

Structural basis for tumor necrosis factor blockade with the therapeutic antibody golimumab

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Abstract: Tumor necrosis factor α (TNF α) is a proinflammatory cytokine, and elevated levels of TNF α in serum are associated with various autoimmune diseases, including rheumatoid arthritis (RA), ankylosing spondylitis (AS), Crohn's disease (CD), psoriasis, and systemic lupus erythematosus. TNF α performs its pleiotropic functions by binding to two structurally distinct transmembrane receptors, TNF receptor (TNFR) 1 and TNFR2. Antibody-based therapeutic strategies that block excessive TNF α signaling have been shown to be effective in suppressing such harmful inflammatory conditions. Golimumab (Simponi®) is an FDA-approved fully human monoclonal antibody targeting TNF α that has been widely used for the treatment of RA, AS, and CD. However, the structural basis underlying the inhibitory action of golimumab remains unclear. Here, we report the crystal structure of the Fv fragment of golimumab in complex with TNF α at a resolution of 2.73 Å. The resolved structure reveals that golimumab binds to a distinct epitope on TNF α that does not overlap with the binding residues of TNFR2. Golimumab exerts its inhibitory effect by preventing binding of TNFR1 and TNFR2 to TNF α by steric hindrance. Golimumab does not induce conformational changes in TNF α that could affect receptor binding. This mode of action is specific to golimumab among the four anti-TNF α therapeutic antibodies currently approved for clinical use.

Keywords: autoimmune disease; competitive inhibition; steric hindrance; therapeutic antibody; golimumab; tumor necrosis factor α (TNF α); X-ray crystallography

Additional Supporting Information may be found in the online version of this article.

Accession numbers: The atomic coordinates and structure factors for TNF α :golimumab Fv complex have been deposited in the Protein Data Bank (<http://www.rcsb.org>) under accession code 5YOY.

A 50-75-word statement, written for a broader audience, outlining the importance and/or impact of the work presented in the manuscript: The anti-TNF α therapeutic antibody golimumab (Simponi®) received FDA approval in 2009 and has demonstrated robust clinical efficacy in the treatment of rheumatic disease; however the structural basis underlying its inhibitory action remains unclear. In this study, we report the crystal structure of the TNF α :golimumab complex and reveal the mode of action of golimumab, which is different from that of the other FDA-approved anti-TNF α antibodies, infliximab (Remicade®), adalimumab (Humira®), and certolizumab pegol (Cimzia®).

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Introduction

Tumor necrosis factor α (TNF α) is a pleiotropic cytokine involved in regulating diverse bodily functions, including cell growth modulation, inflammation, carcinogenesis, viral replication, septic shock and autoimmunity.¹ TNF α performs its functions by binding to two structurally distinct membrane receptors, TNFR1 (also known as p55) and TNFR2 (p75).² TNF α signaling via TNFR1 is predominantly associated with inflammation and apoptosis. In contrast, TNFR2 is mainly implicated in regulation of T-cell migration and tissue regeneration. Because of its pleiotropic actions, the biosynthesis of TNF α is under strict control of multiple and complex regulatory mechanisms, including gene transcription, mRNA turnover, translation and intracellular signaling of the protein. Dysregulation of TNF α production can result in elevated serum TNF α levels and the development of various inflammatory and autoimmune diseases, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), Crohn's disease (CD), psoriasis, and systemic lupus erythematosus.³

Recently, therapeutic strategies that block excessive TNF α signaling have emerged to suppress these harmful inflammatory conditions. To date, the following five molecules have been approved by the FDA as effective TNF α inhibitors for clinical use and led to significant advances in autoimmune inflammatory disease therapy: infliximab (Remicade®; humanized chimeric monoclonal antibody (mAb) of the isotype IgG1), adalimumab (Humira®; fully human mAb of the IgG1/ κ isotype), certolizumab pegol (Cimzia®; PEGylated humanized Fab fragment), golimumab (Simponi®; fully human mAb of the IgG1/ κ isotype), and etanercept (Enbrel®; Fc-TNFR2 fusion protein).⁴ These new biologic agents have changed the paradigm of RA treatment, leading to improvements in controlling the signs and symptoms of RA refractory to classical low-molecular-weight drugs such as methotrexate (MTX), and in slowing joint destruction.⁵ The concentration of golimumab necessary to neutralize TNF α -induced E-selectin expression on human endothelial cells by 50% is reported to be less than those for infliximab and adalimumab.⁶ Nevertheless, patients currently treated with an anti-TNF α agent may remain refractory or become nonresponsive to the treatment.⁷ In addition, a sizable proportion of patients experience adverse events, such as hepatitis B infection and tuberculosis.⁸ Once a primary TNF α inhibitor fails, a patient must switch to another anti-TNF α agent that has a complementary mode of action or differs in its capacity to induce immunogenicity.⁹

The molecular basis underlying the inhibitory mechanisms of infliximab, adalimumab and certolizumab has been elucidated in part by X-ray crystallography.^{10–12} Adalimumab binds a large surface area on

