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ORIGINAL ARTICLE

Structure-based development and optimization of therapy antibody drugs against TNFα

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Abstract Previously, we reported on the crystal structures of the Fab fragments of two food and drug administration approved therapeutic antibodies, Infliximab and Adalimumab, in complex with TNFα. The structurally identified epitopes on TNFα reveal the mechanism of TNFα inhibition by partially overlapping with the TNFα-receptor interface. In this study, we launched a screen of a phage display library to isolate novel anti-TNFα antibodies based on the adalimumab epitope. Structural analysis, the phage display antibody isolation technology, step-by-step antibody optimization, complementarity-determining region residues random mutagenesis, phage ELISA, binding affinity characterization, and cell signaling assays were used for the development and optimization of the novel anti-TNFα antibodies. Moreover, one of the novel antibodies, hAta09, has a superior inhibitory effect on TNFα function and signaling. Taken together, our report established that the novel anti-TNFα antibody hAta09 may achieve clinical efficacy in a TNFα-associated disease.

Keywords TNFα · Targeted therapy · Adalimumab · Epitope · Antibody optimization

Introduction

Targeted therapies have started changing substantially not only basic insights into but also the clinical practice of a number of human diseases, especially in the field of oncology, chronic inflammatory diseases, transplantation, infectious diseases and cardiovascular medicine (Hutchinson 2014). Although the first generation of targeted agents are thought of as low molecular weight inhibitors that could be administered orally, the most effective drugs now involve

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the use of biological macromolecular drugs, such as antibodies, that direct modulate targets expressed on the cell surface or circulated in the blood, such as trastuzumab or pertuzumab (Baselga et al. 2012) in the usage for breast cancer and infliximab (Shealy and Visvanathan 2008) for Ankylosing spondylitis. As monoclonal antibodies were typically produced in mice, the general approaches that are expected to reduce immunogenic in humans could humanize murine antibodies by grafting complementarity-determining regions (CDRs) from the desired mouse antibody onto a recombinant human immunoglobulin backbone; it is also possible to use rodents partially reconstituted with human immunoglobulin genes, human immune cells, or combinatorial phage or yeast display libraries, which can yield high-affinity antibodies without requiring animal immunization at all (Carter 2006).

A high success rate from the first use in humans to regulatory approval has been reported for antibody drugs: 29 % for chimeric antibodies and 25 % for humanized antibodies (Reichert et al. 2005). These data provide the strengths of antibody therapeutics compared with other drug forms; for example, the success rate for small-molecule drugs is approximately 11 % (Kola and Landis 2004). Moreover, the antibody drug is well tolerated by humans based on current usage in clinics, although first-dose reactions are common but usually are slight and well managed. However, much of the development and clinical experience of antibody drugs were from the generation and optimization of one antibody to other antibodies, which is also the reason for the low risks of the newly developed or optimized antibody drugs, and the clinical potential can also readily be increased by improving antibodies' existing properties by endowing them with new activities.(Carter 2001; Presta 2002).

Developing an antibody requires several key steps. The selection of the target antigen is the initial step, which was reviewed elsewhere (Carter et al. 2004). In this step, the concept for therapeutic intervention using an antibody must be carefully checked based on knowledge about the target, including its role in the pathobiology of the disease. This knowledge leads to the antibody design principle, which makes use of crucial or desirable properties to achieve the desired clinical outcome. Next, these principles can help guide the method of antibody screening and generation, which was then followed by identifying the antibodies' desired properties using suitable assays. Panels of candidate antibodies were then evaluated in a number of in vitro/vivo assays to test their therapeutic effects as well as their potency and safety. Antibody optimization and refinement would be the next step to meet the designing standard, and the direct generation of novel antibodies using optimization methods from existing drugs would also be necessary to obtain new clinical candidate antibodies. The repeated optimization and refinement of the candidate antibodies is led by a series of in vitro and in vivo data and then subjected to further preclinical and, if warranted, clinical development.

