Distinct Binding Mode of BAFF antagonist antibodies Belimumab and Tabalumab, analyzed by computer simulation

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Research Article

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Posted Date: October 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-974192/v1

Distinct Binding Mode of BAFF antagonist antibodies Belimumab and Tabalumab, analyzed by computer simulation

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Abstract: B-cell activating factor (BAFF) can binding to specific receptors and activate signaling pathways associated with B cell activation. Belimumab and tabalumab are anti-BAFF monoclonal antibodies, while belimumab was proved by FDA in 2011 as a targeted therapy for systemic lupus erythematosus (SLE) and showed better clinical effect than tabalumab. The combination modes of BAFF-belimumab complex and BAFF-tabalumab complex were simulated to better understand the reason for the difference of the inhibition effect between belimumab and tabalumab. The structures of belimumab and tabalumab were constructed by homology modeling. The combination mode of BAFF-belimumab complex was analyzed by molecular dynamics simulation, and the combination mode of BAFF-tabalumab complex was analyzed by protein-protein docking after molecular dynamics simulation. Both belimumab and tabalumab contacted with BAFF at the same hydrophobic center which the natural receptors of BAFF bind to. I51, F54, K58 D100, D101, L102, L103, P105 of belimumab heavy chain and R27, Y30,

K49, S65 of belimumab light chain contribute to the BAFF-belimumab interaction mainly by hydrogen bond, salt bridge and hydrophobic effect. More important, Belimumab could contact with L83 of BAFF and produced a steric hindrance with the adjacent BAFF trimers, while tabalumab could not. Belimumab had better clinical effect than tabalumab mainly because it could bind to L83 of BAFF and affect the formation of BAFF 60-mer, in addition to inhibition of the interaction of BAFF with its receptors.

Keywords: BAFF, belimumab, tabalumab, computation analysis, systemic lupus erythematosus

1. Introduction

B-cell activating factor (BAFF, also known as BLyS, zTNF4, TNFSF13B, THANK, and TALL-1) is a member of the tumor necrosis factor (TNF) superfamily, and it can activate the signaling pathways by binding specific receptors[1-9]. BAFF is a type II transmembrane protein encoded by human chromosome 13q34, which is mainly expressed in peripheral blood mononuclear cells, lymph nodes, spleen, and thymus, but lowly expressed in the small intestine, pancreas, placenta, and lung[10, 11]. Abundant evidence demonstrated that BAFF is involved in pathogenesis of systemic lupus erythematosus (SLE), which is a prototype autoimmune disease affecting multiple organ systems[12-18]. In these patients, a large number of autoantibodies are produced and the deposition of immune complexes occurs in multiple organs, especially in kidneys[19-26].

BAFF and BAFF is not only biologically activated as a membrane bound protein, but also found in soluble form[27-29]. The soluble BAFF (sBAFF) is formed from the hydrolysis of membrane-type BAFF and can improve the activity of B cells, CD4+T cells, and NK cells to increase the body's immune responses[30]. Three kinds of the receptors of BAFF have been reported, they are B-cell maturation antigen (BCMA), BAFF receptor 3 (BR3), and trans-membrane activator and calcium modulator and cyclophilin ligand interactor (TACI)[2, 31-41]. Although BAFF often functions as a homo-trimer form, a more active BAFF 60-mer has been reported[42]. Twenty soluble BAFF trimers oligomerize into 60-mer through trimer-trimer interaction via a long DE loop (KVHVFGDELS), and this 60-mer is considered to be more active than trimer because the clustering of receptors on the B-cell surface[43, 44]. Since BAFF can bind to B lymphocytes, induce their proliferation, differentiation, and secretion of immunoglobulins[45, 46], and some researches showed that the elevated levels of BAFF are closely related to SLE activity[26], BAFF and its downstream signal transduction factors become ideal targets for the treatment of autoimmune diseases.

Antibodies targeting BAFF can inhibit the proliferation and activation of B cells by blocking the binding of BAFF and its receptors[47]. Anti-BAFF monoclonal antibody can reduce the elevated levels of immunoglobulin and lupus related damage factors in peripheral blood of SLE patients[48]. Belimumab (also known as Benlysata) is a fully human monoclonal IgG1λ antibody, which can neutralize the soluble BAFF[12, 49-55]. It was proved by FDA in 2011 as a targeted therapy for SLE[56-58]. Clinical trials of SLE patients with belimumab has shown decreased the number of circulating naive B cells, activated B cells, and enhanced clinical efficacy[48, 59, 60]. Tabalumab (formerly LY2127399) is a fully human IgG4 monoclonal antibody. Same as natural BAFF

receptors, tabalumab binds both membrane-bound and soluble BAFF, while belimumab binds only soluble BAFF[61, 62]. Tabalumab reduced rheumatoid arthritis (RA) signs and symptoms in subjects naive to biological disease-modifying antirheumatic drugs (bDMARDs) such as TNF inhibitors in an initial dose-ranging study[63]. Telitacicept is a fusion protein constructed from the extracellular part of TACI and Fc part of human IgG1. TACI has a strong affinity for BAFF and A proliferation-inducing ligand (APRIL), a typical type II membrane protein that can stimulate B cell proliferation and tumor cell growth[64, 65]. Therefore, Telitacicept can block the interaction of BAFF and APRIL with their receptors to block the biological activities of BAFF and APRIL. This double-blocking effects may be more effective than blocking BAFF alone, and can block the proliferation of B lymphocytes and treat autoimmune diseases more effectively. On March 12, 2021, Telitacicept was approved by China National Medical Products Administration (NMPA).

In this study, we employed homology modeling, molecular docking, and molecular dynamics (MD) simulation to develop three dimensional (3D) structural model of BAFF-belimumab complex, in order to understand the interaction mechanism of belimumab in atomic detail. After that, we calculated the binding free energy and energy decomposition to each residue to figure out the binding mechanism and alanine mutational analyses to validate our findings. Then we built 3D structure model of tabalumab and performed MD simulation for BAFF-tabalumab complex, to figure out the reason why tabalumab failed in clinical trial[66]. These results give insights into the interaction mechanism between BAFF and its antibody antagonists, and further provide foundation for designing novel BAFF-targeted inhibitors in future.

