Tumor Necrosis Factor and the consequences of its ablation in vivo

G.A. Efimova, A.A. Kruglov, S.V. Tillib, D.V. Kuprash, S.A. Nedospasov

A R T I C L E   I N F O

Keywords:
Anti-cytokine therapy
Autoimmune disorders
TNF receptors

A B S T R A C T

Although TNF has been discovered due to anti-tumor activity, its physiological functions are different. Current knowledge places TNF downstream of many receptors of innate immunity, implying its primary role in host defense and inflammation. When overproduced systemically or locally, TNF may exert deleterious effects on the organism. Anti-TNF therapy is highly efficient in several autoimmune and inflammatory diseases. However, due to TNF unique beneficial functions in immune system, such therapy cannot be entirely free of adverse effects. We review the current status of the field with the focus on drugs and strategies used for TNF ablation in vivo.

© 2009 Elsevier Ltd. All rights reserved.

1. Experiments which led to discovery of TNF

The history of TNF can be traced to as early as XIX century when physicians first described spontaneous tumor regression in patients suffering from bacterial infections. These observations inspired the idea of using bacteria to treat cancer. Coley was among others who implemented this concept by infecting patient bearing malignant tumors (Coley, 1894). However, this strategy proved to be too dangerous and soon he switched to killed bacteria. Coley's idea of using bacteria to treat cancer. Coley was among others who implemented this concept by infecting patient bearing malignant tumors (Coley, 1894). However, this strategy proved to be too dangerous and soon he switched to killed bacteria. Coley's bacterial extracts consisting of Streptococcus pyogenes and Serratia marcescens known as Coley toxins were the first attempt of systemic anti-tumor therapy.

Half a century later Shear et al. (1943) have discovered that the component in bacterial extracts responsible for mounting anti-tumor response is lipopolysaccharide (LPS). Since LPS itself was unable to kill tumors in cell cultures, it was hypothesized that LPS action is indirect and may be mediated by local hypotension and ischemia of the tumor (Algire et al., 1952). Finally, in 1975 Old and co-workers demonstrated that sera from mice primed with BCG and boosted with LPS, was capable of inducing hemorrhagic necrosis of LPS-sensitive mouse sarcoma. They described the serum factor which mediated necrosis of subcutaneously transplanted BALB/c sarcoma Meth A in vivo (hence the name: Tumor Necrosis Factor) and the toxicity on L929 fibrosarcoma cells in vitro (Carswell et al., 1975). Later when TNF gene was cloned and expressed in Escherichia coli (Aggarwal et al., 1985; Pennica et al., 1984; Wang et al., 1985) both in vivo anti-tumor activity and the cytotoxicity against some but not all tumor cell lines were confirmed with recombinant TNF (Pennica et al., 1984).

Although studies in mice were inspiring (Williamson et al., 1983), clinical trials of TNF as anti-cancer drug proved to be disappointing. Effective TNF dose in humans turned out to be much higher than the tolerable dose (Asher et al., 1987). It appeared that mice are more resistant to TNF than humans, as at the same doses they demonstrated only minimal toxic effects. However, TNF as a drug in oncology has found a small niche for treatment of regionally advanced melanoma and inoperable limb sarcoma by isolated perfusions in combination with chemotherapy (Lejeune et al., 1998; Walther et al., 1996).