Previously, we solved the crystal structure of the trimetric TNFα in complex with the therapy antibody infliximab (Liang et al. 2013). We then carefully compared this structure data with our newly solved crystal structure of the TNFα-Adalimumab complex (Hu et al. 2013). Moreover, in our recent report, we have employed the structural comparison data in the antibody screen from the human B cell phage display libraries to help characterize different antibodies (Fu et al. 2014) and used the structural information of the EGFR-cetuximab complex to help find novel EGFR-specific antibodies (Hu et al. 2015). All these data point to the concept that structural observation and data could be used in antibody development and optimization.

Materials and methods

Cell lines, antibodies, and animals

All cell lines were obtained from the American type culture collection (ATCC. Manassas, VA). All cell lines were authenticated twice by morphologic and isoenzyme analyses during the study period. The cell lines were routinely checked for contamination by mycoplasma using Hoechst staining and were consistently found to be negative. The cells were cultured in a DMEM medium supplemented with 10 % fetal calf serum (FCS) in 5 % CO_2 at 37.8 °C in a humidified incubator.

Phage display libraries and selections

Non-immunized human single-chain variable fragment (scFv) phage display libraries were used for lead-scFv isolation. TNFα, at 10 μg ml^{-1} in phosphate buffered saline (PBS), was immobilized on immunotubes (Nunc, Naperville, IL), and TNFα-binding phage was isolated by three sequential rounds of panning (Nakano et al. 2001; Quesnelle and Grandis 2011). The optimized variants were isolated by selection from randomized libraries in solution using biotinylated TNFα captured on streptavidin-coated paramagnetic beads (Dynal, New Hyde Park, NY) (Zhang et al. 2012). The antibody VH CDR3 randomized repertoires were constructed by polymerase chain reaction (PCR) using mutagenic oligonucleotides to replace the last 6 VH CDR3 amino acids with randomized codons. The mutated DNA for both lineages was ligated into the phagemid vector pCANTAB 6 and electroporated into *Escherichia coli* TG1 (Ren et al. 2009). Libraries of 6×10^9 individual clones were generated and immobilized by TNFα, followed by ten rounds of soluble selection

using decreasing concentrations of biotinylated TNFα from 50 nM to 100 pM.

Construction, expression and purification

Residues 77–233 of the human TNFα and the heavy and light chains of the antibody were cloned as described previously (Hu et al. 2014). Antibody screening for a human anti-TNFα antibody is discussed below. The recombinant protein and antibody were purified through protein A affinity chromatography from the serum-free culture supernatant. The antibody concentrations were determined by absorbance at 280 nm, and the purity was confirmed through SDS–PAGE analysis and western blot.

Competitive binding assay

TNFα-expressing cells at 1×10^7 cells ml^{-1} were incubated with a subsaturating concentration of the indicated FITC-conjugated anti-TNFα and increasing concentrations of purified competing antibodies for 1 h at 4 °C. Then, the cells were washed and analyzed by flow cytometry using a FACS can flow cytometer (Becton–Dickinson, San Jose, CA).

Affinity measurement

The affinities of anti-TNFα antibodies for recombinant TNFα were determined as described previously (Citri and Yarden 2006). Briefly, each mAb was incubated with increasing concentrations of recombinant TNFα for an hour. The concentration of free antibody was then measured by ELISA using immobilized recombinant TNFα and was used to calculate the affinity (Kd).

Pharmacokinetics

Groups of 6-week-old BALB/c mice were injected with 5 mg kg^{-1} body weight of anti-TNFα mAb via the tail vein. Blood samples were taken every day from day 1 to day 20 by retro-orbital bleeding and collected in tubes coated with heparin to prevent clotting. Six mice were used every time, and each mouse was bled only once. After centrifugation to remove the cells, the plasma samples were stored at −80 °C until analysis. Serum concentrations of anti-TNFα mAbs were measured by competitive ELISAs. Briefly, serial dilutions of the serum samples were incubated with a subsaturating concentration of adalimumab-biotin on TNFα-coated ELISA plates at 37 °C for 1 h. Detection was carried out with alkaline phosphatase-conjugated avidin. The PK parameters were calculated using a noncompartmental analysis.

