Tumor necrosis factors blocking agents: analogies and differences

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Abstract. Five anti-TNF agents, infliximab, adalimumab, etanercept, golimumab and certolizumab pegol are approved worldwide for the treatment of RA. Anti-TNF agents, bind to and neutralize soluble TNF-α, but exert different effects on transmembrane TNF-α-expressing cells (TNF-α-producing cells). Differences on affinity and avidity for soluble and transmembrane TNF-α were showed. Different activity on cells apoptosis, Complement-dependent cytotoxicity (CDC) antibody dependent cell-mediated cytotoxicity (ACDC) were described. Some dramatic changes in gene expression were seen with all the anti-TNFs. Reviewing the biology of transmembrane TNF-α and its interaction with anti-TNF agents will contribute to understanding the bases of differential clinical efficacy of these promising treatment modalities. (www.actabiomedica.it)

Key words: anti-TNF agents, pharmacokinetic, tumor necrosis factor

Introduction

TNFα was identified in 1975 as the factor in serum isolated from endotoxin-treated mice that induces necrosis of a methylcholanthrene-induced murine sarcoma (1). TNF-α is a potent pro-inflammatory cytokine exerting pleiotropic effects on various cell types and plays a critical role in the pathogenesis of chronic inflammatory diseases, such as RA.

In 1992, the first open-label trial of a TNFα blocking agent was initiated at the Kennedy Institute, Rheumatology Division, United Kingdom; 20 patients with active RA were treated with infliximab, a chimeric antibody specific for human TNF. Treatment with infliximab substantially reduced the signs and symptoms of disease, levels of C-reactive protein (CRP) in the serum and the erythrocyte sedimentation rate (ESR) (2). Five anti-TNF agents have been successfully introduced for the treatment of chronic inflammatory diseases. However, clinical features against granulomatous inflammation are not similar among these agents. For example, all the anti-TNF agents are effective against RA, but not all of them against Crohn's disease.

TNF and receptor system

TNF-α is generated as a precursor form called transmembrane TNF-α, that is expressed as a cell surface type II polypeptide consisting of 233 amino acid residues (26 kDa) on activated macrophages and lymphocytes as well as on other cell types (3-4). After being processed by metalloproteinases such as TNF-α-converting enzyme (TACE) between residues alanine76 and valine77, the soluble form of TNF-α of 157 amino acid residues (17 kDa) is released and mediates its biological activities through type 1 and 2

TNF receptors (TNF-R1 also known as TNFRSF1A, CD120a and TNF-R2 also known as TNFRSF1B, CD120b, respectively) (5-8). Soluble TNF-α is a homotrimer of 17-kDa cleaved monomers and transmembrane TNF-α also exists as a homotrimer of 26-kDa uncleaved monomers (9). Transmembrane TNF-α also binds to TNF-R1 and -R2, but its biological activities are supposed to be mediated mainly through TNF-R2.

Transmembrane TNF

Transmembrane TNF-α is a precursor form of soluble TNF-α that is expressed on TNF-α-producing cells as a homotrimer. After processing by TACE, soluble TNF-α is generated and binds to TNF-R1 or -R2. Transmembrane TNF-α also binds to TNF-R1 and -R2. Upon binding to TNF receptors, both transmembrane and soluble TNF-α mediate pleiotropic effects (apoptosis, cell proliferation and cytokine production). The remaining transmembrane TNF-α, after cleavage with TACE, is further processed by SPPL2b and the intracellular domain is translocated into the nucleus and is supposed to mediate cytokine production. Transmembrane TNF-α on the cell surface of TNF-α producing cells binds to TNF receptors on the target cells and exerts various biological functions that will contribute to the modulation of local inflammation in a cell-to cell contact manner as well as in a cell-type-specific fashion. Expression of transmembrane TNF-α on various cell types would contribute to the physiological as well as pathological responses in health and diseases. The biological activity is induced by the transmembrane TNF-α mediated signal, also called an 'outside-to-inside signal' or 'reverse signal'. In contrast to the well-characterized functions of transmembrane TNF-α as a ligand, the biological functions elicited by outside-to-inside (reverse) signal have not completely been clarified. However, it is supposed that outside-to-inside signalling mediated by transmembrane TNF-α contributes to the pleiotropy of this pro-inflammatory cytokine and its fine-tuning of immune response (10). The biological activities of transmembrane TNF-α as a receptor have been demonstrated in T cells, monocytes/macrophages and NK cells in humans (11-15).