2. Material and methods

2.1 Construction of Molecular Systems for BAFF and Belimumab

The 3D crystal structure of BAFF in complex with belimumab was downloaded from the website of Protein Data Bank (http://www.rcsb.org/) with an access code of 5Y9J. Due to a breakpoint in downloaded 3D structure (S136 to G142 in heavy chain), continuous peptides structure of heavy chain was constructed by SWISS-MODEL (https://swissmodel.expasy.org/) using downloaded structure as template. To build BAFF-belimumab complex, the constructed heavy chain of belimumab were matched to downloaded complex by Chimera Ver1.14.

2.2 Energy Minimization and MD Simulation of BAFF-Belimumab Complex

During the process of molecular dynamics simulation, after building the starting structure, a simulation was carried out to obtain an equilibrated system which is completed structure optimization and energy minimization. The SANDER module of the AMBER 16 package with the AMBER parm99SB force field was used[67]. Counter ions were added to neutralize the charge for the system. TIP3P water box was set to a truncated octahedron periodic box functioning as a kind of solvent to make the system be infiltrated in whose size is 1 nm. Generally, the Particle Grid Ewald (PME) method was used to deal with long-range electrostatic interactions[68, 69]. The SHAKE algorithm which supports atomic vibration in quantum mechanical calculation simulation system was applied to fix all of the covalent bonds. In the simulation, 0.8 nm referring to the cutoff distance was set up when there were the nonbonded interactions[70].

The solvated system which has performed energy minimization was conducted MD simulation. The water-solvent molecules and ions were optimized first while the atoms of the residues of the complex were restrained. Then, for all residues, keep the main chain residues frozen while relaxing the side chains. Finally, all atoms were allowed to move freely. 2 fs was set up for each integration step and the ensemble type was set to NPT (a kind of isothermal isobaric system). The steepest descent method was executed to go on the energy minimization for the first 5000 steps. Then, the conjugated gradient method was used for the subsequent 2500 steps. The temperature was controlled from 0 K to 300 K within the period of 100 ps after the structure was minimized to continue the MD simulation. And the systems were equilibrated in the NPT ensemble (1 atm and 300 K) for 100 ps when the proteins were kept frozen. Next, the complexes were equilibrated for 500 ps without restraint. At last, the whole system was subject to 200 ns Molecular Dynamics under the above NPT ensemble. The PTRAJ module can be used to collect atomic coordinates of the system in detail.

2.3 Binding Free Energy Calculation on BAFF-Belimumab System and Per-Residue Free Energy Decomposition Analysis

After MD simulation, the interactions of the protein complex system were confirmed. The validations were conducted by calculating the binding free energy and decomposing the binding free energy into per residues. The molecular mechanics MM-GBSA algorithms in AMBER 16 were implemented to calculate the binding free energies between BAFF and belimumab. The total binding free

energies in condensed phase can be calculated by the following formula:

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{sol} - T\Delta S$$

$$\Delta E_{MM} = \Delta E_{ele} + \Delta E_{vdw} + \Delta E_{int}$$

$$\Delta G_{sol} = \Delta G_{polar} + \Delta G_{NP}$$

Where ΔG_{bind} is the binding free energy in solution state, ΔE_{MM} is the molecular mechanics energy which is consisted by an electrostatic interaction (ΔE_{ele}), a van der Waals interaction (ΔE_{vdw}) and an internal energy (ΔE_{int}) for a ligand or a protein. ΔG_{sol} is the solvation free energy, consisted by a polar component (ΔG_{polar}) and a nonpolar one (ΔG_{NP}). ΔG_{polar} can be calculated by GB method[71]. ΔG_{NP} is calculated by the solvent-accessibility surface area (SASA) equation $\Delta G_{NP} = \gamma SASA + \beta$, where SASA can be obtained from the MSMS program, and the values of γ and β were set as 0.72 kcal/(mol*nm^2) and 0.00 kcal/mol respectively. A mount of studies showed that entropy does not contribute much to the relative binding free energies[72].

The free binding energy decomposition of residue-inhibitor interaction for each of BAFF and belimumab residues can also be calculated by alanine mutation. The free energy changes ($\Delta\Delta G_{bind}$) were estimated by comparing the delta free energy of the wild type (ΔG_{WT}) with the delta free energy of alanine mutant (ΔG_{mut}), $\Delta\Delta G_{bind} = \Delta G_{WT} - \Delta G_{mut}$, A negative $\Delta\Delta G_{bind}$ indicates that the wild type protein is more favorable as the ΔG_{WT} is more negative, and the opposite is true.

2.4 Construction of BAFF-Tabalumab Complex

Due to the absence of 3D structure of tabalumab, we constructed 3D structure of tabalumab by SWISS-MODEL using 4NPY for heavy chain and 5WT9 for light as template which were recommended by search template results. The constructed 3D structure of tabalumab was performed MD simulation using the same parameters as BAFF-belimumab complex to attain stable structure with low energy. Finally, the interaction between BAFF and tabalumab was analyzed by Chimera Ver1.14.

3. Results and Discussion

3.1 Homology Construction of BAFF-Belimumab System

Homologous construction was done by SWISS-MODEL to smooth the remedy of the break point. To rank the constructed structure, Ramachandran plots were calculated to assess the geometric properties of the backbone conformations of it. 100% of the residues fall within the most favored region for both chains of belimumab. The constructed system was further refined by performing an optimized geometry calculation of the mechanics using the SANDER module.