TNFα mutagenesis and antibodies binding

TNFα site-directed mutants (D32A, R33A, N34A, N36A, R82A, I83A, V85A, S86A, F144A, E146A, and S147A) were created through PCR. All mutations were confirmed by automated DNA sequencing. One μg ml^{-1} of wild-type TNFα-WT or TNFα mutants were added to 96-well plates precoated with 5 μg/ml of adalimumab, followed by incubation at 37 °C for 1 h. The plates were washed, and different concentrations of antibodies or the control antibody were added to each well and incubated at 37 °C for 1 h. After washing, the HRP-conjugated goat polyclonal secondary antibody was added to mouse IgG–H&L, and the plates were further incubated for 1 h at 37 °C. Finally, TMB was added as a substrate, and the absorbance was read at 450 nm.

Immunoblotting

The cells were incubated with the indicated antibodies in a serum-free medium for 30 min at 37 °C. The cells were then treated with TNFα treated for 30 min. After washing, the cells were lysed in an SDS lysis buffer, and the cell lysates were subjected to SDS-PAGE and immunoblotted with antibodies against the indicated protein.

Phage display peptide library screening

The Ph.D.-7 phage display peptide library (PDPL) kit was purchased from New England BioLabs (Beverley, MA). Biopanning of the PDPL with mouse anti-TNFα mAbs was performed according to the manufacturer's instructions. Phage clones were isolated by panning the Ph.D.-7 PDPL with hAta09. After three rounds of panning, the reactivity of the positive phage clones with antibodies was measured by ELISA. All antibody positive phage clones were subjected to sequence analysis.

Phage ELISA

ELISA screening of the phage clones was performed as previously reported (Tao and Maruyama 2008). Briefly, 150 μl of supernatant containing amplified particles from each phage clone were added to 96-well plates precoated with mouse anti-TNFα mAbs. After incubation for 2 h at RT, detection was carried out with HRP-conjugated anti-phage M13 mAb (GE Healthcare). Finally, the positive phage clones were subjected to DNA sequence analysis.

Statistical analysis

Student unpaired t tests were used for the statistical analysis to identify significant differences, unless otherwise

indicated. Differences were considered significant at $P < 0.01$.

Results

Structural insights into antibody inhibitory for the interface data of the antigen–antibody interface

It is well established that antibodies' epitope and their binding interface with the antigens are closely connected with their biochemical properties and inhibitory functions. For instance, as described in our previous report, the anti-TNFα antibodies infliximab and adalimumab have adjacent but different epitopes on TNFα. Infliximab's epitope, which is mainly constituted by the E–F loop of TNFα, did not overlap with the TNFR2 interface, whereas adalimumab directly obstructed the TNFR2 binding interface (Fig. 1). Moreover, a number of residues of TNFα at the TNFα-TNFR2 interface do participate at the TNFα–adalimumab interface. Some of them even play significant role in the strength of the binding affinity, such as $Q21_{TNF}$, $E23_{TNF}$, $E146_{TNF}$ and $Y115_{TNF}$ (Hu et al. 2013) (Fig. 1b). In light of several other preclinical and clinical data, it is possible that an antibody whose epitope directly blocks the receptor binding interface, such as TNFR2-TNFα, may have a better and more predictable clinical effect. Under such a concept, it seems that even adalimumab was not the 'perfect' antibody to block TNFα because it only covers approximately 56 % of the TNFα/receptor binding interface, although it performed better than infliximab in our competitive experiments.

However, the D–E loop and G–F loop of TNFα are free of adalimumab binding, which also played key roles in TNFα binding to its receptor and its inflammatory activation function (Eck and Sprang 1989), which also highlights an opportunity for improvement in the backbone of adalimumab. V85, S86, Y87, and T89 at the D–E loop as well as K128 at the G–F loop all contribute significantly to the receptor binding and TNFα function, but they were not involved in the TNFα–adalimumab interface (Fig. 1a). Thus, the development of novel antibodies may be feasible.