Evolution of anti TNF antibodies

All agents except etanercept are anti-TNF mAbs or fragments thereof. Natural mAbs are derived from single B cells that clonally express copies of a unique heavy (H) chain and a unique light (L) chain that are covalently linked to form an antibody molecule of unique specificity. Engineered mAbs can be structurally identical to natural mAbs but are created by gene splicing and mutation procedures, mimicking natural gene rearrangement and somatic mutation events in B cells (16).

Infliximab, adalimumab and golimumab are full-length, bivalent IgG mAbs, whereas certolizumab is a monovalent Fab1 antibody fragment covalently linked to polyethylene glycol. IgG antibody molecules are composed of 2 H and 2 L polypeptide chains, each of which contains 3 complementarity determining regions in the N-terminal (VH and VL) domains. An IgG molecule is composed of 2 antigen-binding Fab arms, linked to a glycosylated Fc region via a flexible hinge region. The antigen-binding site on each Fab portion of a mAb is generally composed of amino acids from the 6 complementarity determining regions in each H:L chain pair.

Infliximab is a chimeric protein containing 25% mouse-derived amino acids comprising the VH and VL domains and 75% human-derived amino acids comprising the CH1 and Fc constant regions. Certolizumab is a humanized protein containing amino acid sequences in the complementarity-determining regions derived from a mouse anti-TNF mAb and inserted into human VH and VL domain frameworks.

Adalimumab and golimumab are fully human mAbs. The TNF–antagonist mAbs also differ in their IgG isotypes, the Fc regions of which govern effector functions, like complement fixation and Fc receptor-mediated biologic activities. Infliximab, adalimumab and golimumab are IgG1 antibodies, which are capable of complement fixation and Fc receptor binding.

Certolizumab is an Fab1 fragment of an IgG1 mAb and lacks effector functions because it has no Fc region. The hinge region of certolizumab is modified and covalently linked to 2 crosslinked chains of 20 kDa of polyethylene glycol to enhance solubility and half-life in vivo (17).

Etanercept is a genetically engineered fusion protein composed of a dimer of the extracellular portions of human TNFR2 fused to the Fc portion of human IgG1. The TNFR2 portion contains 4 domains, and the C-terminal domain includes a 57-residue region that contains 13 O-glycosylated residues and 11 proline residues (18). The plasma half lives of the antibodies appear to be largely governed by the binding of their Fc regions to the neonatal Fc receptor (FcRn) on endothelial cells (19). Although the amino acid sequences of the Fc regions are identical, the markedly shorter plasma half-life of etanercept versus IgG1 mAbs or other Fc fusion proteins suggests that the conformation or steric accessibility of the Fc region of etanercept may be different from those of the Fc regions of the IgG1 antibodies infliximab and adalimumab. The effect of the glycosylated C-terminal domain of TNFR2 on the structure and function of the adjacent Fc region of etanercept is unclear, as no data have been reported on the binding affinities of etanercept for FcRn or other Fc receptors. In comparison, the long plasma half-lives of infliximab, adalimumab and golimumab suggest that they bind to FcRn like natural IgG1 molecules(20).

Structural Properties of anti TNF Therapeutics

Five anti-TNF agents, infliximab, adalimumab,etanercept, golimumab and certolizumab pegol are approved worldwide for the treatment of RA. Infliximab, adalimumab and golimumab are mAbs against human TNF-α and etanercept is engineered from human TNF receptors.

Infliximab is a chimeric mouse-human anti-TNF-α mAb composed of a murine variable region and a human IgG1 constant region.

Adalimumab and golimumab are fully humanized anti-TNF-α mAbs, which are indistinguishable from the normal human IgG1. Adalimumab binds to TNFα, only TNFα and not other TNF family members; dual mechanism of action, neutralization of TNFα and rapid removal of TNFα from circulation was described.

