated kinase (TAK1) subsequently activates the IKK (inhibitor of NF-κB kinase) complex, which also includes NF-κB essential modulator. Another MAP3K (ASK-1) activates the MAP-kinase pathway. The IKK complex then phosphorylates IκBα, as well as other IκB proteins, resulting in ubiquitination and degradation of IκBα under unstimulated conditions. The free NF-κB subunits translocate into the nucleus and evoke gene transcription (14–16).

NF-κB, the central mediator of the pro-inflammatory effects of TNF, is composed of dimers derived from five different subunits, namely p65 (RelA), RelB, cRel, p50 and p52. Triggering of TNFR1 activates the so-called classical pathway of NF-κB activation, with the p65–p50 heterodimer being the most important set of subunits for transcription. Target genes include cytokines, chemokines, receptors regulating adhesion and migration of cells as well as angiogenesis (17).

In addition, TNFR1 activates the mitogen-activated protein kinase (MAPK) pathway via activation of apoptosis-signalling kinase-1 (ASK-1), which is a MAP3K. Via MAPK kinase 4 (MEK-4) and MEK-6, c-Jun N-terminal kinases (JNKs) and p38 MAPK are activated, resulting in activation of the transcription factor activator protein 1. Phosphorylation of the MEK–ERK (extracellular signal-regulated kinases) pathway is initialized by tumour progression locus 2.

The pro-apoptotic pathway. In addition to these pro-inflammatory signalling pathways, TNFR1 activation is also involved in pro-apoptotic signalling via the Fas-associated death domain (FADD). Micheau and Tschopp showed that TNFR1-induced pro-apoptotic signalling is mediated by the formation of two distinct signalling complexes (18). The first is formed rapidly at the plasma membrane and consists of TNFR1, TRADD, RIP-1, TRAF2, and cellular inhibitor of apoptosis 1 (c-IAP1), and this complex triggers the NF-κB response without affecting apoptosis. The second complex formed lacks the TNFR1 but consists of FADD and procaspases-8 and -10. This complex assembles in the cytoplasm and initiates apoptosis, if the NF-κB signalling does not induce anti-apoptotic proteins.

Tumour necrosis factor receptor 2

The expression of TNFR2, in contrast to the ubiquitous TNFR1, is more restricted and is found on endothelial cells and immune cells, especially monocytes and macrophages but also T cells, B cells and NK cells (1, 2). The primary ligand for TNFR2 is membrane-bound TNF.

In contrast to TNFR1, signalling events mediated exclusively by TNFR2 are not very well understood. As mentioned above, TNFR2 can be fully activated only by membrane TNF and not by soluble TNF. Signalling pathways initiated via TNFR2 involve TRAF1 and TRAF2, c-IAP1 and c-IAP2, leading to activation of NF-κB (19–21). In addition, TNFR2 has been shown to mediate MAPK activation (22). TRAF2 seems to have a central role in this signal-transduction cascade since dominant-negative forms of this molecule block, whereas over-expression enhances, TNFR2-mediated NF-κB activation. In contrast to TNFR1, which activates the classical pathway, TNFR2 has also been shown to activate the non-canonical pathway of NF-κB activation, which triggers p100 processing and the generation of p52-containing heterodimers of this transcription factor (23).

Of note, the intracellular portion of TNFR2 lacks a death domain to interact with, for example, FADD as TNFR1 does. However, there are numerous reports describing an important role of TNFR2 in influencing apoptosis of various cell types, such as T cells and myeloid cells (24, 25). The mechanistic basis of these observations is not known, however.

Overall, compared with the abundance of information concerning signal transduction events downstream of TNFR1, which is one of the best-characterized signalling cascades,
there is little compelling information for TNFR2. This is in part
due to the fact that many experiments were done using solu-
table TNF, which, as mentioned earlier, does not fully activate
TNFR2. In addition, many reports demonstrating a role for
TNFR2 alone in signalling have been performed using systems
in which receptors and/or downstream components were
over-expressed, and there are few data on TNFR2-mediated
signal-transduction events in primary cells. In addition, only
few experiments have been performed in TNFR1-deficient
cells, which could confirm TNFR1-independent effects of
TNFR2. Therefore, there are still open questions regarding
the functional properties of TNFR2.