2. TNF in arthritis

Rheumatoid arthritis (RA) is an autoimmune disease which can be induced by various agents and which is linked to lymphocyte activation in peripheral blood, synovium and synovial fluid (Carter et al., 1981; Corrigan et al., 1979), but the molecular mechanisms of this activation remained unknown. The first cytokine which was discovered in the synovial fluid of RA patients was IL-1 (Fontana et al., 1982). Pivotal role of IL-1 was confirmed in a model disease similar to antigen-induced arthritis after intraarticular injection of IL-1 into rabbit knee joints (Pettipher et al., 1986). First evidence that TNF may also play a role in RA came from the study demonstrating that synovial cell cultures from RA patients upon addition of TNF produced collagenase and prostaglandin E2, which were known to mediate bone destruction in RA (Dayer et al., 1985). Similar activity was also demonstrated for IL-1 (Saklatvala et al., 1985). Moreover, it...
was found that TNF stimulated resorption and inhibited resynthesis of proteoglycan in cartilage explants (Saklatvala, 1986), although less potently than IL-1. TNF is capable of activating osteoclasts to resorb bone in vitro (Thomson et al., 1987). Additionally, TNF is a potent inducer of IL-1 (Dinarello et al., 1986), and it is induced by IL-1 (Philip and Epstein, 1986). Production of IL-1 and GM-CSF in synovial cell cultures from RA patients depended on the presence of active TNF, and its inhibition by monoclonal antibodies downregulated these cytokines (Brennan et al., 1989; Haworth et al., 1991), as well as IL-6 and IL-8 (Butler et al., 1995).

Evidence that TNF was present in the inflamed joints of RA patients was based on detection of both TNF and IL-1 mRNA in mononuclear cells freshly isolated from synovial fluid and on TNF production by these cells when placed in culture (Buchan et al., 1988). Histological data established that TNF in inflamed joint was produced by cells of monocyte/macrophage lineage localized at cartilage–pannus junction (Chu et al., 1991). Additionally, TNF was found in the synovial fluid of patients with RA (Di Giovine et al., 1988; Saxne et al., 1988).

Both types of TNF receptors, TNFR1 (p55) and TNFR2 (p75), were expressed on cell surface of mononuclear cells isolated from synovial membrane of RA patients (Brennan et al., 1992a). Cells expressing both types of TNF receptors comprised 50% of the lining layer cells of synovium and were abundant at the cartilage–pannus junction in the vicinity of TNF producing cells (Deleuran et al., 1992).

All these data suggested the existence of TNF-dependent cytokine cascade in which TNF was somehow coordinating the generation of multiple other cytokines that contributed to pathogenesis of RA (Brennan et al., 1992b; Feldmann et al., 1990). Mouse models also supported the hypothesis regarding the key role of TNF in RA, as collagen-induced arthritis (CIA) was ameliorated by anti-TNF treatment (Piguet et al., 1992; Thorbecke et al., 1992; Williams et al., 1992).

Additionally, transgenic mice bearing human TNF (hTNF) gene with altered regulation due to variation in the 3’ untranslated region demonstrated both overexpression of human TNF and development of spontaneous arthritis, which could be completely blocked by administration of monoclonal antibodies against hTNF (Keffer et al., 1991). A more physiological model of TNF overexpression generated by disruption of AU-rich element in 3’-UTR of TNF gene was later developed by the same group. These mice exhibited spontaneous arthritis and autoimmune colitis proving that overproduction of TNF is sufficient for development of these disorders in mice (Kontoyiannis et al., 2002).

Taken together, these studies suggested that TNF is a potential therapeutic target in RA.

3. Anti-TNF therapy in autoimmune diseases

Polyclonal anti-mouse TNF rabbit serum could be used to passively immunize mice to protect them from LPS induced lethal toxicity (Beutler et al., 1985). Similarly, baboons when administered with murine monoclonal antibody against human TNF survived otherwise lethal dose of E. coli injected intravenously (Tracey et al., 1987).

Vilcek and Lee generated high affinity neutralizing monoclonal antibody A2 against human TNF (reviewed in: Vilcek and Feldmann, 2004) which was later used to engineer the chimeric cA2 (named infliximab, Table 1). This was done by replacing the murine constant regions with human counterparts while retaining the antigen binding regions (Knight et al., 1993).

One of the first obvious applications of anti-TNF therapy was sepsis. However clinical trials failed to show any significant results (Vilcek and Feldmann, 2004), suggesting that when symptoms of sepsis develop, the irreversible signaling cascades are already triggered. At that time the data on TNF involve-
Table 1
Strategies of TNF-inhibition.