Etanercept is composed of the extracellular portion of the two human TNF-R2 (p75 TNF receptor) linked to the Fc portion (CH2and CH3 domains) of human IgG1.

Golimumab (GLM), also known as CNTO148, is a human immunoglobulin (Ig) G1-kappa monoclonal antibody that is specific for TNF-α and binds to both the soluble and transmembrane forms of human TNF-α. Being a fully human monoclonal antibody, GLM resembles adalimumab, the first such product to reach market. However, unlike adalimumab, amino acid sequences of the light and heavy chains of GLM are identical to those of infliximab (20).

CZP is a recombinant, humanized antibody Fab fragment conjugated to polyethylene glycol (PEG), with specificity for human TNF-α. The PEG portion is a bulky hydrophilic inert molecule that increases the plasma half-life of the drug. The chemical structure of CZP is distinctly different from other TNF-monoclonal antibodies approved for use in RA. It does not have an Fc receptor and, therefore, does not activate complement or initiate complement dependent cell lysis or antibody dependent cytotoxicity. The hinge region of certolizumab is covalently linked to two cross-linked chains of 20 kDa of polyethylene glycol, giving certolizumab pegol (21).

Differences in affinity and avidity

We have for different anti TNFα different ka: association constant, kd: dissociation constant: equilibrium dissociation constant (kd/ka): etanercept 4.16 ± 0.29 x 10^6 1, 39 ± 0.37 x 10^{-4} 33.4 pM, certolizumab pegol 1.22 ± 0.09 x 10^6 1.09 ± 0.13 x 10^{-4} 89.3 pM, adalimumab 0.724 ± 0.30 x 10^6 1.14 ± 0.12 x 10^{-4} 157.4 pM, infliximab 1.01 ± 0.06 x 10^6 2.30 ± 0.34 x 10^{-4} 227.2 pM, golimumab 3.4-4.6 x 10^6, 4.3-9.3 x 10^{-5}, 18 pM (17) (Tab. 1).

Infliximab binds to both monomer and trimer forms of soluble TNF-α, whereas etanercept binds only to the trimer form (22). Infliximab forms stable complexes with soluble TNF-α, while etanercept forms relatively unstable complexes (22). Each infliximab molecule is capable of binding to two TNF-α molecules, and up to three infliximab molecules can bind to each TNF-α homotrimer. In contrast, etanercept is supposed to form 1: 1 complex with the TNF-α

Table 1. Characteristics of anti TNF α use in rheumatoid arthritis

Pharmacologic characteristic	Infliximab	Etanercept	Adalimumab	Golimumab	Certolizumab pegol
Half life	8-10 days	3-5,5 days	14 days	12 ± 3 days	13 ± 2,6 days
Target binding	TNF	TNF, limphotoxin	TNF	TNF	TNF
Structure	Chimeric Human-murine anti TNFα monoclonal antibody	Human TNF α receptor p75 fusion protein	Recombinant Human-anti TNFα monoclonal antibody	mAb anti TNFα	Fab pegol anti TNFα
Complement	++	-	+	+	-
Cells lysis toxicity	+	-	+	+	-
Administration and dose	i.v 4-8 q wk 3-10 mg/kg	s.c 50 mg w	s.c 40 mg eow	s.c 50 mg m	s.c 200 mg eow
Ka (M-1s-1)	$1.01 \pm 0.06 \times 10^{6}$	$4.16 \pm 0.29 \times 10^{6}$	$0.724 \pm 0.30 \times 10^{6}$	$3.4\text{-}4.6 \times 10^{6}$	$1.22 \pm 0.09 \times 10^{6}$
Kd (s-1)	$2.30 \pm 0.34 \times 10^{-4}$	$39 \pm 0.37 \times 10^{-4}$	$1.14 \pm 0.12 \times 10^{-4}$	$4.3\text{-}9.3 \times 10^{-5}$	$1.09 \pm 0.13 \times 10^{-4}$

trimer (22). In fact, the mAbs, but not TNF-R2:Ig soluble receptor, form large protein complexes in vitro (23). Overall, all three anti-TNF agents have similar intrinsic binding properties for soluble TNF. Although these kinds of analysis at the molecular level have not been performed, certolizumab pegol showed similar potency in neutralizing soluble TNF-α to infliximab, adalimumab and etanercept (24).