TNFR1–TNFR2 'combined signalling pathways'

Despite the need to understand receptor-specific modalities
of activation and signalling, the biological effects of TNF are
also dependent on TNFR1–TNFR2 cross talk. As both recep-
tors can be cleaved off the surface of cells, the concentration
of soluble receptors can greatly influence TNF-dependent bi-
ological effects. Because of its high-binding affinity to TNF,
the soluble form of TNF can in particular have been reported to
inhibit TNF by scavenging the cytokine, thereby inhibiting
interaction with signalling-competent receptors. Furthermore,
the binding of TNF onto TNFR2 has rapid on and off kinetics.
Therefore, one of the functions of membrane-associated
TNFR2 is to concentrate TNF on the surface of cells and
to act as ligand-passing receptor to TNFR1 and thereby to
augment TNFR1-mediated signals (26).

Function

Host defence

Role of TNFR1. From the perspective of organogenesis, the
TNF–TNF-receptor system has been shown to be importantly
involved in lymphatic tissue development. Mice deficient in
TNFR1 display reduced numbers of Peyer’s patches. In addi-
tion, germinal-centre formation and the development and
localization of follicular dendritic cells are greatly per-
turbed in these mice. TNFR1 seems to be the major receptor
type for transducing TNF signals during lymphoid tissue de-
velopment, even more as the absence of TNFR2 has no in-
fluence on lymphatic organogenesis (27).

Moreover, there is clear experimental evidence that TNFR1
is crucially involved in the immune response to various mi-
crobial challenges.

TNFR1 is essential for the formation of granulomas, which
are composed of differentiated macrophages. These granulo-
mas encapsulate intracellular pathogens such as mycobacte-
ria, thereby preventing their proliferation and dispersion and
subsequent overwhelming infection. This granuloma formation
in mice is critically dependent on TNFR1, whereas TNFR2
seems to be of little importance (28, 29). Clinical evidence
demonstrates that mycobacterial containment is dependent on
constant TNF-derived signals, as patients receiving TNF-
blocking agents may suffer from endogenous reactivation of
latent tuberculosis (30).

TNF, via TNFR1, is also of key importance in controlling
Listeria infection and survival in mouse models of listeriosis
(31). The role of TNFR2 was negligible (32). In addition, an
important role of TNFR1 has been investigated in a variety
of other bacterial infections, e.g. infection with Corynebacteri-
um parvum, Pseudomonas aeruginosa, Salmonella typhi-
murium, Yersinia enterocolitica and Staphylococcus aureus
(33). Also infection of TNFR1−/− mice with the intracellular
protozoal parasite Leishmania major showed that TNFR1-
mediated immune reactions are necessary to restrict and to
resolve infection, whereas TNFR2 seemed to be of little
importance (34).

TNFR1 has also been shown to be the major receptor
involved in host defence against viral infection.

Role of TNFR2. Reports showing direct involvement of
TNFR2 in control of infectious diseases are scarce. Pulmo-
nary inflammation after bacterial challenge, for example is
increased in TNFR2 deficient mice, whereas TNFR1-deficient
mice develop attenuated disease (32).

However, there is a possible role for TNFR2 in T cell co-
stimulation, especially regarding CD8+ T cells. One report
showed that antigen-specific CD8 responses to challenge
with Listeria infections are impaired in TNFR2-deficient ani-
mals (35), which is, however, in contrast to other observa-
ations (32). TNFR2 has been shown to mediate resistance to
ectromelia virus in vivo (36). In addition, TNFR2-deficient ani-
mals were shown to generate increased antigen-specific
CD8+ T cell numbers in response to influenza virus infection.

In a mouse model of polymicrobial septic shock, deletion
of TNFR1 attenuated the disease, whereas deletion of
TNFR2 led to enhanced symptoms and shortened survival
(37). However, loss of TNFR2 has also been demonstrated
to decrease sensitivity to challenge with LPS and recombi-
nant TNF (38).

These data suggest that the two TNF receptors are
involved in different defence situations, with TNFR1 mediat-
ing mainly anti-microbial response, including viral and bac-
terial infections, whereas the preponderant anti-inflammatory
role of TNFR2 may be in viral infections and sepsis.

Autoimmune diseases

There is overwhelming evidence that TNF plays a central
role in the pathogenesis of various autoimmune diseases
(39–42). One of the most prominent examples of such dis-
eases is rheumatoid arthritis (RA), where critical involvement
of TNF was derived from clinical studies (43, 44), which
demonstrated that TNF is over-expressed in synovial fluids
as well as in the synovial membrane of RA patients. More-
over, TNF receptor expression is also up-regulated in the
synovial membrane of RA patients, especially in areas adja-
cent to erosions and increased concentrations of the shed
receptors appear to correlate with disease activity (45–47).

