BIO DESIGN

pISSN 2288-6982 | eISSN 2288-7105

MINI REVIEW P 55-61

# Neutralizing Interaction of Therapeutic Antibodies against TNF Superfamily

**Jee Un Lee, Ji Young Son, Woori Shin, Ki-Young Yoo and Yong-Seok Heo***
Department of Chemistry, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Korea.
*Correspondence: ysheo@konkuk.ac.kr

**The binding of the tumor necrosis factor superfamilies (TNFSF) to their receptors (TNFRSF) initiates many pro-inflammatory immune responses. TNFSF are inflammatory cytokines mediating a diverse range of signaling events within cells that trigger necrosis and apoptosis. Cytokines plays central roles in the communication of the immune systems, and thus selective disrupting of cytokine pathway by specific antibody can alter ongoing inflammation. Monoclonal antibody drugs have been developed for a long time to treat TNFSF-related diseases. Antibody drugs targeting TNFSF have shown dramatic improvement in patients of autoimmune diseases. The essential mechanism of these antibody-based drugs is antagonism of the interaction between TNFSF and TNFRSF. In this review, we describe the interactions of ligand-receptor interaction of TNFSF and structural basis for the neutralizing interactions of the FDA-approved antibody drugs directed to TNFSF.**

## INTRODUCTION

The TNFSF and TNFRSF play critical roles in mammalian biology and mediate pro-inflammatory immune responses (Bodmer et al., 2002). It has been known that there are more than 35 specific ligand-receptor pairs between TNFSF and TNFRSF (Bossen et al., 2006). These ligand-receptor interactions activate signaling pathways of cell growth, survival, and apoptosis and modulate inflammation, host defense, and organogenesis of the immune, ectodermal, and nervous systems (Locksley et al., 2001; Wiens and Glenney, 2011). The TNFSF proteins are trimeric transmembrane proteins that are cleaved releasing soluble trimers from the producing cells. Each protomer of TNFSF trimer is formed by a sandwich of an inner and outer β-sheet (Kishore et al., 2004).

The TNFRSF members are transmembrane proteins structurally defined by pseudorepeats of extracellular cysteine-rich domains. TNFRSF are typically activated by TNFSF-induced trimerization or higher order oligomerization. The trimeric structure of TNFSF promotes efficient clustering of TNFRSF, resulting in initiation of intracellular signaling process. The ligand-receptor interactions induce assemblies formed between adaptor motifs in the cytosolic tails of TNFRSF such as a death domain or TNF-receptor associated factor (TRAF) interacting motifs and downstream signaling components such as Fas-associated protein with death domain (FADD), TNFR1-associated protein with death domain (TRADD), and TRAF (Schrofelbauer and Hoffmann, 2011; Walczak, 2011). TNFRSF with death domains activates caspase protease involved in apoptosis and TNFRSF with TRAF-interacting motifs recruits ubiquitin E3 ligase complexes, leading to activation of serine kinases, which control NF-κB transcription factors and genes involved in cell survival (Pineda et al., 2007). This accumulation of physiological studies suggested that targeting TNFSF could be used to treat autoimmune diseases. There have been many efforts to develop the biologic-based inhibitors of TNFSF and the clinical trials with them have provided new conceptual advances in understanding the immune system and disease pathology in a variety of autoimmune diseases.

Antibody-based therapeutics have achieved unprecedented success and realized the promise of the “magic bullet”. There are more than 30 monoclonal antibody-based drugs approved by FDA and hundreds are in clinical trials for treatment of various diseases including cancers, immune disorders, and infectious diseases (http://clinicaltrials.gov/). These achievements in the field of antibody therapeutics can be due to the major advances in antibody engineering for the generation of safe, specific, high-affinity, and non-immunogenic antibodies during the last few decades. In addition, currently major area of the antibody research and development includes discovery of novel targets and novel antibodies, gradual improvements in the characteristics of existing antibodies, combining antibodies such as bispecific antibodies, conjugating them with small molecule drugs, nanoparticles, other reagents, and developing novel antibody-based scaffolds with superior properties to those already in use.

Currently, there are six FDA-approved antibody-based drugs against TNFSF for treatment of immune disorders (Table 1). Infliximab, adalimumab, certolizumab pegzol, and golimumab are directed to TNFα (TNFSF2). Denosumab and belimumab are against RANKL (TNFSF11) and BAFF (TNFSF13B), respectively. All these antibody drugs share a common mechanism of action, which is to competitively inhibit the binding of TNF to its cognate receptors specifically (Tracey et al., 2008; Kaymakcalan et al., 2009; Scallon et al., 1995). Structural studies during the last decade have provided details for understanding this mechanism of

  

**TABLE 1** | FDA-approved therapeutic antibodies against TNF superfamily (http://clinicaltrials.gov/)

| Antibody Name | Trade Name | Type | Indication | Target | Approval Date |
|---|---|---|---|---|---|
| Infliximab | Remicade | Mo/Hu Chimeric IgG | Autoimmune | TNFα | 1998 |
| Adalimumab | Humira | Fully Human IgG | Autoimmune | TNFα | 2002 |
| Certolizumab pegzol | Cimzia | Humanized, PEGylated Fab | Autoimmune | TNFα | 2008 |
| Golimumab | Simponi | Fully Human IgG | Autoimmune | TNFα | 2009 |
| Denosumab | Prolia | Fully Human IgG | Bone loss | RANKL | 2010 |
| Belimumab | Benylsta | Fully Human IgG | Autoimmune | BAFF | 2011 |

action by investigating the interaction of the target TNF of these antibodies including TNFα, RANKL, and BAFF with their cognate receptors or antibody fragments.