Infliximab, adalimumab, etanercept and certolizumab pegol bind to transmembrane TNF-α on transmembrane TNF-α-transfected cells (24) with similar affinities that were lower (weaker) than for soluble TNF-α (25). As in the case of soluble TNF-α, up to three molecules of infliximab can bind to one transmembrane TNF-α, one etanercept can bind to one molecule of transmembrane TNF-α.

Cells traffic activity and cytokine production

Despite a vast amount of data supporting a role for TNF in lymphoid organization, innate immunity and adaptive immunity, there is relatively little direct evidence that TNF antagonists are immunosuppressive in clinical use. A subset of T cells that is thought to play a central role in the suppression of autoreactivity and regulation of immune responses is the CD4+CD25+ Treg. The normal functions of Tregs, including the suppression of proinflammatory cytokine secretion by activated T cells and monocytes, are reduced in patients with RA compared with healthy individuals (26). Several recent clinical studies have provided evidence that TNF antagonists might normalize immune homeostasis by reversing compromised Treg function. Reduction in anticitrullinated peptide/protein antibody (ACPA) and rheumatoid factor concentrations after infliximab (27), etanercept (28) and adalimumab (29) treatment of patients with RA is compatible with the TNF–Treg connection. The short-term effects of infliximab treatment of patients with RA include an increase in peripheral blood CD4+ and CD8+ T-cell frequencies on Day 1 and a decrease in monocyte frequencies on Day 7, with no significant change in B-cell or NK-cell frequencies (30). Another study demonstrated an increase in CD4+ Th1 cells in the peripheral blood of patients with RA following infliximab treatment (31).

Functional changes include transient increases in proliferation and cytokine responsiveness of T cells to

ex vivo CD28 costimulation, but not to CD3-mediated stimulation. These findings may relate to the separate observation that infliximab treatment rapidly reversed the deficient CD28 expression on CD4+ T cells from patients with RA and restored responsiveness to CD28-mediated T-cell costimulation (32). Similarly, deficient HLA-DR expression on antigen-presenting myeloid cells and the reduced capacity of these cells to stimulate T cells from patients with RA were reversed after infliximab treatment (33).

A number of important proinflammatory cytokines are over-expressed in RA patients, including TNFα, IL-1 IL-12, IFN-γ, IL-18.1 The efficacy of anti-TNF agents in rheumatoid arthritis may be mediated through inhibition of proinflammatory cytokine production.

Some papers compare the production of various cytokines *in vitro* following the pre-incubation of activated, immune-derived cells with either certolizumab pegol, adalimumab, infliximab or etanercept (20).

Formation of immune-complexes

Complexes of TNF-antagonist drugs with sTNF (and LTα3 with etanercept) can vary widely in their composition and stability, depending on the drug and the relative concentrations of drug and TNF. The dynamics of drug distribution throughout the body and drug interaction with TNF or LT in various tissues are influenced by the nature of these complexes. Typically, antigen-antibody complexes are cleared by a combination of Fc receptor- dependent mechanisms in the reticuloendothelial systemin spleen and liver, FcRn-dependent intracellular degradation and filtration through the kidney (19). The amount of circulating TNF increases up to 7-fold in a dosage-related manner after administration ofTNF antagonists, although most of the TNF is in the form of circulating complexes that lack TNF bioactivity (34).

For example, serum TNF concentrations of 15 pg/mL at baseline increased to 35 pg/mL and 105 pg/mL 7 days after administration of 1 mg/kg or 10 mg/kg of infliximab, respectively, to patients with RA (35). These observations have given rise to the concept of the TNF-carrier effect of these drugs. The rates of clearance of TNF antagonist-TNF complexes may differ for etanercept as compared with infliximab or adalimumab. One study comparing the clearance of etanercept, infliximab and adalimumab complexes in transgenic mice expressing human TNF showed that etanercept-TNF complexes circulated for weeks, whereas infliximab-TNF and adalimumab-TNF complexes were cleared quickly (36). Similarly, etanercept-TNF complexes persisted for long periods in humans treated with etanercept (37).