Table I. Crystallographic Data Collection and Refinement Statistics

Protein name	TNF α : golimumab Fv complex
X-ray data collection	
Source,	SPRING-8 BL41XU,
wavelength	1.00000 Å
Resolution (Å) ^a	46.25–2.73 (2.80–2.73)
Space group	$P2_1$
Unit cell	$a = 133.6$, $b = 85.3$,
parameter (Å, °)	$c = 136.0$, $\alpha = 90.0$, $\beta = 101.6$, $\gamma = 90.0$
Unique reflections ^a	77915 (5439)
Redundancy ^a	3.4 (3.5)
Completeness ^a	97.1 (92.8)
R_{merge} (%) ^a	10.7 (98.8)
$CC_{1/2}$ (%) ^a	99.3 (53.9)
$\langle I/\sigma(I) \rangle$ ^a	9.5 (1.5)
Refinement	
Resolution (Å) ^a	46.25–2.73 (2.76–2.73)
$R_{\text{work}}/R_{\text{free}}$ (%) ^{a,b}	19.0/23.4 (35.4/39.9)
R.m.s. deviations	
Bonds (Å)	0.003
Angles (°)	0.672
No. of atoms (average B-factors (Å ²))	
TNF α	7194 (81.8)
golimumab Fv	10836 (75.4)
Water	86 (59.3)
Ramachandran plot	
Favored region (%)	95.6
Allowed region (%)	4.1

^a Values for the highest resolution shells are shown in parentheses.

^b R_{work} was calculated with 95% of the unique reflections used for refinement, whereas R_{free} was calculated with the remaining 5% of the unique reflections.

TNF α , with its epitope directly overlapping the TNFR2 binding area. Infliximab's epitope is located at the “shoulder” on a bell-shaped TNF α trimer and contains a smaller TNF α :TNFR2 interacting surface. Certolizumab binds to the “waist” portion on TNF α and has overlapping segments with the binding footprint of TNFR2. However, the structural basis for the inhibitory action of golimumab remains unclear. Here, we report the crystal structure of the variable domain (Fv) fragment of golimumab in complex with TNF α at a resolution of 2.73 Å, which reveals an idiosyncratic mode of binding to TNF α to compete with TNFRs at the atomic level.

Results and Discussion

Overall structure of the TNF α :golimumab Fv complex

The soluble forms of the human TNF α and the Fv fragment of the golimumab, both of which contain intrachain disulfide bonds, were produced by a Gram-positive bacterial secretory expression. For production of the Fv fragment, the “iRAT (intervening removable affinity tag)” system was used to