## TNFα AND ITS BINDING ANTIBODY DRUGS

TNFα is produced by mononuclear cells. Activation of TLR4 on the surface of mononuclear cells induces activation of NF-κB, MAPK signaling, inducing expression of pro-inflammatory cytokines including, IL-6 and IL-12 (Bodmer et al., 2002). The TNFα protein exists in two trimertic forms: the precursor transmembrane form (tmTNFα, 26 kDa) and the secreted soluble form (sTNFα, 17 kDa), which is proteolytically cleaved from tmTNFα. The secreted soluble TNFα can bind to TNF receptor type 1 (TNFR1) and type 2 (TNFR2) and initiate pro-inflammatory signaling by activation of the MAPKs and NF-κB pathways. The failure of TNFα regulation can lead to the development of inflammatory or autoimmune diseases, including rheumatoid arthritis (RA), Crohn's disease (CD), psoriatic arthritis, and inflammatory bowel disease (IBD)

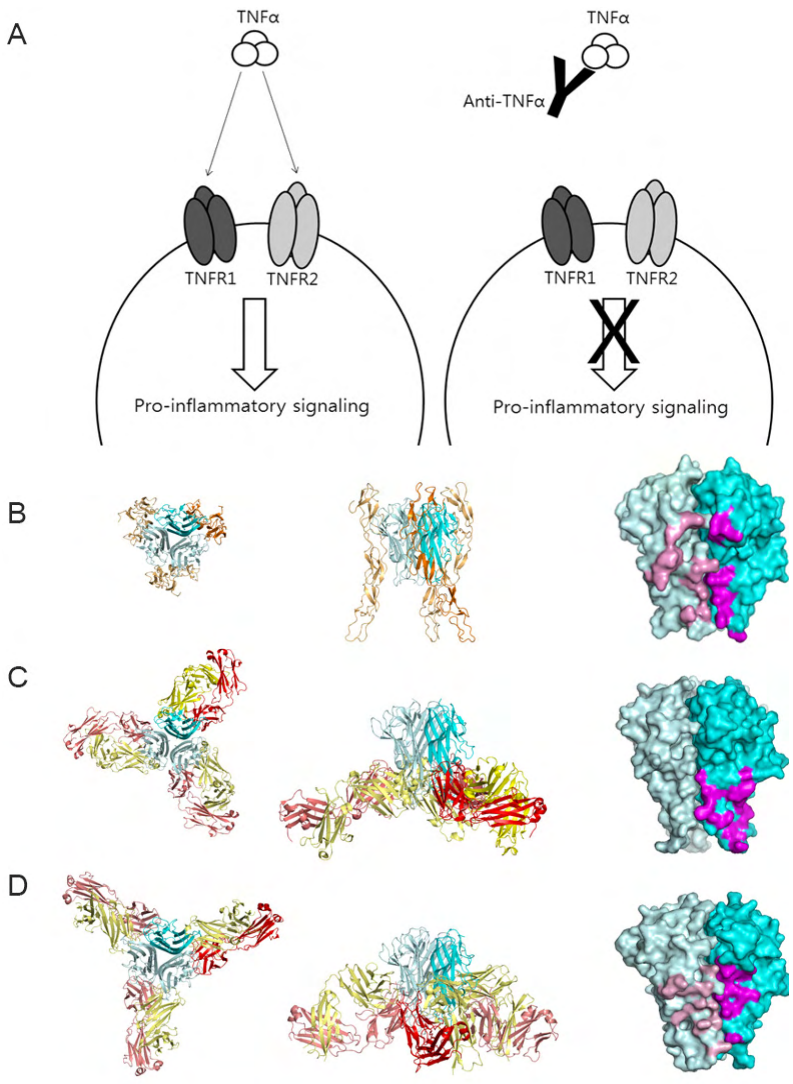


**FIGURE 1** | **TNFα mediated pro-inflammatory signaling and mechanism of anti-TNFα.** (A) The secreted soluble trimer of TNFα can binds to TNFR1 and TNFR2 and initiate pro-inflammatory signaling. Antibody-based drugs bind to trimeric TNFα and inhibit the interaction with TNFRs, and thereby blocking TNFα mediated pro-inflammatory signaling. (B) The top view (left) and side view (center) of the structure of TNFα/TNFR2 complex (PDB entry 3ALQ). The trimeric TNFα is colored cyan and TNFR2 colored orange. The binding interface of TNFα in TNFα/TNFR2 complex is colored violet on the surface model (right). (C) The top view (left) and side view (center) of the structure of TNFα/infliximab Fab complex (PDB entry 4G3Y). The trimeric TNFα is colored cyan and the light and heavy chain of infliximab colored red and yellow. The binding interface of TNFα in TNFα/infliximab complex is colored violet on the surface model (right). (D) The top view (left) and side view (center) of the structure of TNFα/adalimumab Fab complex (PDB entry 3WD5). The trimeric TNFα is colored cyan and the light and heavy chain of adalimimab colored red and yellow. The binding interface of TNFα in TNFα/adalimumab complex is colored violet on the surface model (right). In (B), (C), and (D), the two neighboring protomers of the trimeric TNFα are colored pale for clarity.

  

(Beckham et al., 1992; Nair et al., 2009; Vasilopoulos et al., 2012). Anti-TNFα biologics, including infliximab, adalimumab, golimumab, and certolizumab, have been approved by FDA for the management of RA and other autoimmune diseases. Anti-TNFα drugs bind to homotrimeric tmTNFα and sTNFα, thereby blocking the interaction with two TNFRs to neutralize TNFα-mediated pro-inflammatory signaling (Figure 1A) (Tracey et al., 2008; Kaymakcalan et al., 2009; Scallon et al., 1995).

Infliximab is a chimeric monoclonal antibody against TNFα and approved by FDA for the treatment of autoimmune diseases including psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, and ulcerative colitis. Infliximab was originally developed in mice as a mouse antibody. Because humans have immune reactions to mouse proteins, the mouse common domains were replaced with similar human antibody domains. As a combination of mouse and human antibody amino acid sequences, it is called a chimeric monoclonal antibody (Moreland, 1999).