<table>
<thead>
<tr>
<th>Inhibition principle</th>
<th>Examples</th>
<th>Details</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal antibodies</td>
<td>Infliximab, Adalimumab, golimumub, CDP571</td>
<td>Chimeric mouse–human antibody (Knight et al., 1993)</td>
<td>Demonstrates some immunogenicity</td>
</tr>
<tr>
<td>Soluble receptor</td>
<td>Etanercept, Lenercept</td>
<td>Fusion of two soluble TNF at molecules and Fc fragment (Mohler et al., 1993)</td>
<td>In addition to TNF, inhibits LTA</td>
</tr>
<tr>
<td>Dominant-negative TNF mutants</td>
<td>DN-TNF</td>
<td>Mutant TNF integrating into native trimers and making them inactive (Zalevsky et al., 2007)</td>
<td>Selectively inhibits soluble TNF (sTNF)</td>
</tr>
<tr>
<td>Pre-ligand-assembly domain</td>
<td>Recombinant PLAD of TNFR1</td>
<td>Soluble receptor fragment preventing its assembly and activation (Deng et al., 2005)</td>
<td>Selectively targets TNFR1</td>
</tr>
<tr>
<td>VHH-based constructs</td>
<td>Anti-TNF VHH</td>
<td>Fusion of two anti-TNF VHIs and anti-serum albumin VHIs (Coppier et al., 2006)</td>
<td>Small size</td>
</tr>
<tr>
<td>Vaccination against TNF</td>
<td>KLH complex, Virus-like particles complex</td>
<td>KLH–hTNF heterocomplex (Le Buanec et al., 2006)</td>
<td>Anti-TNF antibodies selectively targeting sTNF</td>
</tr>
<tr>
<td>Natural virus-encoded inhibitors</td>
<td>VARV-CrmB</td>
<td>Recombinant TNFR homologue from variola virus (Gileva et al., 2006)</td>
<td>Binds both TNF and LTA</td>
</tr>
</tbody>
</table>

As already mentioned, while in RA etanercept shows efficacy comparable to that of infliximab (Moreland et al., 1999), it is not effective in Crohn’s disease (Sandborn et al., 2001b) and other granulomatous diseases such as sarcoidosis (Utzi et al., 2003) and Wegener’s granulomatosis (Group, 2005). This notion led to the hypothesis that therapeutic effects of infliximab are associated with induction of apoptosis of immune effector cells, not only with inhibiting TNF per se. Indeed, infliximab was shown to induce apoptosis in monocytes from patients with chronic active Crohn’s disease via a caspase-dependent pathway (Lugering et al., 2001). Large proportion of lamina propria lymphocytes was reported to become apoptotic upon administration of infliximab (ten Hove et al., 2002). However, despite the fact that infliximab can induce apoptosis of cells in vitro, recent in vivo study did not support this idea. Analysis of RA patients 24 h after infliximab injection showed moderate apoptosis in only one case, whereas decrease in numbers of macrophages and T-cells in inflamed synovium was observed in every patient (Wijbrandts et al., 2008).

Activated T-cells are known to express transmembrane TNF (tmTNF) on their surface (Aversa et al., 1993; Kinkhabwala et al., 1990). Peripheral blood lymphocytes from healthy volunteers and lamina propria T-cells from Crohn’s disease patients were compared in their binding of etanercept and infliximab. Infliximab could bind to both cell types with high efficiency, while etanercept exhibited only weak binding visualized by flow cytometry. Moreover, infliximab was able to activate caspase 3 and to induce lymphocyte apoptosis (Van den Brande et al., 2003). Presumably, infliximab simultaneously binds two trimers of tmTNF, while etanercept can only bind one (Evans et al., 1994). It has been shown that infliximab can form complexes Ab–TNF with very high molecular weight, but Enbrel cannot (Bauer et al., 2006; Kohno et al., 2007). Dimerization of tmTNF may result in reverse signaling (Van den Brande et al., 2003), as proposed earlier (Eissner et al., 2000). Such signaling may result in calcium mobilization, cytokine production and E-selectin expression on T-cells (Higuchi et al., 1997; Watts et al., 1999).
Three approved anti-TNF therapeutics, besides their TNF-binding domains, contain Fc portion of human immunoglobulin. The importance of this component should not be underestimated, as antibody Fc region can interact in humans with various receptors. There are two types of Fc receptors for IgG: the FcγR family and FcRn. The first type is expressed on myeloid cells and mediates antibody-dependent effector responses (both activating and inhibitory), including cell activation, degranulation, release of cytokines, ADCC, and inhibition of cell activity. The second type is expressed primarily on endothelial cells, and is involved in IgG recycling and contributes to its serum half-life. Different subclasses of IgGs have different affinity ratios for activating and inhibitory Fc receptor subclasses (Nimmerjahn and Ravetch, 2005). Although all currently available TNF-inhibitors, except certolizumab pegol, are constructed using IgG1 Fc component, etanercept exhibits reduced half-life, which probably can be explained by its lower affinity to FcRn (Tracey et al., 2008). Recent studies suggested that Fc receptor polymorphisms may contribute to the outcome of therapy with TNF blocking agents. For example, homozygosity for point mutation in FcγRIIA isoform of Fc receptor (valine to phenylalanine substitution in position 138) is associated with stronger response to anti-TNF therapy (Tutunjian et al., 2005). Of note, the fact that certolizumab pegol is effective in Crohn’s disease suggests that interactions with Fc receptors may not explain the difference in action of infliximab and etanercept.