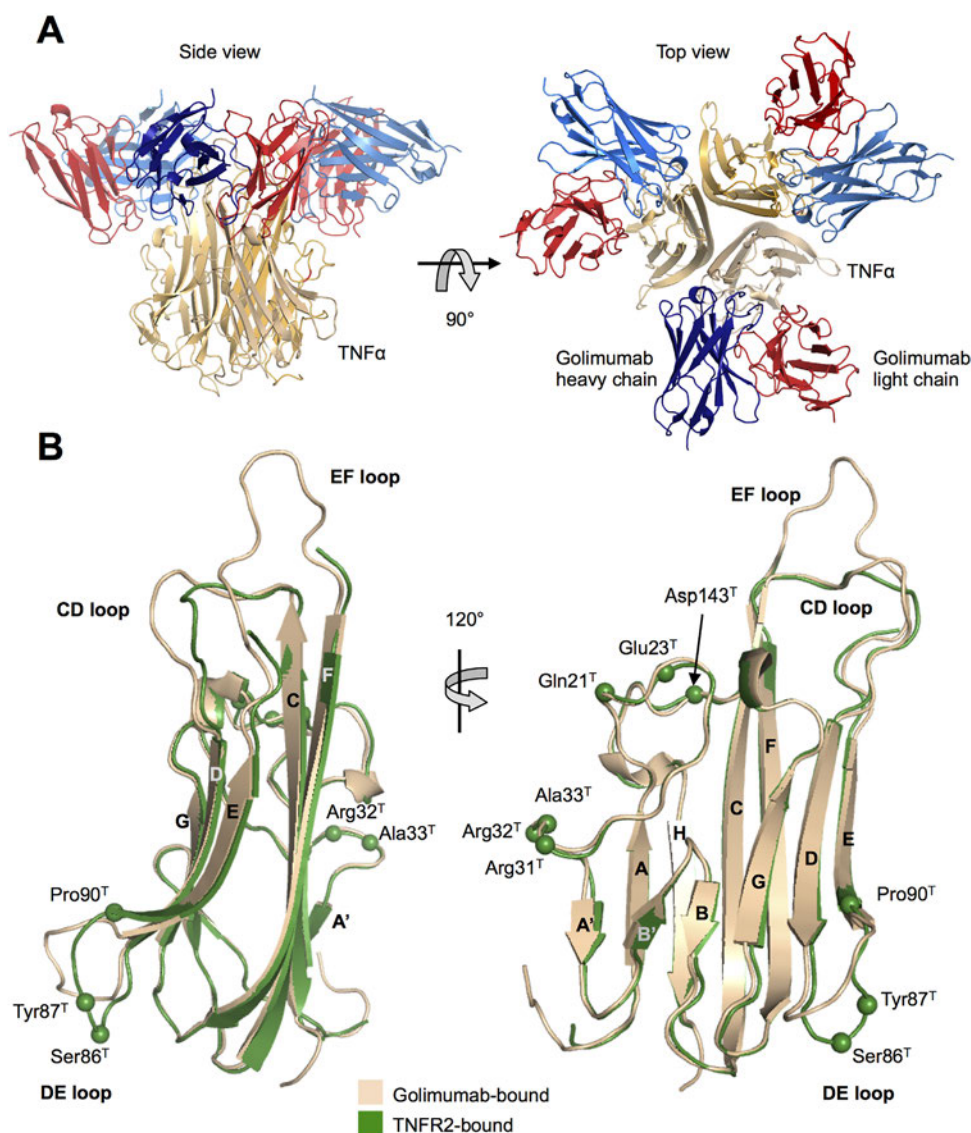


Figure 1. Overall structure of the TNF α :golimumab complex. (A) Ribbon diagrams of the biological unit showing the TNF α homotrimer and three golimumab Fvs binding to each protomer in the side view (left) and top view (right). TNF α , golimumab V_L and golimumab V_H are shown in tan, red and blue, respectively. (B) Structural alignment of the golimumab-bound TNF α protomer (tan) with TNFR2-bound TNF α protomer (green). Canonical designations of β -strands and loops within TNF α are also shown. The TNF α protomer is rotated by 120° around the vertical axis. Residues in TNF α that contact TNFR2 are indicated by green balls.

ensure stoichiometric expression of heavy (V_H)- and light (V_L)-chain variable domains followed by correct folding and assembly.¹³ By employing these recombinant proteins, the TNF α :golimumab Fv complex (subsequently referred to as the TNF α :golimumab complex) was prepared. Analytical gel filtration confirmed that the stoichiometrically saturated 1:3 TNF α homotrimer:golimumab complex (approximately 126 kDa) exists as a monomer in solution [Supplementary Fig. S1(A, B)]. The crystals of TNF α :golimumab complex [Supplementary Fig. S1(C)] appeared in the *P*2₁ space group and diffracted X-rays to 2.73 Å (Table I). The structure was resolved by molecular replacement using the existing TNF α apo structure (PDB code: 1A8M)¹⁴ and

pembrolizumab Fv (PDB code: 5B8C).¹⁵ The asymmetric unit contained two copies of the TNF α homotrimer and six copies of the golimumab Fv, thus providing six copies of the TNF α :golimumab interface related by non-crystallographic symmetry (NCS). The side-chain electron density was sufficiently resolved throughout the TNF α :golimumab interface [Supplementary Fig. S1(D,E)].

The three-dimensional arrangement of the biologically relevant TNF α :golimumab complex has an overall shape that resembles a bell, with the TNF α trimer and the three Fv fragments sitting at the shoulder of the bell [Fig. 1(A, left)]. In this binding orientation, the golimumab would point away from the membrane in the membrane-bound form of

TNF α , with the membrane located below the TNF α molecule in Figure 1(A, left). Therefore, golimumab not only binds the soluble form of TNF α but also may, in principle, be able to bind to TNF α before it is released from its membrane-bound precursor. This structure-based assumption is in line with a previous pharmacological data that golimumab IgG bind to membrane-bound TNF α with an apparent dissociation constant (K_D) of 1890 pM.⁶ When viewed along the intrinsic 3-fold axis of the TNF α trimer, the biologically relevant complex resembles a three-bladed propeller, with each TNF α :Fv protomer forming one blade [Fig. 1(A, right)]. The epitope important for binding and specificity is located within a single TNF α protomer, and the Fv fragments do not bridge protomers in the trimer. The pseudo 2-fold axis of the Fv fragments relating the V_H and V_L domains is not perpendicular to the 3-fold axis, and the Fv fragments are tilted $\sim 45^\circ$ out of the plane [Fig. 1(A)]. Structural alignment of the TNF α :TNFR2 complex¹⁶ with the TNF α :golimumab complex shows no major perturbations in the TNFR2 binding sites except for the DE loop of TNF α [Fig. 1(B)]. The aberrant positioning of the DE loop in the TNF α :golimumab complex might be caused by a crystallization artifact related to the packing contacts between the peripheral regions of molecules rather than a biologically relevant conformational change caused by binding of golimumab. In the crystals, the DE loop of one TNF α molecule interacts with its neighboring TNF α molecule via hydrogen bonds (Supplementary Fig. S2). Furthermore, it cannot be assumed that the DE loop, which is situated distal to the golimumab epitope, specifically undergoes allosteric conformational changes even though proximal regions exhibit no apparent shift upon golimumab binding.