Adalimumab also is a TNFα inhibiting anti-inflammatory drug. Its trade name is derived from the acronym of "human monoclonal antibody in rheumatoid arthritis" (HUMIRA). It is the first fully human monoclonal antibody, while infliximab is a mouse-human chimeric antibody. In rheumatoid arthritis, adalimumab has a response rate similar to methotrexate, and in combination nearly doubles the response rate of methotrexate alone. Because TNFα is part of the immune system that protects the body from infection, treatment with adalimumab may increase the risk of infections (Lapadula et al., 2014).

Certolizumab pegol is a therapeutic monoclonal antibody to TNFα for the treatment of autoimmune disease, mainly Crohn's disease and rheumatoid arthritis. More precisely, it is a PEGylated Fab fragment of a humanized monoclonal antibody (Pasut, 2014). Golimumab is a fully human monoclonal antibody against TNFα which is used as a once monthly subcutaneous treatment for adults with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis (Oldfield and Plosker, 2009).

In the crystal structure of TNFα in complex with its cognate receptor TNFR2, one TNFR2 molecule interacted with two TNF molecules and the total buried surface area is ~2500 $Å^2$ in the interface (Figure 1B) (Mukai et al., 2010). Interestingly, the infliximab Fab in the complex structure interacts with only one TNFα molecule of the trimer and the total buried surface area is ~2000 $Å^2$ with a high shape complementary and its high affinity may be due to this high shape complementary (Figure 1C). The comparison between the interfaces of TNFα-infliximab Fab and TNFα -TNFR2 indicated that the interfaces overlap with each other, thus allowing infliximab to inhibit TNFα function. Moreover, the distinct feature of the E-F loop on TNFα in the complex structure of TNFα -infliximab Fab explained the reason why infliximab binds to TNF α specifically but not TNFβ (TNFSF1) (Liang et al., 2013).

In the TNFα-adalimumab Fab complex, one adalimumab Fab molecule interacts with two adjacent TNFα protomers, such as in the TNFα-TNFR2 complex and the total buried surface area is 2540 $Å^2$ in the interface (Figure 1D) (Hu et al., 2013). This structural feature showed that the adalimumab epitope directly overlaps the TNFR2 binding area with a larger area of the antigen-antibody interface of TNFα-adalimumab, whereas the infliximab epitope is distant from the receptor-binding sites with less interacting surface. Moreover, several residues that are crucial for TNFα receptor binding also participate in the TNFα-adalimumab Fab interface, especially the groove between two protomers in the TNFα trimer.

Clinical investigations showed the advantages of adalimumab treatment compared with infliximab. In RA patients, adalimumab had higher rates of treatment response and disease remission, and longer drug survival rates than infliximab although it is also immunogenic as infliximab in long-term administration, causing a loss of response (Hetland et al., 2010; Lapadula et al., 2014). However, the cause for this distinct efficacy remains elusive. These structural features could explain this clinical result. An antibody molecule whose epitope directly occupies the receptor binding site, such as adalimumab, may have better and more predictable clinical effects than an antibody that has a more distant epitope and uses the steric properties, such as infliximab.

## RANKL AND DENOSUMAB

Osteoporosis is an age-related skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, and thereby increased fragility of bone (Sigl and Penninger, 2014). Denosumab has emerged as a novel and clinically effective therapeutic agent for treatment of osteoporosis (Narayanan, 2013). Denosumab is a fully human monoclonal antibody that neutralizes the receptor activator of nuclear factor κB ligand (RANKL). Bone remodeling is the process by which the body continuously removes old bone tissue and replaces it with new bone. It is driven by various types of cells, most notably osteoblasts (which secrete new bone) and osteoclasts (which break down bone). RANKL is a TNFSF cytokine produced by osteoblasts and a key regulator of the formation and function of bone-resorbing osteoclasts, which activates its cell surface receptor, receptor activator of nuclear factor-κB (RANK), expressed by both osteoclast precursors and mature osteoclasts (Figure 2A).

Denosumab inhibits the binding of RANKL to RANK, thereby decreasing osteoclastogenesis and bone-resorbing activity of mature osteoclasts (Body et al., 2006). Denosumab mimics the natural action of osteoprotegerin, an endogenous RANKL inhibitor, which presents with decreasing concentrations and possibly decreased avidity in patients who are suffering from osteoporosis. Bone loss is a common side effect of cancer treatments. Denosumab can also attenuate bone loss in cancer patients receiving adjuvant aromatase inhibitor therapy and androgen deprivation therapy (Christenson et al., 2012; Peddi et al., 2013; Spencer et al., 2012).

In the crystal structure of RANKL-RANK complex, the receptor bind to a groove at the junction of two protomers in the trimeric ligand with total buried surface of ~2500 $Å^2$ as other TNFSF/