It is important to remember that many mechanistic details of the action of even approved TNF blockers are not completely understood. For example, such differences may be at least partially due to different pharmacokinetics of different drugs (Nestorov, 2005).

6. Other “pathogenic” cytokines and perspectives of their neutralization

Since IL-1 was the first cytokine found to cause inflammation and promote bone resorption in RA (Gowen et al., 1983), it was thought to be a pivotal player in the disease. Later TNF was described to mediate similar effects but to somewhat lesser extent (Saklatvala, 1986), besides TNF was found to be upregulated by IL-1. Thus, quite naturally TNF was placed downstream of IL-1 in the hypothetical pathological cascade in RA.

Subsequently as the data of TNF-inhibition in RA started to accumulate the concept reverted: it was proposed that TNF is responsible for most of IL-1 production in the synovium and thereby inhibition of TNF by itself was sufficient for control of autoimmune responses (Brennan et al., 1989).

Recently, when the success of anti-TNF therapy drew attention to other targets in RA the role of IL-1 was brought into light once again. In 1994, using anti-IL-1 antibodies in experimental arthritis (CIA) van den Berg et al. (1994) demonstrated that simultaneous inhibition of both IL-1α and IL-1β was able to ameliorate the disease after its onset. Even the treatment with anti-IL-1β alone had a significant effect. Potential implications for anti-IL-1 therapy in RA were strengthened by findings that administering anti-TNF antibodies in CIA was able to cure the disease only when given at the early stages while showing little effect in the established CIA. On the contrary, anti-IL-1 treatment was effective at all stages (Joosten et al., 1996).

One of the approaches to ensure IL-1 neutralization is to use naturally occurring IL-1 receptor antagonist which concurrently binds IL-1α and β. After successful preclinical and clinical trials (Bakker et al., 1997; Cohen et al., 2004) such recombinant human IL-1 receptor antagonist anakinra was approved for treatment of RA resistant to one or more disease-modifying drugs. Anakinra is also being evaluated as a potential therapeutic for type 2 diabetes (Larsen et al., 2007) and acute gout (So et al., 2007) with some very promising results. However, the disadvantage of anakinra treatment is that in RA the drug should be administered daily and at high doses (Fleischmann et al., 2006).

Another approach is to use neutralizing monoclonal antibodies, primarily against IL-1β. Two humanized anti-IL-1β antibodies were reported: one demonstrated good activity in mouse CIA model (Owyang et al., 2008); the second successfully passed pilot clinical trial in RA patients (Alten et al., 2008).