Because of the high affinity of golimumab for TNF α , we assume that a stoichiometrically saturated 1:3 TNF α homotrimer: golimumab complex could be formed *in vivo*, although molecular populations of the 1:3 complex might not be dominant, which is similar to the cases of higher-order structures of TNF α :adalimumab and TNF α :infliximab complexes as recently shown by negative stain TEM and cryo-EM techniques.¹⁷ However, formation of the 1:3 complexes is not necessary for inhibition of signaling via TNFRs because this type of signaling requires multiple intact TNFR-binding sites on the TNF α homotrimer, as demonstrated by inhibition of signaling when TNF α exchanges protomers with dominant-negative TNF α variants.¹⁸

Golimumab epitope

To establish the residues that fall within the golimumab epitope on TNF α , we analyzed the interfaces of the TNF α :golimumab complex via PISA.¹⁹ All NCS copies share the same pattern of intermolecular contacts, which involve 13 TNF α residues and bury

surface areas of 905 and 997 Å² on TNF α and golimumab, respectively [Fig. 2(A)]. These are in the typical range of the interaction surface between antibodies and protein antigens.²⁰ The golimumab heavy chain contributed 781 Å² of the buried solvent-accessible area, whereas the light chain contributed 216 Å². The golimumab is bound to a discontinuous epitope composed of residues in the AA' loop (Gly24^T), C strand (Lys65^T, Gln67^T), CD loop (Ser71^T), EF loop (Glu104^T, Thr105^T, Pro106^T, Glu107^T, Gly108^T, Ala111^T), and GH loop (Arg138^T, Asp140^T, Tyr141^T) of TNF α (hereafter, residues of the TNF α and golimumab Fv heavy chain and light chain are designated by the superscript chain identifiers T, H, and L, respectively). Over 18 pairs of interactions are observed, including 11 direct hydrogen bonds between residues, at least three water-mediated hydrogen bonds, three salt bridges, one aromatic side-chain contact and hydrophobic interactions, which indicate a stable interaction network between the two proteins and may explain their high binding affinity (K_D =18 pM when measured using soluble form of TNF α and golimumab IgG).⁶ The recognition of the epitope is mainly achieved by the complementarity-determining region (CDR) loops of the heavy chain. Most prominently, residues located in the EF loop of TNF α provide a key component of the interactions, mainly through hydrogen bonds and salt bridges to the CDR-H1 (Ser31^H) and CDR-H3 (Arg98^H, Arg100^H, and Tyr111^H) of golimumab [Fig. 2(B); Supplementary Fig. S3]. Arg100^H makes the largest contribution to the TNF α :golimumab interaction and presents a binding free energy of -7.7 kcal/mol as calculated with ANCHOR.²¹ From the golimumab light chain, only two residues, Tyr30^L and Tyr32^L in CDR-L1, are involved in the interaction with Gly24^T (AA' loop) and Asp140^T (GH loop), respectively.

Notably, the TNF α residues interacting with golimumab CDRs are distinct from those that engaged TNFR2 [Fig. 2(A) and Supplementary Fig. S3]. A previous crystallographic study of the TNF α :TNFR2 complex (PDB code: 3ALQ) showed that the cysteine-rich domain 2 (CRD2) and CRD3 of TNFR2 have contacts with residues in the AA' loop (Gln21^T, Glu23^T, Arg31^T, Arg32^T, Ala33^T) and GH loop (Asp143^T) of one TNF α protomer, and the DE loop (Ser86^T, Tyr87^T, Pro90^T) of a neighboring protomer.¹⁶ None of these residues participates in polar interactions within the TNF α :golimumab complex; hence, direct competition does not occur between the binding residues of golimumab and TNFR2, which represents an idiosyncratic antagonistic feature exhibited by golimumab that is not shared with the other three anti-TNF α antibodies. The latter three antibody epitopes have overlapping portions with the binding footprint of TNFR2, including buried solvent-accessible surface areas of 26.1 Å² for