Induction of immune cells death

In a recent paper Jurkat T cells stably expressing an uncleavable form of transmembrane TNFα were used for the following study: 1) flow cytometric analysis of binding activities of anti-TNF agents to cell surface transmembrane TNFα, 2) complement-dependent cytotoxicity (CDC), 3) antibody-dependent cell-mediated cytotoxicity (ADCC) by using peripheral blood mononuclear cells, and 4) outside-to-inside (reverse) signal transduction through transmembrane TNFα estimated by apoptosis and cell cycle analysis using flow cytometry. All of the anti-TNFα agents bound to transmembrane TNFα. Infliximab and adalimumab exerted almost equal CDC activities, while etanercept showed considerably lower activity. ADCC activities were almost equal among these 3 agents. Adalimumab and infliximab induced apoptosis and cell cycle arrest in transmembrane TNFα expressing Jurkat T cells, reflecting an outside-to-inside signal transduction through transmembrane TNFα.Three different anti-TNF agents showed different biologic effects on transmembrane TNFα. This finding suggests that CDC and outside-toinside signals by anti-TNF antibodies may explain the successful clinical efficacy of adalimumab and infliximab in Crohn's disease and Wegener's granulomatosis (38).

CDC (Complement-dependent cytotoxicity)

In a system using human Jurkat T cells (38), mouse NS0 myeloma cells (24), mouse Sp2/0 myeloma cells (25) or CHO cells (39), complement-depen-

dent cytotoxicity (CDC) was analysed for the anti-TNF agents. All the reports were in agreement that infliximab and adalimumab induced CDC much more potently than etanercept. In contrast, certolizumab pegol did not have any CDC activity (24), which reflects its absence of the Fc portion of IgG1. From the structural point of view, lack of activation of the complement system by etanercept seems to be reasonable as well. Infliximab, adalimumab and etanercept commonly possess the Fc portion of IgG1, whose CH2 domain activates the first component of complement (C1) activation. However, etanercept does not carry the CH1 domain of IgG1. A narrow region of 23 amino acid residues within the CH1 domain serves as a platform for complement C3 activation (40); it was later confirmed that three amino acid residues within the specific 23 amino acids are involved in the covalent attachment with C3 (41-42). Etanercept is structurally impaired in the appropriate activation of C3, the most important step in complement activation.

Moreover, lack of a hinge region in the Fc portion of etanercept resulted in rigidity compared with the natural antibody and eventually culminated in conformational hindrance to the proper access of complement proteins. It is thus difficult for etanercet to make a membrane attack complex of complement proteins (C5b-C9) for CDC at least in vitro. When activated human peripheral blood mononuclear cells were studied as target cells, none of these three anti-TNF agents induced CDC (25), which may be due to the use of different cell types from the above-mentioned experiments.

ADCC (antibody dependent cell-mediated cytotoxicity)

Infliximab, adalimumab and etanercept showed similar antibody dependent cell-mediated cytotoxicity (ADCC) activitiy using mTNF-transfected Jurkat T cells as target (38), while infliximab and adalimumab showed much more potent ADCC than etanercept in NS0 cells (24) or in CHO cells (39). Certolizumab pegol did not show any ADCC activity (24). The discrepancy in etanercept-induced ADCC is not clear, but may be explained by the different experimental conditions, such as difference in the species of target cell, in the expression level of transmembrane TNF-α. From the structural viewpoint, infliximab, adalimumab and etanercept carry CH2 and CH3 domains of the Fc domain of IgG1, whereas certolizumab pegol does not. These domains of IgG1 are involved in the binding to Fc receptors of NK cells (43), which leads to the lysis of target cells by granzyme B and perforin. The presence or absence of soluble TNF-α in the assay system may also affect ADCC activities. Both mAbs and etanercept weakly bound to Fcg receptors in the absence of soluble TNF-α, but in the presence of soluble TNF-α, there was a marked increase in binding only by mAbs infliximab and adalimumab (39). As for infliximab, induction of both CDC and ADCC has been reported by others (22-44).