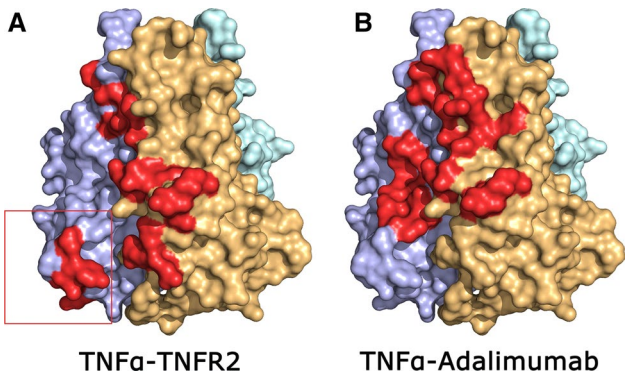

Fig. 1 Comparison of the interface between TNFα and receptors and Adalimumab. TNFα from the complex structures is represented as a colored surface with TNFR2 (**a**) and the adalimumab interface (**b**), highlighted in *red* at one of the three interfaces on the TNFα trimer, which are colored *light orange*, *pale blue* and *pale cyan*. The D–E loop and the G–F loop region are framed (color figure online)

Development of anti-TNFα antibodies

We first use the non-immunized repertoires of human scFv fragments displayed on phage (Hu et al. 2015) for the development of novel anti-TNFα antibodies. Recombinant TNFα were used for the identification of TNFα-binding scFv fragments. To take advantage of the epitope of adalimumab, we randomly combined a light or heavy chain of adalimumab with the heavy or light chain of the isolated scFv fragments. Thus, 565 candidate hybrid candidate scFv fragments were selected for further analysis. The binding affinity to TNFα was determined and 21 hybrid candidate scFv fragments were finally identified with a similar binding affinity to TNFα, comparable with adalimumab.

Optimization of anti-TNFα antibodies by random selection of CDR2 and CDR3 at the V region

Usually, the affinity to a specific antigen for antibodies isolated from the non-immunized phage libraries is relatively low. Thus, the scFv fragments were optimized to obtain better antibodies, especially to CDR2 or CDR3, which normally dominate the main contribution of the affinity. To increase diversity within the VH and VL domains, which directly corresponds to the binding affinity, randomized libraries were generated from all the hybrid candidate scFv fragments in which the terminal six residues of the VH CDR2 or CDR3 or the terminal six residues of the VL CDR2 or CDR3 were randomly replaced. The refined scFvs were next selected in the TNFα binding library. The binding affinity of the selected scFvs to TNFα was determined, and scFvs with an improved affinity were identified. All these scFv fragments were sequenced and constructed to full IgG molecules (IgG1, κ), which were termed as hAta01~hAta14 (Fig. 2).

Characterization of anti-TNFα antibodies

The protein molecular weights of the anti-TNFα full IgGs were checked by SDS-PAGE. Every antibody has two protein bands with molecular masses of ~55 kDa (heavy chain) and ~25 kDa (light chain) in the reducing condition and one band at ~150 kDa under the non-reducing condition. These data implied that these antibodies have a native