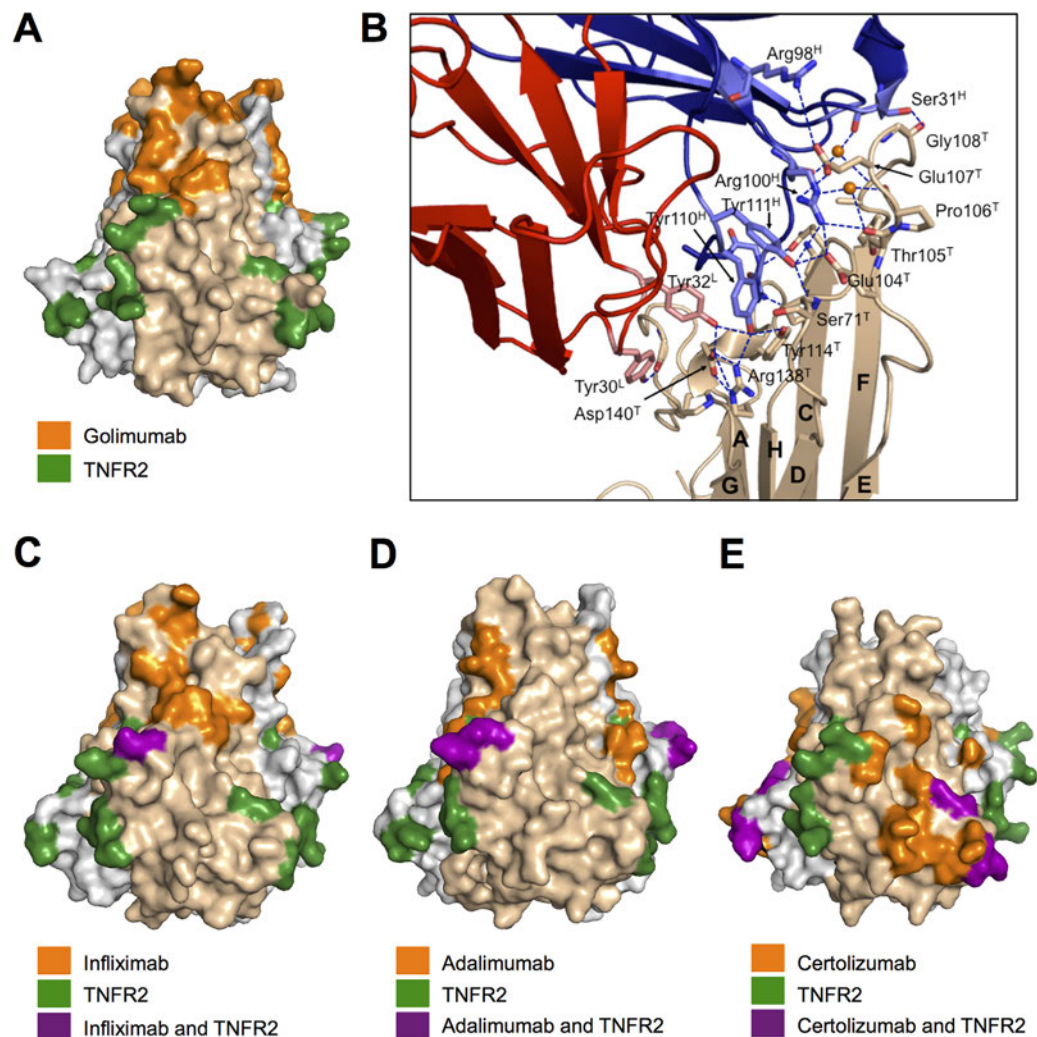


Figure 2. TNF α :golimumab contact residues and comparison with those of the other anti-TNF α mAbs. (A) Surface diagram of TNF α from the TNF α :golimumab complex structure, with the golimumab epitope highlighted in orange. One TNF α protomer is shaded tan, and the other two protomers are shown in grey. The TNFR2-binding sites are outlined in green. (B) Close-up views of interfaces. Residues involved in hydrogen bonds (blue dashes) are shown. The color-coding is the same as in Figure 1(A). Water molecules are shown in orange. Previously published epitopes of infliximab, adalimumab and certolizumab are shown in (C), (D), and (E), respectively. Residues for the polar interactions with anti-TNF α mAbs (orange) and TNFR2 (green) and the overlapping contact residues (purple) are colored differently.

infliximab [Fig. 2(C)], 211.7 Å² for adalimumab [Fig. 2(D)], and 224.9 Å² for certolizumab [Fig. 2(E)].

TNFR steric competition properties of golimumab

Because golimumab inhibition of TNFR binding could not arise from direct competition for receptor-binding residues on TNF α and/or potential golimumab-induced conformational changes in TNF α , steric clashes caused by physical overlap between the bound golimumab and TNFRs could be substantial and critical to golimumab's inhibitory activity. To gain quantitative insights into the effect of inhibitor overlap on blocking TNF α interactions with TNFRs, we calculated the theoretical volumes of atomic overlap between golimumab and TNFR2. First, we performed a structural alignment of the

TNF α :golimumab complex with the TNF α :TNFR2 complex. We then calculated the volumes of atomic overlap between the superimposed golimumab and TNFR2 (Fig. 3). Similar analyses were also performed for infliximab [Supplementary Fig. S4(A,B)], adalimumab [Supplementary Fig. S4(C,D)], and certolizumab [Supplementary Fig. S4(E,F)]. Surprisingly, golimumab exhibits a volume of steric conflict with TNFR2 (30 Å³) that is an order of magnitude smaller than that of the other three anti-TNF α antibodies (infliximab vs. TNFR2 = 1800 Å³; adalimumab vs. TNFR2 = 2090 Å³; and certolizumab vs. TNFR2 = 820 Å³).