Although no direct comparison of anti-TNF with anti-IL-1 therapy has been made, indirect evidence suggests that inhibition of TNF is more beneficial for patients: only 38% of patients receiving anakinra plus methotrexate underwent clinical remission (Cohen et al., 2004) while in the infliximab plus methotrexate study group this value reached 50% (Maini et al., 1998). Additionally, anakinra provides no additional benefits when combined with anti-TNF therapy (Genovese et al., 2004). However, mutual regulation of TNF and IL-1 now appears to be more complex than previously thought. Inhibition of TNF although highly effective is not always sufficient to downregulate IL-1. It is possible that in some cases anti-IL-1 treatment may be more effective than anti-TNF treatment.

Another member of IL-1 family, IL-18, has been found upregulated in synovial fluid and sera of RA patients (Yamamura et al., 2001). Mouse studies showed amelioration of CIA upon anti-IL-18 therapy with both IL-18 binding protein and anti-IL18 antibody (Banda et al., 2003; Plater-Zyberk et al., 2001). The perspectives to block this cytokine in RA patients are currently being explored (Dinarello, 2007). Finally, promising data from clinical trials based on targeting other cytokines implicated in autoimmune pathologies, such as IL-6 and IL-15, are accumulating (Asquith and McInnes, 2007; Rose-John et al., 2007).

7. More blockers and more blockade strategies in development

Due to the current cost of drug production for anti-TNF therapy the field of TNF-inhibitors remains very attractive for pharmaceutical companies. More and more TNF-inhibitors and strategies are undergoing preclinical evaluation.

For example, the notion that TNF requires homotrimeric configuration to gain biological activity, has led to the development of an alternative strategy of TNF-inhibition. Rationally designed dominant negative TNF mutants (DN-TNF, Table 1), with extremely low affinity for TNF receptors, are able to assemble into “mixed” heterotrimers with natural TNF homotrimers and render them inactive (Steed et al., 2003). Importantly, such TNF antagonists are believed to have a selective activity against soluble TNF (sTNF), presumably leaving signaling initiated by membrane-bound tmTNF intact. Inhibition of soluble TNF allows PEGylated DN-TNF to suppress some symptoms of inflammation in two models of arthritis: CIA and antibody-induced arthritis, while innate resistance to L. monocytogenes and M. tuberculosis remains grossly unperturbed (Ollerøs et al., 2009; Zalevsky et al., 2007). Moreover, PEGylated version of DN-TNF showed neuroprotective activity in both in vitro and in vivo models of Parkinson’s disease (PD) (McCoy et al., 2006). PD is characterized by selective loss of dopaminergic neurons, and inflammatory processes have been implicated in the pathogenesis (Hirsch et al., 2005). TNF is thought to be involved in the disease, as PD patients display elevated levels of this cytokine in cerebrospinal fluid and on brain sections (Boka et al., 1994; Hunot et al., 1999; Nagatsu and Sawada, 2005).

Taking into consideration that soluble and tmTNF demonstrate different receptor requirements, one could selectively inhibit TNF-mediated signaling by rationally targeting receptors rather than the ligands. Both TNF receptors consist of four extracellular cysteine-rich domains (CRDs), TNF molecule binds to CRD2 and CRD3 (Banner et al., 1993), while the most distant from cell surface CRD1...
was recently reported to promote ligand-independent receptor assembly (Chan et al., 2000). Pre-ligand-assembly domains (PLADs) mediate trimerization of receptors prior to ligand binding. The receptor complexes associated by PLADs nevertheless remain non-signaling until binding of and activation by the ligand. Existence of such preformed trimers may provide a possibility to prevent signaling initiation by the appropriate ligand mimics. Indeed, soluble proteins containing PLADs of TNFRs could competitively bind to receptor monomers, thus preventing receptor assembly and signaling (Table 1). Such reagents demonstrated high TNF-inhibiting activity in L292 cells cytotoxic assay and in experimental arthritis in mice (Deng et al., 2005).