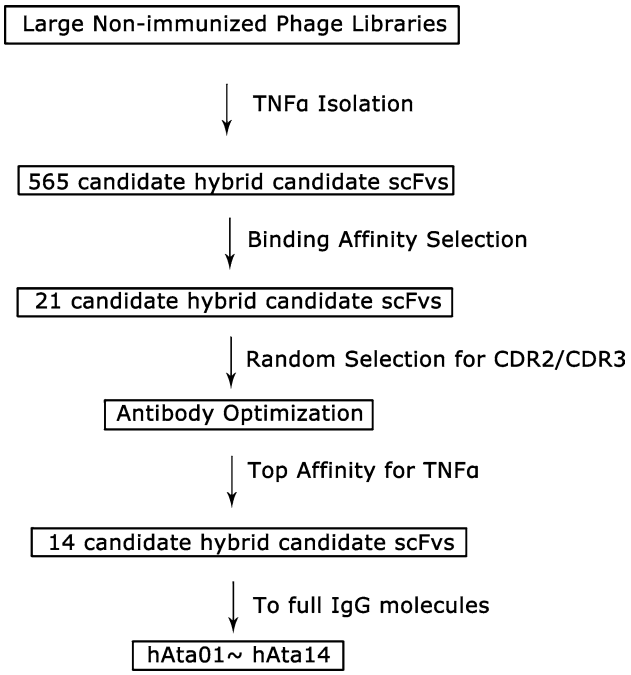

Fig. 2 Schematic representation of the technological process of the development and optimization of novel antibodies against TNFα

IgG-like format, composing of two heavy chains and two light chains linked with disulfide bonds. Next, we used the competitive binding assays to compare these antibodies with infliximab and adalimumab for the epitope control (Fig. 3). In this competitive binding assay, hAta01–hAta07, hAta11, hAta13, and hAta14 all effectively competed with adalimumab or infliximab for TNFα, while hAta008 and hAta12 did not compete with adalimumab or infliximab for TNFα. Moreover, hAta09 had an interesting compete curve compared with adalimumab but not to infliximab, while hAta10 had a unique compete curve for both adalimumab and infliximab, indicating that these antibodies may have a unique epitope or functionary profile. Next, the enzyme-linked immunosorbent assay (ELISA) was used to determine the affinity constant (Kd) of these anti-TNFα antibodies for recombinant TNFα. As shown in Table 1, anti-TNFα antibodies have a similar affinity compared with that of adalimumab and infliximab. Interestingly, the antibodies hAta09 and hAta12 have a higher affinity than those of adalimumab and infliximab.

Next, a single dose was administered intravenously to the mice to determine the serum concentrations of the antibodies with ELISA to measure the pharmacokinetics (PK) of the anti-TNFα antibodies. Briefly, we identified the antibodies in mice serum that would bind to TNFα immobilized on ELISA plates. As shown in Table 2, the main PK parameters of the anti-TNFα antibodies in mice were very close to those of adalimumab (Salfield et al. 1998; Weisman et al. 2003), suggesting that the anti-TNFα antibodies had PK properties similar to those of a conventional IgG molecule, which are highly stable in vivo.

Epitope shifts of anti-TNFα antibodies play a significant role in their ability to block TNFα signaling

To better distinguish these antibodies, the antibody inhibitory activity was measured in a cytotoxicity inhibition assay using L-929 mouse fibro-sarcoma cells in the presence of actinomycin D and TNFα. The inhibition effect was evaluated by MTT assays. Consistent with previous reports and clinical data, adalimumab has a stronger TNFα blocking effect than infliximab. Interestingly, our data showed that among all the anti-TNFα antibodies, hAta09 had the best TNFα inhibition activity, significantly better than adalimumab, and hAta12 also had better TNFα inhibition