Furthermore, the atomic overlapping volumes between anti-TNF α antibodies and TNFR1 were calculated. Because the structure of the TNF α :TNFR1 complex has not been determined, we used the

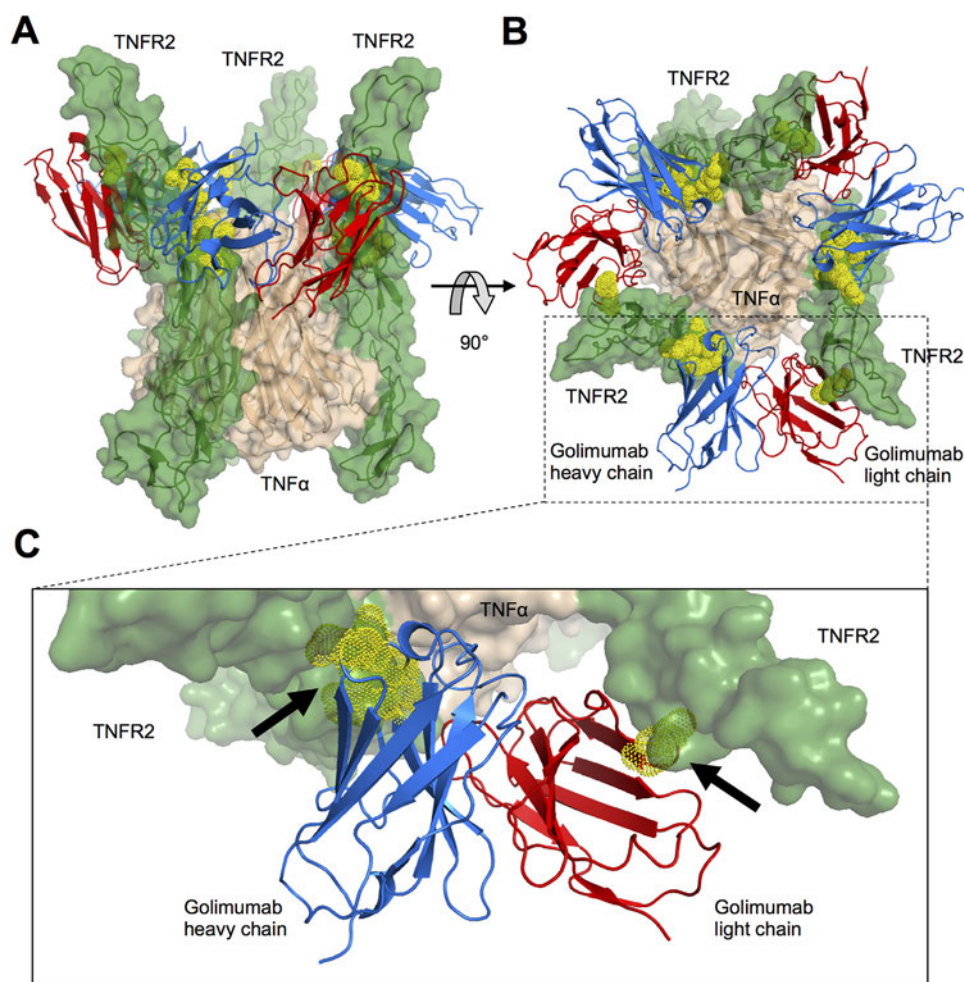


Figure 3. Structural basis of golimumab and TNFR2 competition. Structural alignment of TNF α :golimumab and TNF α :TNFR2 complexes reveals that the golimumab light chain (red) and golimumab heavy chain (blue) penetrate into the volume of TNFR2 (green), which would allow golimumab to block TNFR2 binding. The aligned structure is rotated by 90° around the horizontal axis in (A) and (B). (C) Close-up view of the area shown by the box in dotted lines in (B) showing the steric conflicts (black arrows). Golimumab residues responsible for the steric conflict are shown by dot diagram in yellow.

structure of the lymphotoxin- α (TNF β):TNFR1 complex (PDB code: 1TNR)²² as a template to generate a model of TNF α :TNFR1 complex, which was then superimposed with the TNF α :antibody complexes. This analysis revealed that significant steric clashes occur between golimumab and TNFR1 (360 Å³) (Fig. 4), although they are smaller than those of the other three anti-TNF α antibodies (infliximab vs. TNFR1 = 1820 Å³; adalimumab vs. TNFR1 = 2110 Å³; and certolizumab vs. TNFR1 = 530 Å³) (Supplementary Fig. S5). This steric conflict between golimumab and TNFR1 underlies, in part, the therapeutic efficacy of golimumab, which has strong inhibitory effects on TNF α -binding to TNFR1 and presents an IC₅₀ of 3.9 ng/mL as previously described.⁶