An interesting group of drugs is based on utilization of single-domain variable fragments derived from “heavy chain only” antibodies produced in llamas or camels. Camelid family possesses unique antibody species devoid of only heavy chains (Hamers-Casterman et al., 1993). An extremely long CDR3 loop compensates for the lack of light chain variable region (Muyldermans et al., 1994); thus variable domains of heavy chain only antibody (VHH) are the smallest naturally existing antigen binding folds. Small size brings several advantages as well as some limitations. These antibodies are easier to express, potentially making such therapeutics cheaper in production, and VHHs are more stable (Els Conrath et al., 2001). However, their small size correlates with reduced serum half-life (that can be prolonged by coupling to other proteins). We have recently generated anti-TNF VHH-binders using Camelus bactrianus immunization with purified human TNF (unpublished data) following by cloning and phage display panning procedures (Nguyen et al., 2000, 2001; Rothbauer et al., 2006; Saerens et al., 2004). It is conceivable that homo- or heterodimerization or trimerization of such binders could increase their serum half-life without loss of TNF-blocking capability. A prototype TNF-inhibitor based on VHH was already reported in which case two anti-TNF VHs and anti-serum albumin VHH were combined in a single trivalent, bispecific unit, which surpassed infliximab and adalimumab in TNF-inhibiting activity and possessed a prolonged half-life (Table 1). A mouse version of such molecule showed good therapeutic efficacy in collagen-induced arthritis model (Coppieters et al., 2006).

Another potentially interesting development took advantage of the presence of homologues of soluble TNF receptors in genomes of some large viruses (Table 1). They represent a particular mechanism of immune evasion. For example, cowpox viruses encode several TNFR family homologues (Cunnion, 1999; Hu et al., 1994) some of which, when expressed as recombinant proteins, are effective against LPS-induced toxicity in mice (Gileva et al., 2006). Additionally, several experimental TNF blocking strategies based on immunizations were developed in animal models. It was noted that during the development of experimental autoimmune encephalomyelitis (EAE) in Lewis rats, the levels of anti-TNF antibodies were elevated. To augment such natural anti-TNF response, which by itself is unable to protect animals from TNF-mediated disease, a DNA vaccine encoding for rat TNF was utilized. Upon immunization rats produced high titers of neutralizing anti-TNF antibodies which were able to protect rats from development of EAE (Wildbaum and Karin, 1999). Same approach also worked in adjuvant-induced arthritis (Wildbaum et al., 2000).

Yet another type of anti-TNF vaccination relied on administration of keyhole limpet haemocyanin–ITNF heterocomplex in incomplete Freund adjuvant. Such vaccination elicited neutralizing anti-TNF antibody response, which was protective against TNF/p-galactosamine lethal toxicity (Le Buanc et al., 2006). In ITNF transgenic mice developing a spontaneous arthritis such immunization induced early and long lasting response, protecting animals from TNF-mediated disease (Delavallee et al., 2008).

Finally, virus-like particles, known to be highly immunogenic (Bachmann et al., 1993), were also exploited as TNF carriers. Similar to DN-TNF muteins, this approach was aimed to specifically inhibit only soluble form of TNF, leaving the biological functions mediated by tmTNF intact and thus potentially evading some of the adverse effects, as tmTNF appears to be sufficient for host defense (Saunders et al., 2005). Antibodies against the peptide corresponding to amino acids 4–23 of mouse TNF and predicted to be exposed only on soluble form of TNF, were expected to selectively bind to sTNF. Vaccination of mice with the appropriate viral construct was able to protect them from collagen-induced arthritis. Importantly, animals did not appear to be immunocompromised, showed no increase in susceptibility to Listeria monocytogenes infection and did not reactivate latent Mycobacterium tuberculosis as opposed to mice immunized with full-length TNF molecule (Spohn et al., 2007). A similar approach has been successfully used for IL-1 inhibition in a model system (Spohn et al., 2008). To which extent these findings in mice can be exploited to treat patients is not clear at present, as the levels of neutralizing antibodies may be difficult to regulate in vivo.

8. What happens to beneficial functions of TNF: adverse effects of anti-TNF therapy

The critical and non-redundant role of TNF in host defense became obvious already in late 1980s. When mice were injected with rabbit anti-TNF antibodies 1 or 2 weeks post infection with BCG, a dramatic interference with granuloma formation and bacterial elimination was observed. Furthermore, fully developed granulomas rapidly regressed after anti-TNF treatment (Kindler et al., 1989). Anti-TNF antibodies also exacerbated the L. monocytogenes infection, while administration of recombinant mTNF could protect otherwise lethally challenged mice. In another study depletion of TNF by polyclonal serum from BALB/c mice resulted in reduced survival time, while the infusion of TNF increased resistance against tuberculosis (TB) (Denis, 1991). Upon administration of hamster monoclonal TNF-neutralizing antibodies granuloma formation was delayed and mice quickly succumbed to TB.