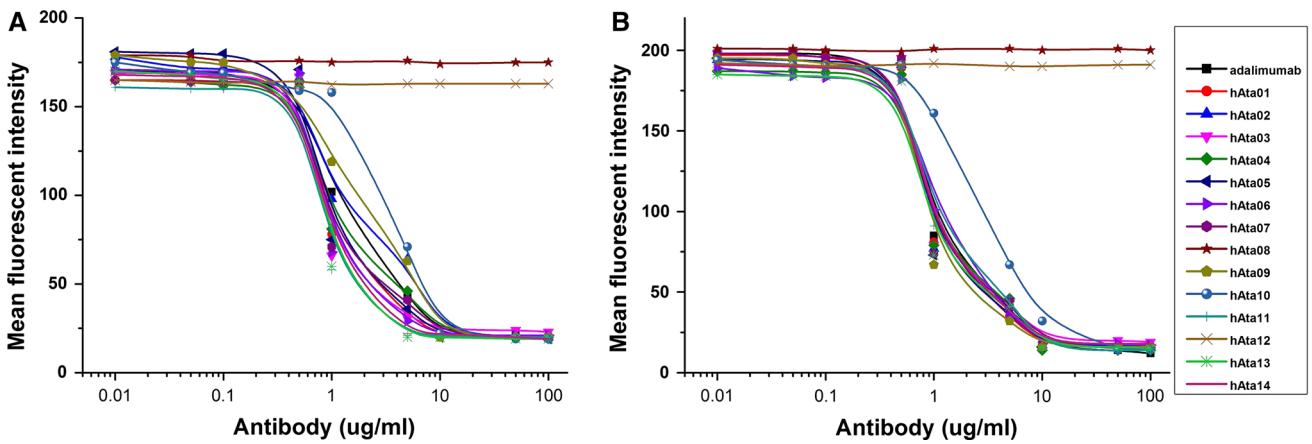

Fig. 3 Characterization of anti-TNFα antibodies for antigen binding. Competitive binding assay. Anti-TNFα antibodies were evaluated for their ability to compete with adalimumab-FITC (**a**) or infliximab-FITC (**b**) for binding to immobilized TNFα

Table 1 Binding affinities of recombinant anti-TNFα antibodies for TNFα

Antibody	Affinity[a] [K_D(M)]
adalimumab	$6.215 \pm 0.336 \times 10^{-10}$
infliximab	$2.661 \pm 0.516 \times 10^{-10}$
hAta01	$3.411 \pm 0.658 \times 10^{-10}$
hAta02	$1.229 \pm 0.638 \times 10^{-8}$
hAta03	$4.331 \pm 0.441 \times 10^{-9}$
hAta04	$6.551 \pm 0.247 \times 10^{-9}$
hAta05	$1.152 \pm 0.812 \times 10^{-10}$
hAta06	$2.058 \pm 0.812 \times 10^{-9}$
hAta07	$7.124 \pm 0.217 \times 10^{-9}$
hAta08	$2.591 \pm 0.481 \times 10^{-9}$
hAta09	$2.481 \pm 0.316 \times 10^{-11}$
hAta11	$2.559 \pm 0.448 \times 10^{-10}$
hAta12	$1.518 \pm 0.457 \times 10^{-11}$
hAta13	$0.991 \pm 0.248 \times 10^{-10}$
hAta14	$2.633 \pm 0.748 \times 10^{-8}$

[a] Affinity was measured by ELISA. Data are representative of six independent experiments

Table 2 Pharmacokinetic parameters of recombinant anti-TNFα antibodies in mice

Parameter[a]	Adalimumab-biotin-based competitive ELISA	
	Adalimumab	HAta01–14
AUC (day × μg × ml^{-1})	391.58	364.67 ± 33.51
$T_{1/2}$ (day)	11.23	9.55 ± 0.83
CL (ml × day^{-1} × kg^{-1})	22.1	19.2 ± 3.31
V_{SS} (ml × kg^{-1})	58.55	62.23 ± 12.23

AUC area under the concentration versus time curve, $t_{1/2}$ half-life, *CL* clearance, *VSS* steady-state volume of distribution

[a] Pharmacokinetic parameters were calculated using a noncompartmental analysis

activity than adalimumab. Next, we used the cell signaling assays to evaluate the anti-TNFα antibodies hAta09, hAta12, adalimumab and infliximab. To assess the inhibitory function of TNF-α mediated JNK activation, we pretreated peripheral blood mononuclear cells (PBMC) with anti-TNFα antibodies before TNFα activation. Interestingly, although all the antibodies could bind directly to TNFα, we observed different inhibitory effects of these antibodies. Prior to TNFα stimulation, adalimumab and hAta12 inhibited the phosphorylation of JNK/c-Jun with an IC50 value of 2.55 and 1.94 μg ml^{-1}, while infliximab inhibited the phosphorylation of JNK/c-Jun with an IC50 value of 1.77 μg ml^{-1} (Fig. 4). However, hAta09 inhibited the phosphorylation of JNK/c-Jun with an IC50 value

