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Identify the key amino acid of BAFF binding with TACI

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ABSTRACT

B-cell activating factor (BAFF) has been used as a therapeutic target. To develop BAFF-specific small molecular inhibitors, it is necessary to know the key amino acid in the BAFF binding with its receptor. The key binding amino acid of BAFF interacting with its receptor TACI (trans-membrane activator and calcium modulator and cyclophilin ligand interactor) was analyzed based on the computer-guided molecular modeling method. According to theoretical prediction, a series of key amino acid mutants of BAFF, including M204 (Lys^{204} to Ala), M208 (Met^{208} to Ala), M209 (Gly^{209} to Ala), M210 (His^{210} to Ala), M234 (Gln^{234} to Ala), M236 (Met^{236} to Ala), and M237 (Pro^{237} to Ala) were designed and evaluated with biological experiments. The results show that M208, M209, M236, and M237 of BAFF were the key amino acids and in accord with the theoretical results. The results highlight clues for the further development of BAFF-specific small molecular inhibitors.

1. Introduction

B-cell activating factor (BAFF: also known as BLyS, TALL-1, THANK, zTNF4 or TNFSF13b) was identified as an important member of the TNF family of cytokines in 1999 [1,2]. BAFF exerts its effect by binding three different receptors: BAFF-receptor (BAFFR/BR3), B-cell maturation Ag (BCMA), and trans-membrane activator and calcium modulator and cyclophilin ligand interactor (TACI) [3]. The pathogenic role of BAFF in SLE was revealed early in BAFF Tg mice that developed a lupus-like illness with the production of anti-DNA antibodies and the development of glomerulonephritis [4]. Increasing data show that overproduction of BAFF is associated with human autoimmune diseases. BAFF has been regarded as a local and systemic target in autoimmune diseases [5].

Several strategies including anti-BAFF (Belimumab) and TACI-Ig (Atacicept) have been developed to block BAFF *in vivo*. Several lines of evidence indicate that TACI may be the most versatile receptor responsible for BAFF-mediated function [6–9]. TACI is emerging as an unusual TNF receptor-like molecule with a sophisticated mode of action [10,11] TACI is reported to attenuate T cell-independent and -dependent B-cell responses induced by B-cell activating factor (BAFF). The soluble protein TACI was shown to alleviate the autoimmune phenotype of NZBWF1 and MRL-lpr/lpr mice, [12] and its fusion protein (TACI-Fc) was used as a novel antagonist to treat relapsing multiple sclerosis induced by BAFF and APRIL. Thus, interaction of TACI and BAFF may be used to provide hints for the development of BAFF antagonists.

BCMA, TACI and BAFF-R show perfect structural conservation in the L-hairpin structure. This constitutes the conserved core of the interaction with BAFF. The hairpin structure is followed by a helix-loop-helix motive that is strikingly different among receptors: its spatial orientation differs in BCMA and TACI, independently of receptor binding, and BAFF-R contains only the first helix [13]. It is said that the hairpin is crucial for ligand binding and that the C-terminal domain defines ligand specificity.

The 3′ genomic organization of TACI is similar to that of BCMA and BAFF-R, but the ligand-binding region is duplicated in an extra 5′ exon. Although TACI contains two CRDs, only the second one is necessary and sufficient for high affinity binding to BAFF and APRIL [14]. Determining the true affinities of BAFF for their receptors is not trivial. Indeed, measures performed with dimeric receptor-Ig proteins are subject to significant but unpredictable avidity effects that increase the apparent affinity.

With the development of computational biology, it has become possible to understand the physical basis of protein–protein interaction and constrain the molecular basis of their specificity based on computational prediction [15,16]. In the present study, the

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key amino acid of BAFF in binding with TACI was identified. The key binding amino acid of BAFF interacting with its receptor TACI was analyzed based on the computer-guided molecular modeling method. According to theoretical predictions, a series of key amino acid mutants of BAFF were designed and evaluated with biological experiments.

2. Materials and methods

2.1. Mice

Six–eight-week-old C57BL/6 mice (Chinese Academy of Medical Sciences, Beijing, China) were bred in our animal facilities under specific pathogen-free conditions. This study was approved by the Animal Care and Use Committee of Beijing Institute of Basic Medical Sciences.