Interestingly, golimumab's physical overlapping volumes with the two TNFRs are drastically different. Such a disparity is not observed for the other three anti-TNF α antibodies. However, given that even a small overlapping volume between the

receptor and the antibody can cause steric repulsion, it is clear that golimumab exhibits competitive inhibitory action against both TNFR1 and TNFR2. Previous functional analyses of TNFRs have demonstrated the predominant role of TNFR1 in the pathogenesis and exacerbation of inflammation.^{23,24} Moreover, TNFR2 has been shown to be crucial for the proliferation, activation and antigen presentation of T-cells, which are essential in cell-mediated immune responses against bacteria and viruses.²⁵ Blocking all effect of TNFRs signaling can therefore be counter-productive. Clinical trials have assessed the side effects of golimumab treatment and reported an increased risk of infections, reactivation of tuberculosis, invasive fungal infections, and other opportunistic infections.^{26,27} These adverse drug reactions can be explained in part by our finding that golimumab sterically hinders TNFR2 as well as TNFR1 from associating with TNF α (Figs. 3 and 4).

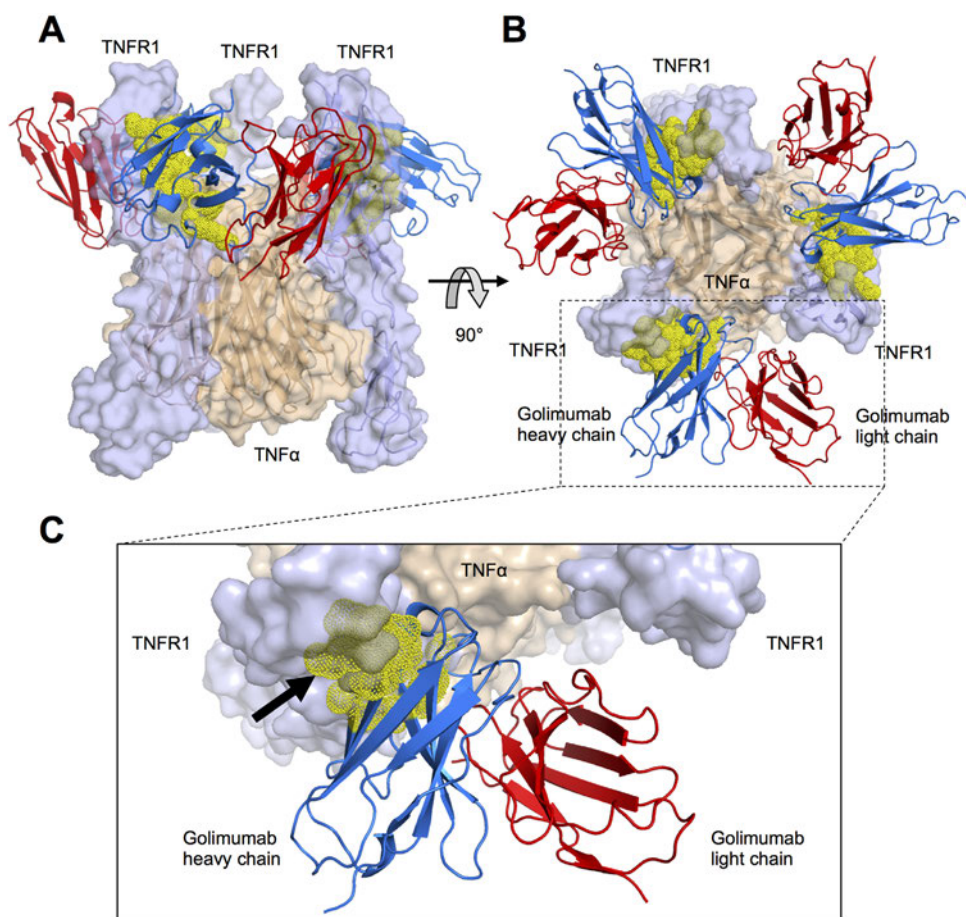


Figure 4. Structural model for golimumab and TNFR1 competition. Structural alignment of the TNF α :golimumab complex and the TNF α :TNFR1 complex model reveals that the golimumab heavy chain (blue) penetrates into the volume of TNFR1 (light blue), which would allow golimumab to block TNFR1 binding. The aligned structure is rotated by 90° around the horizontal axis in (A) and (B). (C) Close-up view of the area shown by the box in dotted lines in (B) showing the steric conflicts (black arrow). Golimumab residues responsible for the steric conflict are shown by dot diagram in yellow.

In summary, structural analysis of the TNF α :golimumab complex and its comparison to the TNF α :TNFR2 complex revealed that the binding footprints of golimumab and TNFR2 on TNF α do not overlap at all; however, steric clashes between the bound golimumab and TNFRs are critical to its inhibitory activity. Golimumab does not induce conformational changes in TNF α that could affect receptor binding. This mode of action of golimumab is different from that of the other currently approved anti-TNF α therapeutic antibodies, infliximab, adalimumab, and certolizumab pegol. Our findings may provide structure-based insights to guide engineering efforts to create even more efficient versions of anti-TNF α biologics.