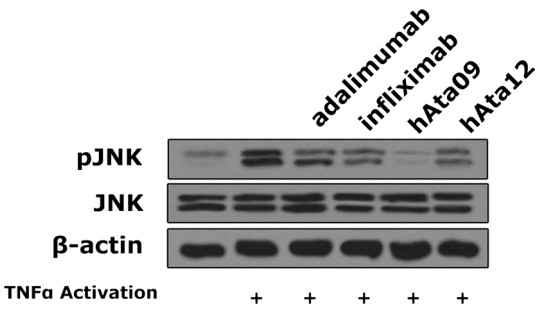

Fig. 4 hAta09 has superior anti-TNFα activity in cell signaling assay. Immunoblots examining the ability of 1 μg ml^{-1} of the indicated antibodies to inhibit JNK signaling. *Western blotting* for p–c-Jun N-terminal kinase (*JNK*) and JNK are shown

of 0.33 μg ml^{-1}, suggesting that it had the best inhibitory function for TNF-α mediated JNK activation.

Although hAta09 was developed based on the sequence of adalimumab, the epitope of hAta09 had the possibility to change. We next explored the epitope recognized by hAta09 with the random peptide display library as well as TNF-α mutagenesis study. Four sections of the Ph.D.-7 phage display peptide library were selected, and peptide ELISA was used to determine the binding of the candidate phage clones to hAta09 after selection. Three distinct amino acid sequences were identified with a sequence analysis of the YAH627-positive phage clones (Table 3). Remarkably, after carefully checking these sequences with the TNFα sequence, these consensus motifs can be aligned with the a–a′ loop, d–e loop and g–h loop of TNFα. Next, the mutagenesis and ELISA characterization of hAta09 binding to recombinant TNFα showed that binding was detected for residues R33, N34, R82, V85, S86, F144, and S147 of the TNFα construct, which conformed to the epitope of hAta09 (Fig. 5). However, compared with Adalimumab, the c–d loop was not directly covered in the epitope of hAta09, although these loops did not have a precise receptor activation function.

Discussion

TNFα is an inflammatory cytokine that is predominantly produced by activated macrophages and lymphocytes. This cytokine plays a key role in acute inflammation and is responsible for a diverse range of signaling events within cells that lead to necrosis or apoptosis (Carter et al. 2001; Idriss and Naismith 2000). During the past decades, preclinical and clinical studies have demonstrated that the inhibition of TNFα plays a central role in inflammation and is a validated and favorable method for treating several important human diseases, especially in TNFα-associated autoimmune diseases. Based on this situation, a number

Table 3 Epitope mapping results from phage display

Consensus motif	Corresponding segments of TNFα	Location of motif
DCNPN	(32) DRNAN (36)	A–A′ loop
IIAAS	(82) RIAVS (86)	D–E loop
FAESE	(144) FAESG (148)	G–H loop

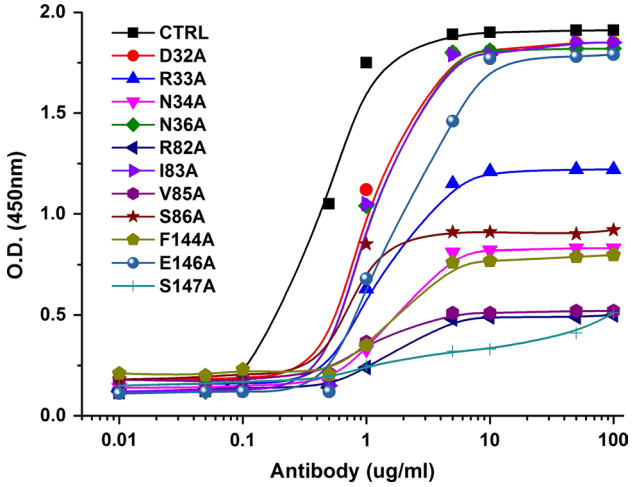

Fig. 5 The epitope of hAta09. Effect of the alanine substitutions of the residues at the binding interface on hAta09 binding to TNFα

of therapeutic biological macromolecular drugs, most of which are based on an antibody scaffold, have been developed and are being applied with great success in clinics (Wiens et al. 2010).