Also LTα, which binds to TNFR1 and TNFR2 (in addition to
its ‘private’ receptor herpesvirus entry mediator, HVEM), has
been demonstrated to be expressed in inflamed joints of hu-
mans RA patients. Moreover, a recent report showed that
pathogenic Tp17 and Tp1 cells preferentially express LTα
and that removing these cells via an antibody targeting
LTα ameliorated arthritis in an animal model of RA (48). LTα
is also can activate synovial fibroblasts and transform them into
an aggressive phenotype (49). Nevertheless, unlike TNF,
the role of LTα in the pathogenesis of inflammatory arthri-
des is still enigmatic (49). Indeed, one of the therapeutic
compounds targeting TNF, etanercept, a TNFR2 construct, also binds and inhibits LTα (Table 1), but its efficacy in RA is very similar to that of monoclonal antibodies only targeting TNF and so is the adverse event profile.

As for TNF, further evidence for its pivotal involvement in arthritis development came from genetically altered mice, where over-expression of TNF suggests that TNF directly contributes to the pathogenesis of the disease: hTNFtg mice (which over-express human TNF) develop spontaneous RA-like lesions in their joints, which show the formation of a hyperplastic synovial membrane in combination with destruction of cartilage and bone (50). Increased expression of TNF can also be found in other inflammatory diseases such as psoriasis (skin and, if associated with arthritis, also in the synovial fluid) (51), ankylosing spondylitis, juvenile idiopathic arthritis (52) and inflammatory bowel disease (IBD) (53). Most importantly, inhibiting the biological activity in patients suffering from these diseases has substantially improved the outcomes of patients and revolutionized their treatment (54). Currently, there are five different drugs targeting TNF (three antibodies, one Fab directed against TNF and one recombinant fusion protein of TNFR2 with the Fc part of human IgG1) licenced for the treatment of various autoimmune diseases (Table 1). Interestingly, only the monoclonal antibodies are effective in IBD, while all are similarly efficacious in the other disorders mentioned (53).

Role of TNFR1. Despite the dominant role of TNF in autoimmunity, information about its two receptors in the initiation and maintenance of autoimmune diseases is less abundant. Using animal models of inflammatory arthritis, TNFR1 has unequivocally been identified as the driving force in arthritis development: TNFR1-deficient mice show reduced development of collagen-induced arthritis (CIA) (55) and in hTNFtg mice lack of TNFR1 completely protects these animals from arthritis. Moreover, reintroduction of TNFR1 on mesenchymal cells is sufficient to allow for the development of full-blown TNF-dependent arthritis (56). Furthermore, TNFR1 is a driving force of local bone destruction by enhancing the generation of osteoclasts (57, 58). In addition, as mentioned above, there are multiple large clinical studies demonstrating effectiveness of blocking TNF in various diseases.

Interestingly in CIA, an anti-inflammatory effect of TNFR1 was even observed, under special circumstances. Lack of TNFR1 on haematopoietic cells led to increased signs and symptoms of arthritis, arguing for anti-inflammatory effects of TNF on haematopoietic cells in this model (59). Notley et al. (60) reported that TNF, via TNFR1, inhibits the development of pathogenic T<sub>H</sub>17 cells in CIA, and blocking TNFR1 increased the frequency of T<sub>H</sub>17 cells. In line with this, increased levels of IL-17 were noted in patients who had received TNF-blocking therapies (61).

Role of TNFR2. There are many other reports demonstrating anti-inflammatory and anti-arthritogenic properties of TNF as well as its receptors, especially for TNFR2 (Fig. 2).

In the hTNFtg mouse model of arthritis, TNFR2-deficient mice develop aggravated arthritis and joint destruction compared with wild-type mice (62). In addition, we recently demonstrated that the expression of TNFR2 on haematopoietic cells reduces the severity of TNF-driven arthritis (63). Loss of TNFR2 on haematopoietic cells leads to increased