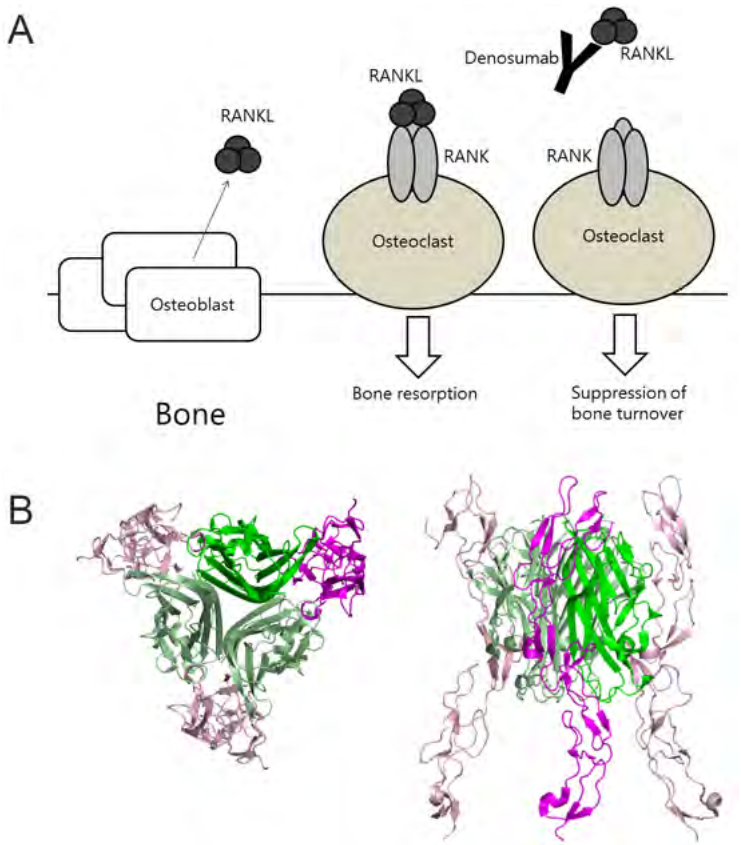


**FIGURE 2 | RANKL-RANK interaction and mechanism of denosumab.** (A) RANKL is secreted from osteoblasts and binds to the RANK receptor on osteoclasts and promotes osteoclast differentiation and activity. Denosumab is a monoclonal antibody that binds to RANKL and inhibits osteoclasts maturation, activity, and survival by preventing RANKL from binding RANK receptor on osteoclast, thereby decreases bone resorption and suppresses bone turnover. (B) The top view (left) and side view (right) of the structure of RANKL/RANK complex (PDB entry 3QBQ). The trimeric RANKL is colored green and RANK colored violet.

TNRSF complexes (Figure 2B). But RANK has distinctive conformational features and ligand-binding mode that confer the binding specificity for RANKL, providing a framework for rational design of RANKL inhibitors (Ta et al., 2010). Structural study of denosumab-RANKL complex is required for understanding the exact mechanism of action and the binding specificity of denosumab.

## BAFF AND BELIMUMAB

B-cell activating factor (BAFF), also called B-lymphocyte stimulator (BLyS), is required for the development and survival of B cells. B cells are one of the immune cells responsible for the damage in autoimmune disease (Vincent et al., 2013). B cells develop in the bone marrow and continue to mature peripherally in secondary lymphoid organs. When autoimmune B cells attack the body's own tissues, they are normally destroyed by apoptosis. Systemic lupus erythematosus (SLE) is caused when autoimmune B cells proliferate, and survive from apoptosis (Moore et al., 1999; Schneider et al.,1999). In SLE, BAFF is overexpressed (Stohl and Hilbert 2012). Given the central role for B cells in the pathogenesis of SLE and the function of BAFF as a vital B-cell survival factor, BAFF emerged as a logical candidate target for control SLE.

A proliferation inducting ligand (APRIL, TNFSF13) was identified as a cell growth stimulator in various cancer cell lines and shares two receptors with BAFF, TACI and BCMA. Additionally, BAFF binds strongly to BAFF receptor (BAFF-R) (Figure 3A) (Gross et al., 2000; Knight et al., 2007). These three main receptors, BAFF-R, TACI, and BCMA have distinct expression patterns based on B cell development stages. Although APRIL has little effect on the size of the mature B-cell pool, it does importantly contribute to survival of plasmablasts and plasma cells. Moreover, it was known that BAFF and APRIL could form heterotrimers, and circulating levels of such heterotrimers were elevated in at least some SLE patients.

Belimumab is the first and sole FDA-approved biologic for SLE. Belimumab is a fully human antibody that binds to BAFF, preventing BAFF from binding to its cognate receptors on B cells or plasma cells (Furie et al., 2011; Navarra et al., 2011). Without the binding of BAFF to the receptors, B cells commit suicide, and no longer contribute to the autoimmune damage of SLE. Unfortunately, it is known that 40~60% of lupus patients failed to significantly respond to belimumab (Stohl and Hilbert, 2012; Treml et al., 2009). Thus, it is suggested that neutralization of BAFF plus APRIL could have a greater effect than neutralization of BAFF alone (Roschke, 2002).

In the crystal structure of BAFF/BAFF-R complex, the ligand-receptor interaction is much different from the general mode of other TNFSF/TNFRSF complex, in which the receptors are highly elongated and bind to the crevice between the two neighboring protomers of the trimeric ligands. One BAFF-R binds to only one protomer of trimeric BAFF with total buried surface of 922 $Å^2$, which is less than those of other TNFSF/TNFRSF complex (Figure 3B) (Kim et al., 2003). No structural data has been reported about the binding of belimumab to BAFF. The precise epitope, which can be revealed by structural studies, can explain the more precise mechanism of action of belimumab and provide useful information for structure-based improvement of anti-BAFF antibodies.

## ANTIBODY-BASED BIOLOGICS TARGETING OTHER TNFSF

A disease in which significant subgroups of patients do not respond to an anti-TNF suggests that other mechanisms of inflammation and immune responses may communicate pathogenesis.