<table>
<thead>
<tr>
<th>Genetic defect</th>
<th>Mouse strains</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF null</td>
<td>TNFΔΔ/ΔΔ (Korner et al., 1997; Marino et al., 1997; Pasparakis et al., 1996; Taniguchi et al., 1997), TNFΔA/A (Kuprash et al., 2005)</td>
<td>Defects in host defense, antibody responses, and in lymphoid organ development and maintenance. Some phenotypic features vary between strains.</td>
</tr>
<tr>
<td>sTNF null</td>
<td>TNFΔA/ΔA, K11E (Ruuls et al., 2001), TNFΔA/ΔΔ (Alexopoulos et al., 2006)</td>
<td>Lack of primary B cell follicles in spleen, defective germinal centers and TFC development can be used in combination with various Cre transgens to obtain cell-type-specific and/or inducible TNF ablation.</td>
</tr>
<tr>
<td>TNF conditional</td>
<td>TNFΔΔΔ/ΔΔΔ (Grevinekoul et al., 2005; Kruglov et al., 2008)</td>
<td>Phenotypes of TNFR1Δ−/− and TNFΔ−/− mice are similar. Unique biological role of TNFR2 is not completely defined.</td>
</tr>
<tr>
<td>TNFR null</td>
<td>TNFR1Δ−/− (Pfeffer et al., 1993; Rothe et al., 1993), TNFR2Δ−/− (Erickson et al., 1994)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Genetic mouse models of in vivo TNF ablation.
tionally, granulomas formed in the absence of TNF appeared less well organized and the majority of lung granulomas in infected mice was necrotizing and contained overwhelming bacillary numbers.

The availability of various TNF-deficient mice (including mice with conditional gene deficiency, Table 2) has tremendously helped in elucidation of TNF physiological functions. Unlike blockade of TNF signaling in patients, in gene-modified mice TNF ablation is usually complete and/or irreversible. In particular, studies in mice have uncovered previously unsuspected role of TNF in the development of structural features of the immune system, such as intricate organization of peripheral lymphoid tissues (Kruglov et al., 2008; Pasparakis et al., 1996). Some of these findings have been recently confirmed in RA patients undergoing anti-TNF therapy in which case germinal centers and FDC in tonsils were impaired apparently due to the action of etanercept (Anolik et al., 2008). Based on mouse studies one may expect certain extent of immunodeficiency in patients placed on continuous TNF blockade due to defective organization of peripheral lymphoid tissues. On the other hand, results with pharmacological ablation of TNF signaling in mice suggest that some TNF-dependent features in lymphoid organs are plastic, and they can be restored once the blocker is withdrawn (Browning, 2008).

Significance of TNF for protection against TB and its specific role in granulomas maintenance were nicely confirmed in knockout mice (Flynn et al., 1995). Additionally, in aerosol infection model of drug-induced latent and reactivated murine tuberculosis TNF-deficient mice developed reactivation of infection and eventually succumbed to disease, while wild type mice were able to control infection after cessation of chemotherapy (Botha and Ryffel, 2003). It should be pointed out that the exact mechanistic role of TNF signaling in granuloma maintenance is not completely defined. TNF may be part of functional collaboration between macrophages and T-cells, constituting these structures (Egen et al., 2008; Wallis and Ehlers, 2005).

In the aggregate, all mouse studies indicated that intrinsic potential risks of anti-TNF therapy should be anticipated.

The direct evidence that inhibition of TNF can reactivate latent TB infection came from clinical trials (Keystone, 2005). Incidence of TB is largely influenced by age, socioeconomic status and geography. Most cases of TB reactivation are reported from Europe, where the frequency of latent TB infection is considerably higher than in US (Keane et al., 2001). In developing countries risks of reactivation might be significantly higher.