Materials and Methods

Complete amino-acid sequence of the expression constructs

The cloned *Homo sapiens* TNF α sequence contains residues 77–233 of the 233 total residues (UniProt accession number: P01375), with the FLAG-tagged

at the N-terminus underlined and additional C-terminal residues retained after restriction site cloning or tobacco etch virus (TEV) cleavage shown in italics (refer to the following section for cloning details):

DYKDDDDKVRRSSRTPSDKPVAVHVVANPQAEG
QLQWLNRRANALLANGVELRDNQLVVPSEGLYLIY
SQVLFKGQGPCSTHVLLTHTISRIAVSYQTKVNLLS
AIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGD
RLSAEINRPDYLDFAESGQVYFGHIALTSENLYFQ

The golimumab light chain variable region (V_L), with the additional N- and C-terminal residues retained after restriction site cloning or TEV cleavage shown in italics is as follows:

AGSEIVLTQSPATLSLSPGERATLSCRASQSVYS
YLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSG
SGTDFTLTISSLEPEDFAVYYCQQRSNWPPFTFGP
GTKVDIKTSENLYFQ

The golimumab heavy chain variable region (V_H), with the additional N-terminal residues retained after restriction site cloning or TEV cleavage shown in italics is as follows:

SKLQVQLVESGGGVVQPGRSLRLSCAASGFISS
YAMHWVRQAPGNGLEWVAFMSYDGSNKKYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARD
RGIAAGGNYYYYGMDVWGQGTTVTVSS

Protein expression and purification

The soluble form of TNF α was produced by secretion from the Gram-positive bacterium *Brevibacillus choshinensis*.¹³ The artificially synthesized codon-optimized cDNA of TNF α was inserted downstream of and in frame with the secretion signal sequence of the plasmid pNY326 (Clontech). To facilitate the detection and purification of the secreted proteins, sequences for the TEV protease cleavage site and a His₆ tag were placed at the C-termini of the TNF α cDNAs. *B. choshinensis* cells harboring the TNF α -expression plasmid were grown at 30°C with shaking at 200 rpm in 2SY medium (soytone 40 g/L, yeast extract 5 g/L, glucose 20 g/L, and CaCl₂ 0.15 g/L) supplemented with 50 mg/L neomycin for 65–70 h. The recovered culture supernatant was adjusted to a final ammonium sulfate concentration of 60% saturation. The precipitate was pelleted, dissolved in TBS buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl), and dialyzed overnight against the same buffer. The dialyzed sample was purified with Ni-NTA resin, mixed with TEV-His₆ and dialyzed overnight again against TBS buffer. The cleaved His₆ tag and TEV-His₆ were removed using a HisTrap column. The flow-through fractions were further purified with a HiLoad 16/60 Superdex 75 column (GE Healthcare).

The golimumab Fv was expressed and purified using the iRAT system as previously described.¹³

Crystallization

The protein complex was prepared by incubating TNF α with golimumab Fv at a molar ratio of 1:6 for 1 h on ice. The complex was subjected to size exclusion chromatography (Superdex 200 10/300 column, GE Healthcare). Peak fractions containing the TNF α :golimumab Fv complex were concentrated to approximately 10 mg/mL by ultrafiltration (Millipore, MWCO 50 kDa) and used for crystallization experiments. The crystals of the complex used for structural determination were grown at 20°C by hanging drop vapor diffusion. A 400 μ L reservoir containing 0.1 M sodium acetate (pH 4.6) and 1.4 M NaCl was equilibrated against a 2 μ L drop containing a 1:1 mixture of the complex and reservoir solution. After 14–21 days of growth, the crystals were cryo-protected in 25% ethylene glycol in the mother liquor and then flash-frozen in liquid nitrogen.

Data collection, structure determination and analysis

The diffraction data were collected at 100 K at the SPring-8 beamline BL41XU (Japan) using a PILATUS3

6M detector. The data were then integrated and scaled using XDS.²⁸ The structure of the TNF α :golimumab Fv complex was determined by molecular replacement with the program MR-PHASER²⁹ using the atomic coordinates of the human TNF α apo structure (PDB code: 1A8M) and the pembrolizumab Fv (PDB code: 5B8C) as the search models. The model was further rebuilt in COOT³⁰ and refined with phenix.refine³¹ (Table I). In the final Ramachandran plot, 95.6% and 4.1% of residues were in the favored and allowed regions, respectively. The refined structures were visualized with PyMOL (<http://www.pymol.org/>). The PISA server¹⁹ was used to identify protein-protein interactions and estimate the solvent-accessible surface area. The model complex of TNF α :TNFR1 was constructed based on the structural alignment of the LT α (TNF β):TNFR1 complex (PDB code: 1TNR)²² and the TNF α :TNFR2 complex (PDB code: 3ALQ)¹⁶ using a superimposing program in UCSF Chimera.³² The volumes of physical overlap of each anti-TNF α antibody with TNFRs were calculated by the volumes tool in Chimera.

Acknowledgments

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Conflicts of Interest Statement

The authors declare no conflict of interest associated with this manuscript.

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