  

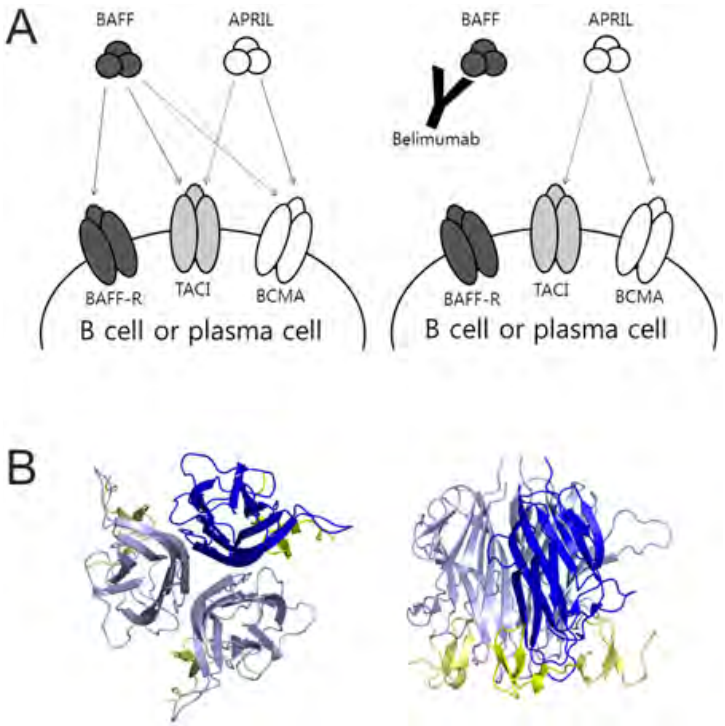


**FIGURE 3 | Mechanism of action of belimumab.** (A) BAFF and APRIL are expressed as membrane-bound trimers, which are proteolytically cleaved to form soluble trimers. BAFF binds strongly to BAFF-R and TACI, and weakly to BCMA. APRIL binds strongly to BCMA, weakly to TACI. BAFF-R is primarily expressed on all B cells, TACI on innate-like B cells, and BCMA on plasmablasts and plasma cells. Belimumab binds BAFF and blocks engagement of BAFF with BCMA, TACI and BAFF-R. Belimumab does not bind APRIL, so engagement of APRIL with BCMA or TACI is not affected. (B) The top view (left) and side view (right) of the structure of BAFF/BAFF-R complex (PDB entries 1POT and 1OTZ). The trimeric BAFF is colored blue and BAFF-R colored yellow.

Targeting other members of the TNFSF has provided proof of principle that other TNF cytokine pathways may be involved in pathogenesis (Table 2). For example, the Fn14/TWEAK (TNFSF12) pathway mediates key pathologic processes underlying rheumatoid arthritis and lupus nephritis (Michaelson et al., 2012). A phase II trial with BIIB023, an anti-TWEAK neutralizing antibody, is ongoing to assess efficacy in the patients of these autoimmune diseases (Wisniacki et al., 2013). Antibodies to LTα (TNFSF1) or LIGHT (TNFSF14) are also in clinical development for autoimmune disease.

A humanized antibody to LTα (MLTA3698A) possessing antibody-dependent cytotoxicity (ADCC) activity was used to deplete T cells in a xenogenic GVHD model with disease amelioration (Chiang et al., 2012), and a phase I study in RA showed good safety with mild/moderate adverse events (Emu et al., 2012). A fully human antibody to LIGHT (SAR252067) competitively inhibits LIGHT binding to all of its three receptors, HVEM, LTβR and DcR3, showing inflammatory bowel disease may be an appropriate clinical indication (Browning, 2008; Cohavy et al., 2005; Doherty et al., 2011; Gatumu et al., 2009; Shaikh et al., 2001;Ware, 2009).

The OX40/OX40L (TNFSF4) interaction contributes to an optimal T cell response following allergic stimuli and plays an important role in the maintenance and reactivation of memory T effector cells (Mahmood and Yang, 2012). Anti-OX40L monoclonal antibody (Oxelumab) is in a phase II study to test allergen-induced responses in subjects with asthma (Gwyer Findlay et al., 2012; Kaur and Brightling, 2012). Antibody drug conjugate (ADC) approaches with monomethyl auristatin phenylalanine or duocarmycin are also promising in anti-CD27L (TNFSF7) biologics for treatment of cancer (Ryan et al., 2010; Alley et al., 2009; Oflazoglu et al., 2008; Thevanayagam et al., 2013).

## CONCLUSION

Antibody-based TNFSF inhibitors have revealed new insights into human immune and inflammatory systems and the related human disease mechanisms. And therapeutic antibodies against TNFSF are used clinically to treat several chronic autoimmune diseases. However, such treatment sometimes results in serious

**TABLE 2** | TNF superfamily targeting antibodies in clinical development (http://clinicaltrials.gov/)

| TNF | Antibody Name | Type | Indication |
|---|---|---|---|
| BAFF | Tabalumab | Fully Human IgG | Autoimmune |
| CD27L | SGN-75 | Antibody Drug Conjugate | Cancer |
| CD27L | MDX-1411 | Fc modified Human IgG | Cancer |
| CD27L | MDX-1203 | Antibody Drug Conjugate | Cancer |
| LTα | MLTA3698A | Humanized IgG | Autoimmune |
| LIGHT | SAR252067 | Fully Human IgG | Inflammatory bowel disease |
| OX40L | Oxelumab | Fully Human IgG | Asthma |
| TWEAK | BIIB023 | Humanized IgG | Autoimmune |

side effects, which are partly caused by the blocking of signals from other TNFRs. Once a new antibody is identified as having promising results in preclinical studies, a structural study to determine its precise epitope may help us in making strategic decisions before proceeding with costly clinical drug trials as the affinity and the epitope are the crucial elements in evaluating antibody drugs. In addition, accumulation of structural knowledge for the recognition of TNF by each receptor or antibody molecules would be invaluable in designing selective and potent drugs as they can provide useful information for antibody optimization by understanding the precise molecular mechanism of TNF inhibition.


## ACKNOWLEDGMENTS

This work was supported by a NRF grant (NRF-2012R1A1A2008197), funded by the Korean government.


## AUTHOR INFORMATION

The authors declare no potential conflicts of interest.