3.2 MD Simulation and Free Binding Energy Calculation

To ensure the proper conformational space sampling, we performed a 200 ns MD simulation for the BAFF-belimumab complex. The atomic Root Mean Square Deviation (RMSD) fluctuations of the backbone atoms (CA, C and N atoms) of BAFFs and Belimumabs were separately calculated and outlined into a function of time (Fig. 1).

It can be concluded that the BAFF and two chains of belimumab have relatively smooth curves in the last 20 ns period with the average RMSD values of 0.200 nm for heavy chain, 0.127 nm for light chain of belimumab and 0.135 nm for BAFF. The system becomes equilibrated as judged by RMSDs.

The individual energy terms of the binding free energies of the BAFF-belimumab complex were calculated by the MM-GBSA method. Different terms of energy values are listed in Table 1. The enthalpy of the BAFF-belimumab complex was estimated at -48.68 kcal/mol by the MM-GBSA approach.

Table 1 The binding free energies of the BAFF-belimumab complex (kcal/mol)

Component*	Complex		BAFF		Belimumab		Delta	
	Mean	**STD.**	**Mean**	**STD.**	**Mean**	**STD.**	**Mean**	**STD.**
E_{vdw}	-4317.95	26.25	-3153.03	20.69	-1073.85	11.75	-91.07	6.11
E_{ele}	-40584.57	101.12	-29863.14	92.27	-10345.54	43.09	-375.89	18.17
E_{int}	0	0	0	0	0	0	0	0
G_{polar}	-5867.00	74.46	-4236.61	76.49	-2061.96	36.20	431.57	14.1
G_{NP}	180.05	2.36	138.72	1.74	54.62	0.82	-13.29	0.55
E_{MM}	-44902.52	99.74	-33016.17	89.87	-11419.39	41.77	-466.96	15.65
G_{sol}	-5686.95	73.44	-4097.89	76.00	-2007.34	36.12	418.28	14.04
Total	-50589.47	63.97	-37114.06	52.17	-13426.73	28.69	-48.68	4.09

*E_{vdw}: the van der Waals contribution from molecular mechanics; E_{ele}: the electrostatic energy as calculated by the molecular mechanics force field; G_{polar}: the electrostatic energy to the solvation free energy; G_{NP}: the energy of nonpolar components contributing to the solvation free energy; E_{MM}: the free energy of the complex in vacuum which equals to E_{vdw}, E_{ele} and E_{int}; G_{sol}: the free energy of the complex in solvation

states which equals to G_{polar} and G_{NP}; Total: the whole binding free energy including E_{MM} and G_{sol}; Delta: the sum of BAFF and belimumab minus complex

In order to identify which of the interaction have a significant impact on the system, the binding free energy was divided into polar and nonpolar interactions. It was clear that the nonpolar interaction function as the dominant force, and the polar energy provided an unfavorable contribution for the formation of BAFF-belimumab complex by comparing values of the two types of energy. The absolute value of the nonpolar energy is about twice as much as the polar one. In addition, the polar interaction in the gas phase provided a favorable energy component, but it is mainly unfavorable in the solution phase.

In order to confirm the binding ability of belimumab and BAFF, the ΔG_{bind} of belimumab-BAFF complex and the complex of BAFF and a natural receptor BCMA were compared (Table 2). Our study only simulated the interaction between belimumab and a single chain of BAFF, while the previous study simulated the interaction between BAFF trimer and BCMA as a result, we divided the previous binding energy data by three to compare them. It showed that nonpolar force contributes to the complex binding much more for both two systems, this is consistent with the interaction between BAFF and its other natural receptors we reported before[70, 73]. Compared with the natural receptor BCMA, the binding energy of belimumab with BAFF is higher in absolute value, which means that Belimumab-BAFF complex interact more strongly and bind more closely with each other. It is the reason why belimumab can inhibit the binding of BAFF and their natural receptors and the function of the belimumab binding with BAFF to inhibit the function of BAFF can be realized.

Table 2 The comparison of binding energies between belimumab-BAFF complex and BCMA-BAFF complex (kcal/mol)

Energy	Belimumab-BAFF	BCMA-BAFF
ΔE_{vdw}	-91.07	-63.96
ΔE_{ele}	-375.89	-94.23
ΔE_{int}	0	0
ΔG_{polar}	431.57	123.09
ΔG_{NP}	-13.29	-10.13
ΔE_{MM}	-466.96	-158.19
ΔG_{sol}	418.28	112.96
ΔG_{bind}	-48.68	-45.23

3.3 The Binding Mode Between BAFF and Belimumab

To figure out the binding site and interact mechanism, we analyzed the binding mode between BAFF and belimumab (Fig. 2). BAFF can interact with both heavy and light chain of belimumab mainly by hydrophobic effect, hydrogen bond and salt bridge. The major binding site comprised of eight residues (I51, F54, K58, D100, L101, L102, L103 and P105) in the heavy chain of belimumab. The

hydrophobic part of belimumab heavy chain was in close contact with Y65, L70, I92 and P123 of BAFF and $_{heavy}$K58/$_{BAFFE}$125 and $_{heavy}$D100/$_{BAFF}$R124 formed salt bridge, respectively (Fig. 2a).

BAFF formed three hydrogen bonds with the light chain of belimumab, they are formed by backbone of $_{light}$R27 with backbone of $_{BAFF}$S85, hydroxyl of $_{light}$S65 with backbone of $_{BAFF}$G80, backbone of $_{light}$G93 with backbone of $_{BAFF}$S21. K49 of belimumab light chain and D81 of BAFF formed salt bridge, and the hydrophobic effect of the light chain and BAFF is mainly existed by Y30 of light chain and L83 of BAFF (Fig. 2b).