Outside-to Inside Signal

This is a novel mechanism for the inhibition of transmembrane TNF-α-bearing cells by anti-TNF antibodies. In the absence of NK cells or complement, adalimumab or infliximab induces G0/G1 cell cycle arrest and apoptosis, which inhibits TNF-α-producing cells and leads to an anti-inflammatory response. A number of molecules (p21WAF1/CIP1, Bax, Bak and ROS) were involved in these intracellular signalling events through the intracellular domain of transmembrane TNF-α. These signalling molecules are supposed to be associated with p53 activation. Three serine residues in the intracellular domain of transmembrane TNF-α are essential for the activities. Bak and Bax are proapoptotic multidomain molecules; tmTNF: transmembrane TNF-α (45).

Selective gene expression

Selected set of gene expression changes with different TNF blockers in LPS-stimulated monocytes.

Some dramatic changes in gene expression were seen with all the anti-TNFs. Etanercept changed the level of the fewest genes. Infliximab, adalimumab and certolizumab pegol had a similar profile. The effects

on some genes were unique for each anti-TNF. Using a genome-wide strategy some authors have identified and validated the association of 7 genetic loci with response to anti-TNF treatment in RA (45-46).

Conclusion

New insights into the mechanisms of action of TNF antagonists and related distinctions between the agents will undoubtedly emerge as greater numbers of diseases are treated by TNF blockade. Another intriguing about the mechanism question is why most patients who fail to respond, have lost response or are intolerant of one TNF antagonist respond well when switched to another TNF antagonist. Pharmacokinetic analyses of drug concentrations in some patients who are nonresponders or who lost response to a particular TNF antagonist have revealed the presence of antidrug antibodies, which form complexes and promote the rapid clearance of the drug. Immunogenicity is most prevalent with infliximab and has been linked to the relatively high rate of acquired resistance to infliximab relative to etanercept or adalimumab, which are less immunogenic. In addition, immune-mediated inflammatory disease patient populations are heterogeneous and, even within a single disease, TNF may play a greater pathogenic role in some patients than in others. Biomarkers that can reliably identify different pathogenic subsets associated with response or lack of response to TNF antagonist therapy need to be found (47).

Five TNF blockers are available for treating patients with systemic rheumatic disease. All drugs are approved for treating RA patients, but not for granulomatosus disease. These drugs differ in structure, morphology, pharmacokinetic propriety and activity. New mechanisms of actions have been described in the last few years explaining the differences among these drugs.

References

1. Carswell EA, et al. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA* 1975; 72: 3666-70.
2. Elliott MJ, et al. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum* 1993; 36: 1681-90.
3. Kriegler M, Perez C, DeFay K, Albert I, Lu SD. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell* 1988; 53: 45-53.
4. Luettiq B, Decker T, Lohmann-Matthes ML. Evidence for the existence of two forms of membrane tumor necrosis factor: an integral protein and a molecule attached to its receptor. *J Immunol* 1989; 143: 4034-8.
5. Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. *N Engl J Med* 1996; 334: 1717–25.
6. Moss ML, Jin SL, Milla ME, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. *Nature* 1997; 385: 733-6.
7. Black RA, Rauch CT, Kozlosky CJ, et al. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature* 1997; 385: 729-33.
8. Vandenabeele P, Declercq W, Beyaert R, Fiers W. Two tumour necrosis factor receptors: structure and function. *Trends Cell Biol* 1995; 5: 392-9.
9. Tang P, Hung M-C, Klostergaard J. Human pro-tumor necrosis factor is a homotrimer. *Biochemistry* 1996; 35: 8216-25.
10. Eissner G, Kolch W, Scheurich P. Ligands working as receptors: reverse signaling by members of the TNF superfamily enhance the plasticity of the immune system. *Cytokine Growth Factor Rev* 2004; 15: 353-66.
11. Harashima S, Horiuchi T, Hatta N, et al. Outside-to-inside signal through the membrane TNF-α induces E-selectin (CD62E) expression on activated human CD4+ T cells. *J Immunol* 2001; 166: 130-6.
12. Vudattu NK, Holler E, Ewing P, et al. Reverse signalling of membrane-integrated tumour necrosis factor differentially regulated alloresponses of CD4+ and CD8+ T cells against human mirovascular endothelial cells. *Immunology* 2005; 115: 536-43.
13. Eissner G, Kirchner S, Lindner H, et al. Reverse signaling through transmembrane TNF confers resistance to lipopolysaccharide in human monocytes and macrophages. *J Immunol* 2000; 164: 6193-8.
14. Xin L, Wang J, Zhang H, et al. Dual regulation of soluble tumor necrosis factor-a induced activation of human monocytic cells via modulating transmembrane TNF-α-mediated "reverse signaling". *Int J Mol Med* 2006; 18: 885-92.
15. Yu M, Shi W, Zhang J, et al. Influence of reverse signaling via membrane TNF-α on cytotoxicity of NK92 cell. *Eur J Cell Biol* 2009; 88: 181-91.
16. Salfeld J, Kaymakcalan Z, Tracey D, Robert A, Kamen R. Generation of fully human anti-TNF antibody D2E7. *Arthritis Rheum* 1998; 41: S57.
17. Weir N, Athwal D, Brown D, et al. A new generation of high-affinity humanized PEGylated Fab' fragment anti-tumor necrosis factor-(alpha) monoclonal antibodies. *Therapy* 2006; 3: 535–45.