Original Submission: May 12, 2014
Revised Version Received: May 22, 2014
Accepted: May 22, 2014


## REFERENCES


Alley, S.C., Zhang, X., Okeley, N.M., Anderson, M., Law, C.L., Senter, P.D., and Benjamin, D.R. (2009). The pharmacologic basis for antibody-auristatin conjugate activity. *J Pharmacol Exp Ther* **330**, 932-938.

Beckham, J.C., Caldwell, D.S., Peterson, B.L., Pippen, A.M., Currie, M.S., Keefe, F.J., and Weinberg, J.B. (1992). Disease severity in rheumatoid arthritis: relationships of plasma tumor necrosis factor-alpha, soluble interleukin 2-receptor, soluble CD4/CD8 ratio, neopterin, and fibrin D-dimer to traditional severity and functional measures. *J Clin Immunol* **12**, 353–361.

Bodmer, J.L., Schneider, P., and Tschopp, J. (2002). The molecular architecture of the TNF superfamily. *Trends Biochem Sci* **27**, 19–26.

Body, J.J., Facon, T., Coleman, R.E., Lipton, A., Geurs, F., Fan, M., Holloway, D., Peterson, M.C., and Bekker, P.J. (2006). A study of the biological receptor activator of nuclear factor-kappaB ligand inhibitor, denosumab, in patients with multiple myeloma or bone metastases from breast cancer. *Clin Cancer Res* **12**, 1221–1228.

Bossen, C., Ingold, K., Tardivel, A., Bodmer, J.L., Gaide, O., Hertig, S., Ambrose, C., Tschopp, J., and Schneider, P. (2006). Interactions of tumor necrosis factor (TNF) and TNF receptor family members in the mouse and human. *J Biol Chem* **281**, 13964–13971.

Browning, J.L. (2008). Inhibition of the lymphotoxin pathway as a therapy for autoimmune disease. Immunol Rev 223, 202–220.

Chiang, E.Y., Kolumam, G., McCutcheon, K.M., Young, J., Lin, Z., Balazs, M., and Grogan, J.L. (2012). In vivo depletion of lymphotoxin-alpha expressing lymphocytes inhibits xenogeneic graft-versus-host-disease. *PLoS ONE* **7**, e33106.

Christenson, E.S., Jiang, X., Kagan, R., and Schnatz, P. (2012). Osteoporosis management in post-menopausal women. *Minerva Ginecologica*, **64**, 181–194.

Cohavy, O., Zhou, J., Ware, C.F., and Targan, S.R. (2005). LIGHT is constitutively Expressed on T and NK cells in the human gut and can Be induced by CD2-mediated signaling. *J Immunol* **174**, 646–653.

Doherty, T.A., Soroosh, P., Khorram, N., Fukuyama, S., Rosenthal, P., Cho, J.Y., Norris, P.S., Choi, H., Scheu, S., Pfeffer, K., Zuraw, B.L., Ware, C.F., Broide, D.H., and Croft, M. (2011). The tumor necrosis factor family member LIGHT is a target for asthmatic airway remodeling. *Nat Med* **17**, 596–603.

Emu, B., Luca, D., Offutt, C., Grogan, J.L., Rojkovich, B., Williams, M.B., Tang, M.T., Xiao, J., Lee, J.H., and Davis, J.C. (2012). Safety, pharmacokinetics, and biologic activity of pateclizumab, a novel monoclonal antibody targeting lymphotoxin alpha: results of a phase I randomized, placebo-controlled trial. *Arthritis Res Ther* **14**, R6.

Furie, R., Petri, M., Zamani, O., Cervera, R., Wallace, D.J., Tegzova, D., Sanchez-Guerrero, J., Schwarting, A., Merrill, J.T., Chatham, W.W., Stohl, W., Ginzler, E.M., Hough, D.R., Zhong, Z.J., Freimuth, W., et al. (2011). A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum* **63**, 3918–3930.

Gatumu, M.K., Skarstein, K., Papandile, A., Browning, J.L., Fava, R.A., and Bolstad, A.I. (2009). Blockade of lymphotoxin-beta receptor signaling reduces aspects of Sjogren's syndrome in salivary glands of non-obese diabetic mice. *Arthritis Res Ther* **11**, R24.

Gross, J.A., Johnston, J., Mudri, S., Enselman, R., Dillon, S.R., Madden, K., Xu, W., Parrish-Novak, J., Foster, D., Lofton-Day, C., Moore, M., Littau, A., Grossman, A., Haugen, H., Foley, K., (2000). TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature* **404**, 995-999.

Gwyer Findlay, E., Danks, L., Madden, J., Cavanagh, M.M., McNamee, K., McCann, F., Snelgrove, R.J., Shaw, S., Feldmann, M., Taylor, P.C., Horwood, N.J., and Hussell, T. (2014). OX40L blockade is Therapeutic in arthritis, despite promoting osteoclastogenesis. *Proc Natl Acad Sci USA* **111**, 2289-2294.

Hetland, M.L., Christensen, I.J., Tarp, U., Dreyer, L., Hansen, A., Hansen, I.T., Kollerup, G., Linde, L., Lindegaard, H. M., Poulsen, U.E., Schlemmer, A., Jensen, D.V., Jensen, S., Hostenkamp, G., and Østergaard, M. (2010). Direct comparison of treatment responses, remission rates, and drug adherence in patients with rheumatoid arthritis treated with adalimumab, etanercept, or infliximab: results from eight years of surveillance of clinical practice in the nationwide Danish DANBIO registry. *Arthritis Rheum* **62**, 22-32.

Hu, S.1., Liang, S., Guo, H., Zhang, D., Li, H., Wang, X., Yang, W., Qian, W., Hou, S., Wang, H., Guo, Y., and Lou, Z. (2013). Comparison of the inhibition mechanisms of adalimumab and infliximab in treating tumor necrosis factor α-associated diseases from a molecular view. *J Biol Chem* **288**, 27059-27067.