Effective communication with TNF receptors was required for the correct functioning of TNFα. Currently, there are two identified distinct TNFRs, TNFR1 and TNFR2 (Chan et al. 2000). The direct binding information was disclosed and revealed the mechanism of TNFRs' function, which makes the intervention of the TNFα function feasible. Three generations of reagents, including Etanercept (a soluble TNFR2-Fc chimera), Infliximab (a mouse–human chimeric mAb), and Adalimumab (a fully humanized mAb), have been approved by the US Food and Drug Administration (FDA) (Hasegawa et al. 2001). To address the molecular mechanism of how Infliximab and Adalimumab inhibit the TNFα function, we previously solved the crystal structures of TNFα in complex with the Infliximab and Adalimumab Fab fragments. Although the two complexes are obtained in different space groups, i.e., TNFα–Infliximab Fab in H3 and TNFα–Adalimumab Fab in I312, there is only one complex molecule in the asymmetric unit. However, both structures revealed a central TNFα trimer bound by three symmetrically arranged Infliximab Fab or adalimumab Fab molecules related through a crystallographic three-fold axis. The Infliximab Fab interacts with only one TNFα molecule of TNFα trimer in the complex structure through a highly complementary interface, in which the total buried surface area is 1977 $Å^2$ with a high shape complementarity (Sc) value 14 of 0.72. The epitope on TNFα is primarily composed of the C–D and E–F loop residues, as well as several key residues in strands C and D, which interact with CDRs in the heavy chain of the Infliximab Fab.

Unlike the interaction between Infliximab Fab and TNFα, Adalimumab Fab is in contact with two adjacent TNFα promoters of a TNFα trimer. The epitope of Adalimumab Fab on TNFα is composed of a number of discontinuous segments. The CDRs L2 and H2 of Adalimumab Fab play a central role in the interaction with the residues from strands A and C and the E–F and G–H loops of the TNFα molecule, while CDRs L1, L3, H1 and H3 make additional contributions (Fig. 2b). $_{Ada}$D1 and $_{Ada}$R93 of CDR L3 form three hydrogen bonds and a salt bridge with $_{TNFα}$P20, $_{TNFα}$Q21 and $_{TNFα}$E23 at the beginning of strand A and the A–A' loop of TNFα. Moreover, CDR L1 and L2 of Adalimumab Fab additionally contribute to the interactions with strand C and the E–F loop of TNFα. However, two loops that play a significant role in the TNFα function are not within both of the antibodies. All in all, the structural evidence concerning the TNFα–Infliximab Fab and Adalimumab Fab interfaces provides direct information for the understanding of the precise epitopes and working mechanism of these two widely used anti-TNFα mAbs. The structures could facilitate efforts to optimize anti-TNFα mAbs drugs with higher efficacy and lower adverse effects.

In the present study, we employed structural analysis, the phage display antibody isolation technology, step-by-step antibody optimization, CDR residues random mutagenesis, phage ELISA, binding affinity characterization, and cell signaling assays to develop a novel antibody to TNFα. We provide evidence that an optimized antibody, hAta09, generated based on the backbone of adalimumab, has a better inhibitory effect on TNFα function. To our knowledge, this is the first study to describe such a method to develop and optimize an anti-TNFα antibody. Interestingly, hAta09 was more active than all anti-TNFα antibodies in all in vitro models and biochemical assays. In a conclusion, our data greatly support the hypothesis that the structural information could be used to develop and optimize novel antibodies. The unique potential of hAta09 to have the best affinity and inhibitory effect warrants its consideration as promising anti-TNFα therapies in the clinic.

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Conflict of interest The authors declare that they have no conflict of interest.

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