18. Kohno T, Tam L, Ting T, Bass RB, Stevens SR. Adalimumab and infliximab bind to Fc-receptor and C1q and generate immunoprecipitation: a different mechanism from etanercept. *Arthritis Rheum* 2005; 52 (9): S562–S563.
19. Lobo ED, Hansen RJ, Balthasar JP. Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci* 2004; 93: 2645–68.
20. Tracey D, Klareskog L, Sasso EH, et al. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* 2008; 117 (2): 244-79.
21. Shealy D, Cai A, Staquet K, et al. Characterization of golimumab, a human monoclonal antibody specific for human tumor necrosis factor alpha. *MAbs* 2010; 8; 2 (4).
22. Scallon B, Cai A, Solowski N, et al. Binding and functional comparisons of two types of tumor necrosis factor antagonists. *J Pharmacol Exp Ther* 2002; 301: 418-26.
23. Kohno T, Tam LT, Stevens SR, Louie JS. Binding characteristics of tumor necrosis factor receptor-Fc fusion proteins vs anti-tumor necrosis factor mAbs. *J Investig Dermatol Symp Proc* 2007; 12: 5-8.
24. Nesbitt A, Fossati G, Bergin M, et al. Mechanism of action of certolizumab pegol (CDP870): in vitro comparison with other anti-tumor necrosis factor alpha agents. *Inflamm Bowel Dis* 2007; 13: 1323-32.
25. Kaymakcalan Z, Sakorafas P, Bose S, et al. Comparisons of affinities, avidities, and complement activation of adalimumab, infliximab, and etanercept in binding to soluble and membrane tumor necrosis factor. *Clin Immunol* 2009; 131: 308-16.
26. Ehrenstein MR, Evans JG, Singh A, et al. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. *J ExpMed* 2004; 200: 277–85.
27. Alessandri C, Bombardieri M, Papa N, et al. Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNFalpha therapy infliximab in rheumatoid arthritis is associated with clinical improvement. *Ann Rheum Dis* 2004; 63: 1218-21.
28. Chen HA, Lin KC, Chen CH, et al. The effect of etanercept on anti-cyclic citrullinated peptide antibodies and rheumatoid factor in patients with rheumatoid arthritis. *Ann Rheum Dis* 2006; 65: 35–9.
29. Atzeni F, Sarzi-Puttin, P, Dell'Acqua D, et al. Adalimumab clinical efficacy is associated with rheumatoid factor and anti-cyclic citrullinated peptide antibody titer reduction: a oneyear prospective study. *Arthritis Res Ther* 2006; 8: R3.
30. Lorenz HM, Antoni C, Valerius T, et al. In vivo blockade of TNF-alpha by intravenous infusion of a chimeric monoclonal TNF-alpha antibody in patients with rheumatoid arthritis short term cellular and molecular effects. *J Immunol* 1996; 156: 1646-53.
31. Maurice MM, Van Der Graaff WL, Leow A, Breedveld FC, Van Lier RAW, Verweij CL. Treatment with monoclonal anti-tumor necrosis factor α antibody results in an accumulation of Th1 CD4+T cells in the peripheral blood of patients with rheumatoid arthritis. *Arthritis Rheum* 1999; 42: 2166-73.
32. Bryl E, Vallejo AN, Matteson EL, Witkowski JM, Weyand CM, Goronzy JJ. Modulation of CD28 expression with anti-tumor necrosis factor alpha therapy in rheumatoid arthritis. *Arthritis Rheum* 2005; 52: 2996–3003.