Kaur, D., Brightling, C. (2012). OX40/OX40 ligand interactions in T-cell regulation and asthma. *Chest* **141**, 494-499.

Furie, R., Petri, M., Zamani, O., Cervera, R., Wallace, D.J., Tegzova, D., Sanchez-Guerrero, J., Schwarting, A., Merrill, J.T., Chatham, W.W., Stohl, W., Ginzler, E.M., Hough, D.R., Zhong, Z.J., Freimuth, W., et al. (2009). Comparisons of affinities, avidities, and complement activation of adalimumab, infliximab, and etanercept in binding to soluble and membrane tumor necrosis factor. *Clin Immunol* **131**, 308–316.

Kim, H.M., Yu, K.S., Lee, M.E., Shin, D.R., Kim, Y.S., Paik, S.G., Yoo, O.J., Lee, H., and Lee, J.-O. (2003). Crystal structure of the BAFF-BAFF-R complex and its implications for receptor activation. *Nat Struc Biol* **10**, 342-348

Kishore, U., Gaboriaud, C., Waters, P., Shrive, A.K., Greenhough, T.J., Reid, K.B., Sim, R.B., and Arlaud, G.J. (2004). C1q and tumor necrosis factor superfamily: modularity and versatility. *Trends Immunol* **25**, 551–561.

Knight, A. K., Radigan, L., Marron, T., Langs, A., Zhang, L., and Cunningham-Rundles, C. (2007). High serum levels of BAFF, APRIL, and TACI in common variable immunodeficiency. *Clin Immunol* **124**,182-189.

Lapadula, G., Marchesoni, A., Armuzzi, A., Blandizzi, C., Caporali, R., Chimenti, S., Cimaz, R., Cimino, L., Gionchetti, P., Girolomoni, G., Lionetti, P., Marcellusi, A., Mennini, F.S., and Salvarani, C. (2014). Adalimumab in the treatment of immune-mediated diseases. *Int J Immunopathol Pharmacol* **27**, 33-48.

Liang, S.Y., Dai, J.X., Hou, S., Su, L., Zhang, D., Guo, H., Hu, S., Wang, H., Rao, Z., Guo, Y. J., and Lou, Z.Y. (2013). Structural basis for treating tumor necrosis factor alpha (Tnfalpha)-associated diseases with the therapeutic antibody infliximab. *J Biol Chem* **288**, 13799-13807.

Locksley, R. M., Killeen, N., and Lenardo, M.J. (2001). The TNF and TNF receptor superfamilies: Integrating mammalian biology. *Cell* **104**, 487–501.

Mahmood, T., and Yang, P.C. (2012). OX40L-OX40 Interactions: A Possible Target for Gastrointestinal Autoimmune Diseases. *N Am J Med Sci* **4**, 533-536.


  

Michaelson, J.S., Wisniacki, N., Burkly, L.C., and Putterman, C. (2012) Role of TWEAK in lupus nephritis: a bench-to-bedside review. *J Autoimmun* **39**,130-142.

Moore, P.A., Belvedere, O., Orr, A., Pieri, K., Lafleur, D.W., Feng, P., Soppet, D., Charters, M., Gentz, R., Parmelee, D., Li, Y., Galperina, O., Giri, J., Roschke, V., Nardelli, B., et al. (1999) BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science* **285**, 260–263.

Moreland, L.W. (1999). Inhibitors of tumor necrosis factor for rheumatoid arthritis. *J Rheumatol Suppl* **57**, 7-15.

Mukai, Y. 1., Nakamura, T., Yoshikawa, M., Yoshioka, Y., Tsunoda, S., Nakagawa, S., Yamagata, Y., and Tsutsumi, Y. (2010). Solution of the structure of the TNF-TNFR2 complex. *Sci Signal* **3**, ra83.

Moots, R.J., and Naisbett-Groet, B. (2012). The efficacy of biologic agents in patients with rheumatoid arthritis and an inadequate response to tumour necrosis factor inhibitors: a systematic review. *Rheumatology (Oxford)* **51**, 2252–2261.

Nair, R.P., Duffin, K.C., Helms, C., Ding, J., Stuart, P.E., Goldgar, D., Gudjonsson, J.E., Li, Y., Tejasvi, T., Feng, B.J., Ruether, A., Schreiber, S., Weichenthal, M., Gladman, D., Rahman, P., et al. (2009). Genomewide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet* **41**, 199–204.

Narayanan, P. (2013). Denosumab: A comprehensive review. *South Asian J Cancer* **2**, 272-277.

Navarra, S.V., Guzman, R.M., Gallacher, A.E., Hall, S., Levy, R.A., Jimenez, R.E., Li, E.K., Thomas, M., Kim, H.Y., Leon, M.G., Tanasescu, C., Nasonov, E., Lan, J.L., Pineda, L., Zhong, Z.J., et al. (2011). Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* **377**, 721–731.

Oflazoglu, E., Stone, I.J., Gordon, K., Wood, C.G., Repasky, E.A., Grewal, I.S., Law, C.L., and Gerber, H.P. (2008). Potent anticarcinoma activity of the humanized anti-CD70 antibody h1F6 conjugated to the tubulin inhibitor auristatin via an uncleavable linker. *Clin Cancer Res* **14**, 6171-6180.

Oldfield, V., and Plosker, G.L. (2009). Golimumab: in the treatment of rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. *BioDrugs* **23**, 125-135.

Pasut, G. (2014) Pegylation of biological molecules and potential benefits: pharmacological properties of certolizumab pegol. *BioDrugs* **28**, 15-23.

Peddi, P., Lopez-Olivo, M.A., Pratt, G.F., and Suarez-Almazor, M.E. (2013). Denosumab in patients with cancer and skeletal metastases: a systematic review and meta-analysis. *Cancer Treat Rev* **39**, 97–104.