Currently, before being placed on anti-TNF therapy patients undergo screening for latent TB. This precaution has already resulted in substantial reduction of the incidence of TB (Carmona et al., 2005; Schiff et al., 2006).

The roles of other members of TNF family, in particular, lymphotoxins (LT), in lymphoid organs development and in supporting their microarchitecture were also revealed in mice (see Tumanov et al., 2003; Ware, 2005, for review). A recent study suggested that anti-TNF therapy in particular with etanercept, which can block both TNF and LTα (Table 1), may disrupt this regulation. RA patients on etanercept therapy demonstrate reduction in memory B–cells count in peripheral blood and alterations in germinal centers (GC) structures, scarcity of follicular dendritic cells (FDC) networks in tonsils (Anolik et al., 2008). Interestingly, blockade of these specific TNF and LT-mediated functions may contribute to the efficacy of therapy in RA, as FDC have been implicated in pathogenesis of arthritis (Vicartados and Kollias, 2008). Additionally, numbers of regulatory T–cells were restored due to infliximab treatment in RA patients (Nadkarni et al., 2007). In order to clarify the contribution of TNF, rather than of LTα, inhibition it would be important to collect similar data in RA patients treated with infliximab.

TNF signaling was also implicated in congestive heart failure (CHF). Two clinical trials for CHF treatment with etanercept did not show any difference from placebo group (Anker and Coats, 2002). However, small pilot trial for Infliximab suggested that whereas medium doses had no effect, high dosage treatment increased the risk of death or hospitalization (Chung et al., 2003).

One of the earlier theories about TNF functions linked it to immunosurveillance mechanisms. If so, systemic TNF-inhibition might increase the risk of malignancies. However, the incidence of solid tumors in patients on anti-TNF therapy equals those of sex, age and race-matched population. Significance of elevated lymphoma frequency is more complex, as lymphomas are also more frequent in patients with RA in general. Some reports indicated a slight increase in the incidence of lymphoma in patients receiving infliximab or etanercept (Wolfe and Michaud, 2004). However, recent mouse study failed to demonstrate any correlation between TNF-deficiency and increased risk of malignancies in lymphoma-prone mouse models (Kuprash et al., 2008).

9. Perspectives

In spite of tremendous success of anti-TNF therapy in patients with several autoimmune diseases, some adverse effects are unavoidable due to unique and non-redundant beneficial functions of TNF in immunity. Interference with host defense may become the most serious problem, as the current modes of anti-TNF therapy are being extended to developing countries. Since anti-TNF therapy is relatively young, its long-term beneficial effects have yet to be fully evaluated. It is conceivable that the existing protocols utilizing already approved TNF blockers can be further improved to allow for retention of more residual protective TNF signaling in the system. In this regard, quantitative estimations of specific “thresholds” associated with distinct functions of TNF in vivo will be useful. Another possible improvement is the development of more selective TNF blockers that can inhibit predominantly pathogenic TNF, but spare TNF signaling required for structural maintenance of lymphoid organs or bactericidal granulomas. Experimentation will require more advanced animal models in which all blockers of human TNF and various protocols of their administration can be compared side-by-side, e.g. in mice with “humanized” TNF system. Finally, the expected clinical success with other inhibitors of inflammatory responses, such as blockers of IL1 or IL6 systems, may allow to temporarily substitute TNF-inhibition in patients with other drugs and to restore TNF-mediated protective functions.

Acknowledgement

The work was supported by grant 07-04-12208 from the Russian Foundation for Basic Research and by EC FP6 grant (TB REACT). S.A.N. is International Research Scholar of the Howard Hughes Medical Institute.

References


Haworth, C., Brennan, F.M., Chantry, D., et al., 1991. Expression of granulocyte-


G.A. Efimov et al. / Molecular Immunology 47 (2009) 19–27

103, 19442–19447.

927–932.

731–740.

56, 731–740.

56, 731–740.

268, 67–75.

268, 67–75.

2005. Novel tumor necrosis factor-