33. Mueller RB, Skapenko A, Grunke M, et al. Regulation of myeloid cell function and major histocompatibility complex class II expression by tumor necrosis factor. *Arthritis Rheum* 2005; 52: 451–60.
34. Barrera P, Joosten LA, den Broeder AA, van de Putte LB, van Riel PL, van den Berg WB. Effects of treatment with a fully human antitumour necrosis factor alpha monoclonal antibody on the local and systemic homeostasis of interleukin 1 and TNF-alpha in patients with rheumatoid arthritis. *Ann Rheum Dis* 2001; 60: 660–9.
35. Charles P, Elliott MJ, Davis D, et al. Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF-alpha therapy in rheumatoid arthritis. *J Immunol* 1999; 163: 1521-8.
36. Kaymakcalan Z, Beam C, Salfeld J. Murine model for assessing adalimumab, infliximab, and etanercept to prevent polyarthritis. *Ann Rheum Dis* 2003; 62 (Suppl 1): 136–7.
37. Wee S, Pascual M, Eason JD, et al. Biological effects and fate of a soluble, dimeric, 80-Kda tumor necrosis factor receptor in renal transplant recipients who receive OKT3 therapy 1. *Transplantation* 1997; 63: 570–7.
38. Mitoma H, Horiuchi T, Tsukamoto H, et al. Mechanisms for cytotoxic effects of anti-tumor necrosis factor agents on transmembrane tumor necrosis factor alpha-expressing cells: comparison among infliximab, etanercept, and adalimumab. *Arthritis Rheum* 2008; 58 (5): 1248-57.
39. Arora T, Padaki R, Liu L, et al. Differences in binding and effector functions between classes of TNF antagonists. *Cytokine* 2009; 45: 124–31.
40. Shohet JM, Pemberton P, Carroll MC. Identification of a major binding site for complement C3 on the IgG1 heavy chain. *J Biol Chem* 1993; 268: 5866-71.
41. Sahu A, Pangburn MK. Covalent attachment of human complement C3 to IgG. Identification of the amino acid residue involved in ester linkage formation. *J Biol Chem* 1994; 269: 28997-9002.
42. Vidarte L, Pastor C, Mas S, et al. Serine 132 is the C3 covalent attachment point on the CH1 domain of human IgG1. *J Biol Chem* 2001; 276: 38217-23.
43. Siberil S, Dutertre C-A, Boix C, et al. Molecular aspects of human FcgR interactions with IgG: functional and therapeutic consequences. *Immunol Lett* 2006; 106: 111-8.
44. Scallon BJ, Moore MA, Trinh H, Knight DM, Ghrayeb J. Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. *Cytokine* 1995; 7: 251-9.
45. Horiuchi T, Mitoma H, Harashima S, Tsukamoto H, Shimoda T. Transmembrane TNF-alpha: structure, function and interaction with anti-TNF agents. *Rheumatology* (Oxford) 2010; 49 (7): 1215-28.
46. Plant D, Bowes J, Potter C, Hyrich KL, Morgan AW, Wilson AG, Isaacs JD; Wellcome Trust Case Control Consortium; British Society for Rheumatology Biologics

Register, Barton A. Genome-wide association study of genetic predictors of anti-tumor necrosis factor treatment efficacy in rheumatoid arthritis identifies associations with polymorphisms at seven loci. *Arthritis Rheum* 2011; 63 (3): 645-53.
47. Isaacs JD, Ferraccioli G. The need for personalised medicine for rheumatoid arthritis. Ann Rheum Dis 2011; 70 (1): 4-7.

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