3.4 The Binding Free Energy Decomposition of Per Residue and Computational Alanine Scanning

To further analyze the binding mechanism, binding free energy decomposition was performed into a per-residue contribution spectrum. The criterion of $|\Delta G_{bind}|$ >1 kcal/mol was employed to identify the important residues which make contributions to the free energy. According to this standard, seven residues in heavy chain of belimumab (I51, F54, D100, D101, L102, L103 and P105), four residues in light chain of belimumab (R27, Y30, K49 and S65), and eight residues in BAFF (Y65, L70, G80, L83, L85, I92, P123 and R124) were selected. Total free energy of each key residue was illustrated as Fig. 3. For belimumab, D100 in heavy chain and K49 in light chain mainly provided electrostatic interaction, while the side chains of L101, L102 and L103 in heavy chain formed a hydrophobic site and mainly provided van der Waals interactions. These interaction results are consistent with our previous docking model, however, K58 of belimumab heavy chain and E125 was on the surface of protein, the desolvation step led to the decrease in the absolute value of binding energy although they formed salt bridge. The ΔG_{bind} value of $_{heavy}$K58 and $_{BAFF}$E125 was -0.90 kcal/mol and 1.59 kcal/mol, respectively. And the same situation existed in D81 of BAFF with the ΔG_{bind} value of 0.19 kcal/mol. The hydrogen bond interaction between G94 of belimumab light chain and G20 of BAFF was weak and unstable in MD simulation, which can be concluded by their ΔG_{bind} value of -0.80 kcal/mol and -0.82 kcal/mol, respectively.

In order to confirm whether the side chain of specific residues plays an important role in the binding of BAFF-belimumab complex or not, seventeen residues were selected to perform the computational alanine scanning (Table 3). P105 of belimumab heavy chain, G80 and P123 of BAFF were not mutated into alanine because the significant skeleton structure will change when these side chains were replaced. In addition to R27 of belimumab light chain and L85 of BAFF, the binding energies of other residues become less

negative after computational alanine scanning. It indicated that any one of these amino acids mutated to alanine would weaken the binding affinity of BAFF-belimumab complex. Among these residues which had effect on the binding affinity, D100 of belimumab heavy chain and R214 of BAFF showed the largest $\Delta\Delta G_{bind}$ value, more than 21.48 kcal/mol and 16.66 kcal/mol, respectively, which means that they played the most important role on the binding affinity. For both of them, the changes of electrostatic interactions contribute the most to their $\Delta\Delta G_{bind}$.

Table 3 The computational alanine scanning results of the key residues for the BAFF-belimumab complex (kcal/mol)

Residue (chain)	$\Delta\Delta G_{vdw}$	$\Delta\Delta G_{ele}$	$\Delta\Delta G_{polar}$	$\Delta\Delta G_{NP}$	$\Delta\Delta G_{MM}$	$\Delta\Delta G_{sol}$	$\Delta\Delta G_{bind}$
I51 (heavy chain)	1.75	-0.14	8.53	0.23	1.61	8.75	1.29
F54 (heavy chain)	4.9	2.7	4.23	0.75	7.6	4.97	3.49
D100 (heavy chain)	-2.2	37.1	-4.61	0.28	34.9	-4.33	21.48
L101 (heavy chain)	2.56	0.68	6.71	0.40	3.25	7.11	10.36
L102 (heavy chain)	8.18	0.04	5.26	1.05	8.21	6.3	5.43
L103 (heavy chain)	1.94	0.28	7.14	0.27	2.22	7.41	9.64
R27 (light chain)	2.89	34.02	-27.88	0.35	36.91	-27.54	0.29
Y30 (light chain)	2.77	-2.72	11.82	0.41	0.05	12.23	3.19
K49 (light chain)	0.74	129.16	-119.1	0.67	129.9	-118.44	2.37
S65 (light chain)	0.12	3.25	9.07	0.15	3.36	9.22	3.50
Y65 (BAFF)	7.04	2.55	5.01	1.04	9.58	6.05	6.55
L70 (BAFF)	2.76	0.1	7.71	0.37	2.85	8.08	1.85
L83 (BAFF)	7.15	-0.46	6.42	0.84	6.68	7.26	4.86
L85 (BAFF)	1.51	0.27	7.79	0.21	1.78	7.99	0.69
I92 (BAFF)	2.12	-0.07	7.95	0.35	2.05	8.3	1.26
R124 (BAFF)	1.22	35.35	-14.13	3.31	36.57	-10.83	16.66
E125 (BAFF)	0.85	120.52	-110.97	0.55	121.37	-110.43	1.86

$\Delta\Delta G_{bind} = \Delta G_{bind}(alanine\ mutant) - \Delta G_{bind}(wild\ type)$

$\Delta\Delta G_{bind} = \Delta\Delta G_{MM} + \Delta\Delta G_{sol}$

$\Delta\Delta G_{MM} = \Delta\Delta G_{ele} + \Delta\Delta G_{vdw} + \Delta\Delta G_{int}$

$\Delta\Delta G_{sol} = \Delta\Delta G_{polar} + \Delta\Delta G_{NP}$

3.5 The Comparison of Binding Mode Between BAFF-Tabalumab Complex, BAFF-Belimumab Complex and BAFF-BR3 Complex

The constructed tabalumab could interact with BAFF mainly by heavy chain through hydrophobic effect, hydrogen bond and salt bridge. BAFF formed two hydrogen bonds with the heavy chain of tabalumab, they are formed by $_{heavy}$N58 with $_{BAFF}$E125 and $_{heavy}$Y100 with backbone of $_{BAFF}$R124. In addition, DXL motif (D101, I102, L103) of tabalumab heavy chain is involved in binding with BAFF, $_{heavy}$D101 and $_{BAFF}$R90 formed salt bridge. The hydrophobic effect of tabalumab and BAFF is mainly existed by I102, L103 of tabalumab heavy chain and L70, V86 of BAFF (Fig. 4).

As shown in Fig. 5, The hydrophobic center of BAFF was the active site which could contact with its natural receptors such as BCMA and BR3 at the DxL motif[74], and a structure similar to DxL motifs could also be found in CDR3 of heavy chains in both

belimumab and tabalumab (Fig. 6). As a result, the mechanisms of inhibition of BAFF by belimumab and tabalumab were concerned to be competitive inhibition with natural receptors.