### Table 1. Currently licenced TNF inhibitors

<table>
<thead>
<tr>
<th>Agent</th>
<th>Molecule</th>
<th>Application</th>
<th>Licenced for</th>
</tr>
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<tbody>
<tr>
<td>Infliximab (Remicade®)</td>
<td>Chimeric monoclonal antibody (IgG1) targeting TNF</td>
<td>Intravenous</td>
<td>RA, Pso, PsA, AS, IBD</td>
</tr>
<tr>
<td>Etanercept (Enbrel®)</td>
<td>Soluble human TNFR2 fused to Fc part of human IgG1 binding TNF and LT</td>
<td>Subcutaneous</td>
<td>RA, Pso, PsA, AS, JIA</td>
</tr>
<tr>
<td>Adalimumab (Humira®)</td>
<td>Human monoclonal antibody (IgG1) targeting TNF</td>
<td>Subcutaneous</td>
<td>RA, Pso, PsA, AS, JIA</td>
</tr>
<tr>
<td>Certolizumab (Cimzia®)</td>
<td>PEGylated Fab’ fragment of a monoclonal antibody targeting TNF</td>
<td>Subcutaneous</td>
<td>RA, IBD</td>
</tr>
<tr>
<td>Golimumab (Simponi®)</td>
<td>Human monoclonal antibody (IgG1) targeting TNF</td>
<td>Subcutaneous</td>
<td>RA, PsA, AS</td>
</tr>
</tbody>
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Abbreviations: Pso, psoriasis; PsA, psoriatic arthritis; AS, ankylosing spondylitis; JIA, juvenile inflammatory arthritis.
recruitment of inflammatory cells to the synovial membrane. Furthermore, we observed enhanced osteoclastogenesis in vitro as well as in vivo in mice lacking TNFR2, thereby aggravating TNF-driven arthritis, especially with regard to local bone destruction (63). In line with this, an increased osteoclastogenic potential of TNFR2-deficient osteoclast precursor cells in various inflammatory conditions in vitro and in vivo has been observed by other groups (64).

Interestingly, in experimental colitis, the absence of TNFR2 expression specifically on CD4+ T cells led to an accelerated onset of disease and to more-severe signs of inflammation.

Also in other models of inflammation and autoimmunity divergent roles for the two TNF-receptors have been noted, with a prominent anti-inflammatory component for TNFR2. Suvannavejh et al. (65) reported exacerbated experimental autoimmune encephalitis (EAE) in TNFR2 deficient mice, whereas TNFR1-deficient mice were resistant. Two other studies described cardioprotective effects delivered via TNFR2 in TNF-induced heart failure and myocardial infarction, whereas TNFR1 was responsible for cardiotoxic effects (66, 67).

Therefore, there is increasing evidence in a variety of experimental autoimmune pathologies suggesting that anti-inflammatory signals are delivered via TNFR2. One possible mechanism that has been proposed and validated is shedding of TNFR2, thereby scavenging soluble TNF and blocking its pro-inflammatory properties, but there are also other possible mechanisms.

One cell type has emerged as possible mediator of the anti-inflammatory effects of TNFR2. A number of studies have shown an important role of TNF and its receptors in regulatory T cell (Treg) biology (68–70). TNFR2 is preferentially expressed by Tregs with high-suppressive capacity. TNF modulates the function of Tregs as well as the ability of responder T cells to be suppressed (68, 71). Therefore, conflicting results about the function of TNF and its receptors in Treg biology have been reported. Tregs from TNFR2-deficient mice have been reported to function normally in vitro (72). However, a recent study showed a reduced ability of TNFR2-deficient Tregs in preventing experimental colitis in vivo, demonstrating a functional role of TNFR2 on Tregs (73). Although the exact modalities and circumstances of the regulation of TNF and its two receptors on Tregs have not been defined yet, the role of TNFR2 in Treg biology might be of key importance in understanding the anti-inflammatory properties of the TNF–TNF-receptor system in inflammation and immunity.

Summary

TNF-blocking agents have successfully been used to treat systemic inflammatory disorders since the 1990s. They have revolutionized the prospects of many patients suffering from RA and other autoimmune diseases and are today a cornerstone in their treatment. However, not all patients respond to therapy and therefore there is still a need for improving efficacy as well as safety of current therapeutic regimens. Given the numerous observations regarding differential effects of the two TNF receptors in models of inflammation and autoimmunity, especially experimental arthritis, it is tempting to speculate about selective blockade of TNFR1. Blocking specifically TNFR1 would target the main arthritogenic pathway initiated by TNF but might leave signals delivered via TNFR2 intact. TNFR2-mediated signals, as mentioned above, have been repeatedly shown to be of anti-inflammatory nature and might also be involved in particular anti-infective defence. Thus, targeting TNFR1 might provide better anti-arthritic efficacy and maybe even better safety. However, the reality of this hypothesis will have to be tested in appropriate clinical trials.

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