Pineda, G., Ea, C.K., and Chen, Z.J. (2007). Ubiquitination and TRAF signaling. *Adv Exp Med Biol* **597**, 80–92.

Prajapati, R., Plant, D., and Barton, A. (2011). Genetic and genomic predictors of anti-TNF response. *Pharmacogenomics* **12**, 1571–1585.

Roschke, V., Sosnovtseva, S., Ward, C.D., Hong, J.S., Smith, R., Albert, V., Stohl, W., Baker, K.P., Ullrich, S., Nardelli, B., Hilbert, D.M., and Migone, T.S. (2002). BLyS and APRIL form biologically active heterotrimers that are expressed in patients with systemic immune-based rheumatic diseases. *J Immunol* **169**, 4314-4321.

Ryan, M. C., Kostner, H., Gordon, K.A., Duniho, S., Sutherland, M.K., Yu, C., Kim, K.M., Nesterova, A., Anderson, M., McEarchern, J.A., Law, C.L., and Smith, L.M. (2010). Targeting pancreatic and ovarian carcinomas using the auristatin-based anti-CD70 antibody-drug conjugate SGN-75. *Br J Cancer* **103**, 676-684.

Scallon, B.J., Moore, M.A., Trinh, H., Knight, D.M., and Ghrayeb, J. (1995). Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. *Cytokine* **7**, 251–259.

Schneider, P., MacKay, F., Steiner, V., Hofmann, K., Bodmer, J.L., Holler, N., Ambrose, C., Lawton, P., Bixler, S., Acha-Orbea, H., Valmori, D., Romero, P., Werner-Favre, C., Zubler, R.H., Browning, J.L., et al. (1999). BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J Exp Med* **189**, 1747-1756.

Schrofelbauer, B., and Hoffmann, A. (2011). How do pleiotropic kinase hubs mediate specific signaling by TNFR superfamily members? *Immunol Rev* **244**, 29–43.

Shaikh, R.B., Santee, S., Granger, S.W., Butrovich, K., Cheung, T., Kronenberg, M., Cheroutre, H., and Ware, C.F. (2001). Constitutive expression of LIGHT on T cells leads to lymphocyte activation, inflammation, and tissue destruction. *J Immunol* **167**, 6330–6337.

Sigl, V., andPenninger, J.M. (2014). RANKL/RANK - From bone physiology to breast cancer. *Cytokine Growth Factor Rev* **25**, 205-214.

Spencer, S., Marini, B.L., and Figg, W.D. (2012). Novel approaches in the pharmacotherapy of skeletal-related events in metastatic castrate-resistant prostate cancer. *Anticancer Res* **32**, 2391–2398.

Stohl, W., and Hilbert, D.M. (2012). The discovery and development of belimumab: the anti-BLyS-lupus connection. *Nat Biotechnol* **30**, 69–77.

Ta, H.M., Nguyen, G.T., Jin, H.M., Choi, J.K., Park, H., Kim, N.S., Hwang, H.Y., Kim. K.K. (2010). Structure-based development of a receptor activator of nuclear factor-kappab ligand (Rankl) inhibitor peptide and molecular basis for osteopetrosis. *Proc Natl Acad Sci USA* **107**, 20281-20286.

Thevanayagam, L., Bell, A., Chakraborty, I., Sufi, B., Gangwar, S., Zang, A., Rangan, V., Rao, C., Wang, Z., Pan, C., Chong, C., Cardarelli, P., Deshpande, S., and Srinivasan, M. (2013). Novel detection of DNA-alkylated adducts of antibody-drug conjugates with potentially unique preclinical and biomarker applications. *Bioanalysis* **5**, 1073-1081.

Tracey, D., Klareskog, L., Sasso, E.H., Salfeld, J.G., and Tak, P.P. (2008). Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* **117**, 244–279.

Treml, J.F., Hao, Y., Stadanlick, J.E., and Cancro, M.P. (2009). The BLyS family: toward a molecular understanding of B cell homeostasis. *Cell Biochem Biophys* **53**, 1–16.

Vasilopoulos, Y., Manolika, M., Zafiriou, E., Sarafidou, T., Bagiatis, V., Kruger-Krasagaki, S., Tosca, A., Patsatsi, A., Sotiriadis, D., Mamuris, Z., and Roussaki-Schulze, A. (2012). Pharmacogenetic analysis of TNF, TNFRSF1A, and TNFRSF1B gene polymorphisms and prediction of response to anti-TNF therapy in psoriasis patients in the Greek population. *Mol Diagn Ther* **16**, 29–34.

Vincent, F.B., Saulep-Easton, D., Figgett, W.A., Fairfax, K.A., and Mackay, F. (2013) The BAFF/APRIL system: emerging functions beyond B cell biology and autoimmunity. *Cytokine Growth Factor Rev* **24**, 203-215.

Walczak, H. (2011). TNF and ubiquitin at the crossroads of gene activation, cell death, inflammation, and cancer. *Immunol Rev* **244**, 9–28.

Wallis, R.S. (2011). Biologics and infections: lessons from tumor necrosis factor blocking agents. *Infect Dis Clin North Am* **25**, 895–910.

Ware, C.F. (2009). Targeting the LIGHT-HVEM pathway. *Adv Exp Med Biol* **647**, 146–155.

Wiens, G.D., and Glenney, G.W. (2011). Origin and evolution of TNF and TNF receptor superfamilies. *Dev Comp Immunol* **35**, 1324–1335.

Wisniacki, N., Amaravadi, L., Galluppi, G.R., Zheng, T.S., Zhang, R., Kong, J., and Burkly, L.C. (2013). Safety, tolerability, pharmacokinetics, and pharmacodynamics of anti-TWEAK monoclonal antibody in patients with rheumatoid arthritis. *Clin Ther* **35**, 1137-49.