It was reported that the dissolved BAFF trimer could combine to form a 60-mer, and this 60-mer was thought to be more active than BAFF trimer. In addition, L83 of BAFF is concerned that specifically contributes to the formation of BAFF 60-mer[75]. As a result, whether the antibody can interact with L83 of BAFF became the key point of the inhibition of BAFF. For BAFF-belimumab complex, the heavy chain of belimumab contact with L83 of BAFF by hydrophobic interaction. This hydrophobic effect brings the light chain close to BAFF, and causes hindrance that prevent the D loop of BAFF interact with adjacent BAFF homo-trimer, blocks the formation of BAFF 60-mer, and inhibit soluble BAFF more specifically and effectively compared with natural TNF receptors. But for BAFF-tabalumab complex, the light of tabalumab could not combine with L83 of BAFF, so that no steric hindrance existed between the light chain of tabalumab and the adjacent BAFF trimer. As a result, tabalumab could not prevent the formation of BAFF 60mer, and it seems to be the reason why tabalumab failed in clinical trials.

By alignment of the sequences of belimumab and tabalumab (Fig. 6), it is noted that R30 is in $_{light}$CDR1 of tabalumab, instead of Y30 in belimumab. It is interesting that the difference in single amino acid of CDR1 make the two antibodies function differently. The amino acids corresponding to R27 in $_{light}$CDR1 and G94 in $_{light}$CDR3 could not be found in tabalumab, which made the light chain of tabalumab unable to form hydrogen bond with BAFF. Both the $_{heavy}$CDR3 of belimumab and tabalumab had DxL motif. They could bind to the active site of BAFF and competitively inhibit the effect of BAFF, which showed that the heavy chain of belimumab and tabalumab had a similar mechanism in the process of inhibiting BAFF activity.

4. Conclusions

In summary, we successfully constructed the theoretical complex of BAFF with belimumab and tabalumab by homologous construction. The binding model between BAFF and belimumab was analyzed through MD simulation. The amino acids that played a key role in the interaction were determined, and the corresponding binding energy values were calculated through the energy decomposition. I51, F54, K58 D100, D101, L102, L103, P105 of belimumab heavy chain and R27, Y30, K49, S65 of belimumab light chain contribute to the BAFF-belimumab interaction mainly by hydrogen bond, salt bridge and hydrophobic effect. Both

belimumab and tabalumab had a DxL motif which is the main binding site of the natural receptors with BAFF, and could function competitive inhibition with the natural receptors. The light chain of belimumab could contact with L83 of BAFF which is the key residue for the formation of BAFF 60-mer, and then the steric hindrance between belimumab light chain and adjacent BAFF trimers cause it unable to form BAFF 60-mer. Tabalumab contact with BAFF at the similar site as belimumab and the natural receptors while its light chain could not contact with L83 of BAFF, could not affect the formation of BAFF 60-mer and had a less inhibitory effect. Therefore, it is reasonable that belimumab showed better clinical therapeutic efficacies than Tabalumab and was approved by FDA for SLE in 2011. In summary, our computation studies may provide foundation for designing novel therapeutic antibodies in future.

***Declarations：**

Funding: This study was supported by the National Natural Science Foundation of China (Grant No.81273308).

Conflicts of interest/Competing interests: The authors declare no conflict of interest, financial or otherwise.

Availability of data and material: Not applicable.

Code availability: Not applicable.

Authors' contributions: Sun J and Wei J conceived and designed the experiments. Jiang Y performed the experiments. Wei J and Jiang Y analyzed the binding modes. Jiang Y and Sun J wrote the paper. All authors have read and approved the final manuscript to be published.

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FIGURE LEGENDS:

Fig. 1 Time dependence of RMSD values along the MD trajectories for the complex of BAFF and belimumab, the heavy chain of belimumab is shown in red, the light chain of belimumab is shown in blue, and BAFF is shown in black

Fig. 2 Protein-protein docking schematic and interaction analysis of BAFF-belimumab complex for **a** the view of protein-protein docking schematic and interaction analysis of BAFF and the heavy chain of belimumab, **b** the view of protein-protein docking schematic and interaction analysis of BAFF and the light chain of belimumab, tan: BAFF, sky blue: heavy chain of belimumab, plum: light chain of belimumab, amino acid contacts in 0.5 nm were indicated, hydrogen bond was shown in blue straight line, the name and number of the interactive amino acids on BAFF and both chains of belimumab were also indicated

Fig. 3 Decomposition analyses for belimumab heavy chain (blue), belimumab light chain (yellow) and BAFF (grey)

Fig. 4 Protein-protein docking schematic and interaction analysis of BAFF-tabalumab complex, tan: BAFF, sky blue: heavy chain of belimumab, plum: light chain of belimumab, amino acid contacts in 0.5 nm were indicated, hydrogen bond was shown in blue straight line, the name and number of the interactive amino acids on BAFF and both chains of belimumab were also indicate

Fig. 5 The comparison of protein-protein docking schematic between BAFF-tabalumab complex and BAFF-belimumab complex for **a** the section included in the red square was the steric hindrance between the light chain of belimumab and the adjacent BAFF trimer after the combination of belimumab and BAFF, **b** the section included in the red square was the DxL motif where belimumab, tabalumab and BR3 binding and acting with BAFF, **c** the interaction between Y30 of belimumab light chain and L83 of BAFF, tan: local structure of BAFF60-mer, sky blue: heavy chain of belimumab, plum: light chain of belimumab, light green: tabalumab, red: the nature receptor of BAFF (BR3), the section included in the red square was the steric hindrance between the light chain of belimumab and the adjacent BAFF trimer after the combination of belimumab and BAFF

Fig. 6 The alignment result for both light chain and heavy chain of belimumab and tabalumab for **a** the alignment result of light chain, **b** The alignment result of heavy chain, the section included in the yellow square was the CDR1/2/3 of belimumab and tabalumab according to the label, the section included in the yellow square was the DxL motif

Fig. 1

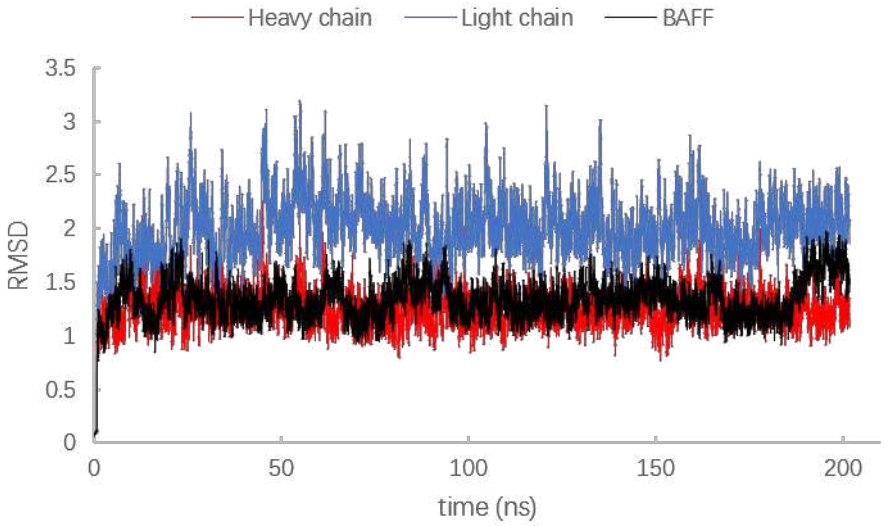
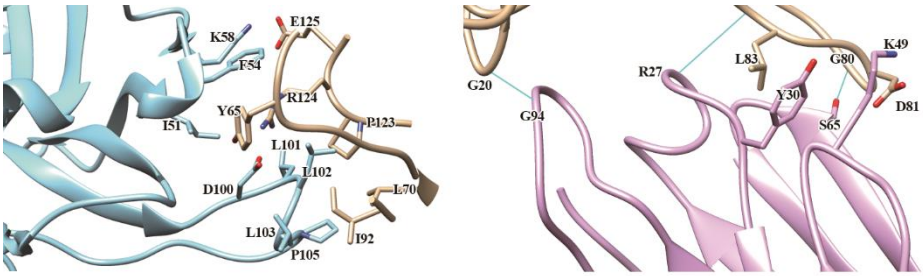

Heavy chain
Light chain
BAFF
RMSD
3.5
3
2.5
2
1.5
1
0.5
0
0
50
100
150
200
time (ns)

Fig. 2


E125
K58
F54
R124
Y65
I51
P123
L101
L102
D100
L70
L103
I92
P105
G20
G94
R27
L83
G80
K49
Y30
D81
S65

A B

Fig. 3

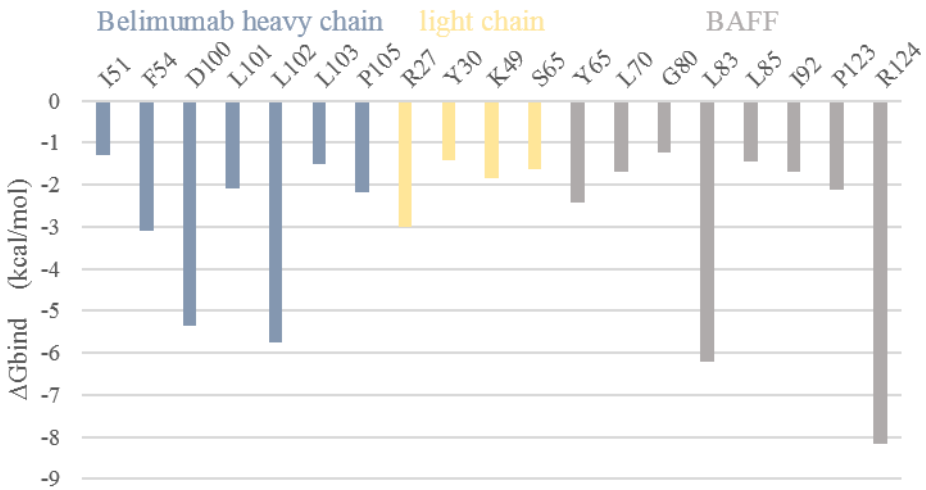

Belimumab heavy chain
light chain
BAFF
I51
F54
D100
L101
L102
L103
P105
R27
Y30
K49
S65
Y65
L70
G80
L83
L85
I92
P123
R124
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
ΔGbind (kcal/mol)

Fig. 4

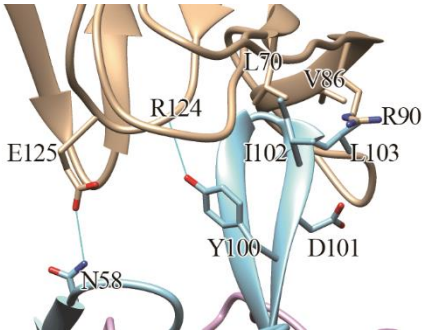


Fig. 5

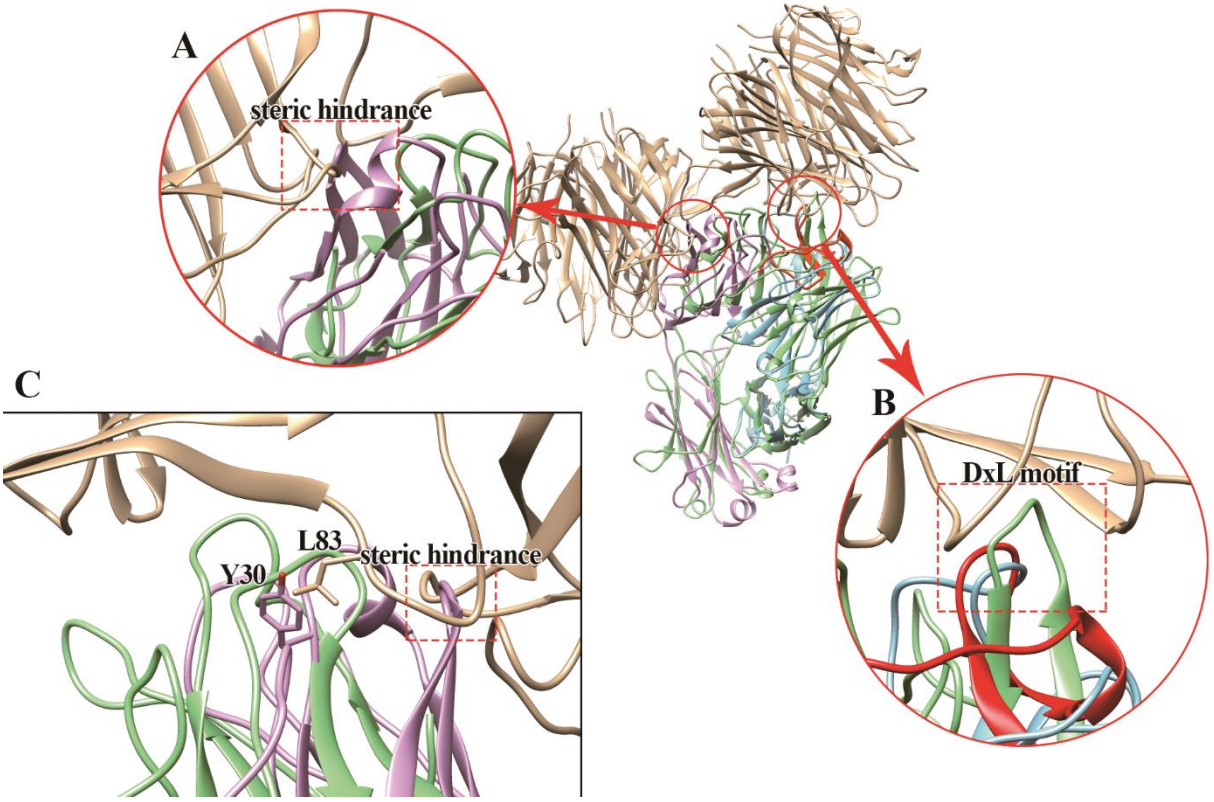
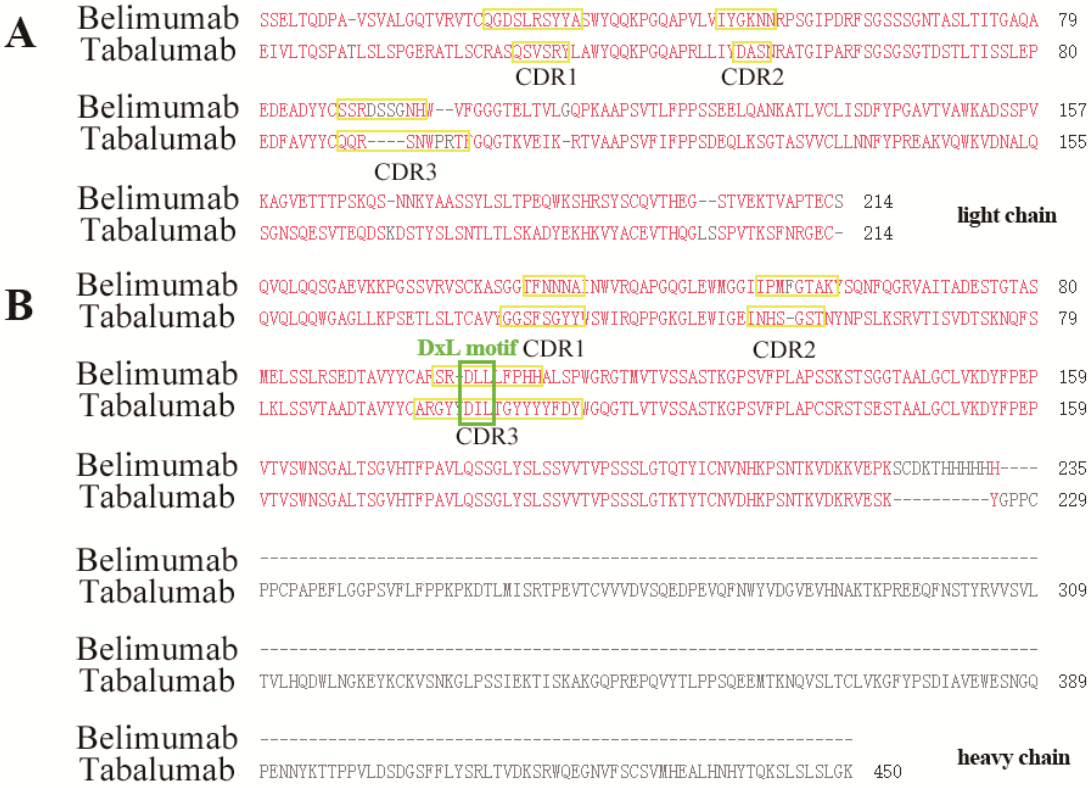


Fig. 6


A
Belimumab SSELTQDPA-VSVALGQTVRVTCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQA 79
Tabalumab EIVLTQSPATLSLSPGERATLSCRASQSVSRYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDSTLTISSLEP 80
CDR1
CDR2
Belimumab EDEADYYCSSRDSSGNHV--VFGGGTELTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPV 157
Tabalumab EDFAVYYCQQR----SNWPRTFGQGTKVEIK-RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ 155
CDR3
Belimumab KAGVETTTPSKQS-NNKYAASSYLSLTPEQWKSHRSYSCQVTHEG--STVEKTVAPTECS 214
Tabalumab SGNSQESVTEQDSKDSTYSLSNTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC- 214
light chain
B
Belimumab QVQLQQSGAEVKKPGSSVRVSCKASGGTFNNNAINWVRQAPGQGLEWMGGIIPMFGTAKYSQNFQGRVAITADESTGTAS 80
Tabalumab QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYVSWIRQPPGKGLEWIGEINHS-GSTNYNPSLKSRVTISVDTSKNQFS 79
DxL motif
CDR1
CDR2
Belimumab MELSSLRSEDTAVYYCARSR-DLLLFPHHALSPWGRGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP 159
Tabalumab LKLSSVTAADTAVYYCARGYSDILTGYYYYFDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEP 159
CDR3
Belimumab VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHHHHHH---- 235
Tabalumab VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESK----------YGPPC 229
Belimumab ----------
Tabalumab PPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL 309
Belimumab ----------
Tabalumab TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ 389
Belimumab ----------
Tabalumab PENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK 450
heavy chain

Tables:

Table 1 The binding free energies of the BAFF-belimumab complex (kcal/mol)

Component*	Complex		BAFF		Belimumab		Delta	
	Mean	**STD.**	**Mean**	**STD.**	**Mean**	**STD.**	**Mean**	**STD.**
E_{vdw}	-4317.95	26.25	-3153.03	20.69	-1073.85	11.75	-91.07	6.11
E_{ele}	-40584.57	101.12	-29863.14	92.27	-10345.54	43.09	-375.89	18.17
E_{int}	0	0	0	0	0	0	0	0
G_{polar}	-5867.00	74.46	-4236.61	76.49	-2061.96	36.20	431.57	14.1
G_{NP}	180.05	2.36	138.72	1.74	54.62	0.82	-13.29	0.55
E_{MM}	-44902.52	99.74	-33016.17	89.87	-11419.39	41.77	-466.96	15.65
G_{sol}	-5686.95	73.44	-4097.89	76.00	-2007.34	36.12	418.28	14.04
Total	-50589.47	63.97	-37114.06	52.17	-13426.73	28.69	-48.68	4.09

*E_{vdw}: the van der Waals contribution from molecular mechanics; E_{ele}: the electrostatic energy as calculated by the molecular mechanics force field; G_{polar}: the electrostatic energy to the solvation free energy; G_{NP}: the energy of nonpolar components contributing to the solvation free energy; E_{MM}: the free energy of the complex in vacuum which equals to E_{vdw}, E_{ele} and E_{int}; G_{sol}: the free energy of the complex in solvation states which equals to G_{polar} and G_{NP}; Total: the whole binding free energy including E_{MM} and G_{sol}; Delta: the sum of BAFF and belimumab minus complex

Table 2 The comparison of binding energies between belimumab-BAFF complex and BCMA-BAFF complex (kcal/mol)

Energy	Belimumab-BAFF	BCMA-BAFF
ΔE_{vdw}	-91.07	-63.96
ΔE_{ele}	-375.89	-94.23
ΔE_{int}	0	0
ΔG_{polar}	431.57	123.09
ΔG_{NP}	-13.29	-10.13
ΔE_{MM}	-466.96	-158.19
ΔG_{sol}	418.28	112.96
ΔG_{bind}	-48.68	-45.23

Table 3 The computational alanine scanning results of the key residues for the BAFF-belimumab complex (kcal/mol)

Residue (chain)	$\Delta\Delta G_{vdw}$	$\Delta\Delta G_{ele}$	$\Delta\Delta G_{polar}$	$\Delta\Delta G_{NP}$	$\Delta\Delta G_{MM}$	$\Delta\Delta G_{sol}$	$\Delta\Delta G_{bind}$
I51 (heavy chain)	1.75	-0.14	8.53	0.23	1.61	8.75	1.29
F54 (heavy chain)	4.9	2.7	4.23	0.75	7.6	4.97	3.49
D100 (heavy chain)	-2.2	37.1	-4.61	0.28	34.9	-4.33	21.48
L101 (heavy chain)	2.56	0.68	6.71	0.40	3.25	7.11	10.36
L102 (heavy chain)	8.18	0.04	5.26	1.05	8.21	6.3	5.43
L103 (heavy chain)	1.94	0.28	7.14	0.27	2.22	7.41	9.64
R27 (light chain)	2.89	34.02	-27.88	0.35	36.91	-27.54	0.29
Y30 (light chain)	2.77	-2.72	11.82	0.41	0.05	12.23	3.19
K49 (light chain)	0.74	129.16	-119.1	0.67	129.9	-118.44	2.37
S65 (light chain)	0.12	3.25	9.07	0.15	3.36	9.22	3.50
Y65 (BAFF)	7.04	2.55	5.01	1.04	9.58	6.05	6.55
L70 (BAFF)	2.76	0.1	7.71	0.37	2.85	8.08	1.85
L83 (BAFF)	7.15	-0.46	6.42	0.84	6.68	7.26	4.86
L85 (BAFF)	1.51	0.27	7.79	0.21	1.78	7.99	0.69
I92 (BAFF)	2.12	-0.07	7.95	0.35	2.05	8.3	1.26
R124 (BAFF)	1.22	35.35	-14.13	3.31	36.57	-10.83	16.66
E125 (BAFF)	0.85	120.52	-110.97	0.55	121.37	-110.43	1.86

$\Delta\Delta G_{bind} = \Delta G_{bind}(alanine\ mutant) - \Delta G_{bind}(wild\ type)$

$\Delta\Delta G_{bind} = \Delta\Delta G_{MM} + \Delta\Delta G_{sol}$

$\Delta\Delta G_{MM} = \Delta\Delta G_{ele} + \Delta\Delta G_{vdw} + \Delta\Delta G_{int}$

$\Delta\Delta G_{sol} = \Delta\Delta G_{polar} + \Delta\Delta G_{NP}$

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