2.2. Computer-guided molecular modeling and docking

Based on the crystal structure of BAFF (PDB code: 1kd7) and its receptor TACI (PDB code: 1xu1), using the 3-D modeling complex structure of BAFF-TACI, the identified key amino acid residues of BAFF by TACI were predicted. The 3-D optimized complex structure of BAFF mutant and TACI was obtained with Insight II (2005) software on IBM workstation. Using molecular minimization and dynamics stimulation method, the complex was optimized. Furthermore, the interaction energies between the mutants (i.e., the key amino acid residue were replaced with alanine) of BAFF and TACI were evaluated. According to the theoretical 3-D complex structure of key amino acids of BAFF and TACI, based on the difference of the solvent-accessible area of the monomer and complex, considering the intermolecular hydrogen bond forming, the interaction mode between BAFF and TACI was analyzed. The binding energy was calculated as following: $\Delta E = E_{\text{complex}} - E_{\text{TACI}} - E_{\text{BAFFmutant}}$. The E_{complex}, E_{TACI}, $E_{\text{BAFFmutant}}$ were the mean energy of the complex, the TACI and BAFF mutant, respectively, which was obtained using molecular minimization methods.

2.3. Recombinant BAFF and BAFF mutant preparation

The cDNA encoding extracellular domain of human BAFF was obtained by reverse transcriptase polymerse chain reaction (RT-PCR) using RNA isolated from peripheral blood leukocytes, inserted into pET-32a to construct recombinant plasmid pET-32a/BAFF. According to the predicted key amino acid of BAFF identified by TACI, the residues were substituted with alanine by overlapped PCR to construct recombinant plasmids of BAFF mutants. Seven amino acid mutants of BAFF [M204 (Lys^{204} to Ala), M208 (Met^{208} to Ala), M209 (Gly^{209} to Ala), M210 (His^{210} to Ala), M234 (Gln^{234} to Ala), M236 (Met^{236} to Ala), and M237 (Pro^{237} to Ala)] were generated using overlapped PCR methods by introducing the GCT (coding alanine) into the mutation site. All the plasmids constructed above were confirmed by DNA sequencing.

2.4. Protein purification

The recombinant plasmids mentioned above were transformed into *Escherichia coli* BL21. The bacteria carrying the recombinant plasmids were induced with IPTG (0.1 mM) at 20 °C overnight. After centrifuged at 5000 rpm for 5 min, the pellets were resuspended in 20 mmol/L sodium phosphate buffer (PB) plus 500 mmol/L sodium chloride (NaCl, pH8.0). The bacteria were broken by sonication and centrifuged at 9000 rpm for 15 min to remove cell debris. The supernatant of cell lysate was purified with nickel-chelated affinity column by standard methods. LPS were removed after recombinant protein purification from bacterial lysate prior to in vitro cellular assays. Then the purified proteins were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

2.5. ELISA analysis

ELISA was performed using plates coated with 200 nM of Trx, rhBAFF, rhBAFF mutants, and human BAFF (Peprotech), respectively. BAFF receptor TACI-IgG (0.01, 0.1, 1 and 10 μg/mL) was used for the first antibody and prepared with dilution buffer was then added to each well. The peroxidase-conjugated anti-IgG was used as the secondary antibody. Binding signals were visualized using TMB substrate and analyzed at 450 nm. The OD value for recombinant BAFF was set as 100% binding activity. The binding capability of BAFF mutant is calculated by $OD_{\text{BAFF mutant}}/OD_{\text{rhBAFF}}$.

2.6. Western blot analysis

Anti-BAFF antibody (Cell signaling Technology, Danvers, MA) was used to test 200 nM Trx, rhBAFF, rhBAFF mutants and human BAFF (Peprotech), respectively. The signals were detected with HRP conjugated-secondary F(ab')2 (Zymed Laboratories, San Francisco, CA) using the ECL system (Amersham, Arlington Heights, IL).

2.7. Binding kinetics assays

The binding kinetics of the TACI-Ig to BAFF and its mutants were measured using Bio-Layer Inter-Ferometry on Octet RED (ForteBio, USA). All interaction analyses were conducted at 30 °C in PBS buffer unless stated otherwise. Sensor tips were pre-wet for 5 min in buffer immediately prior to use. The microplates used in the Octet were filled with 200 μl of sample or buffer per well and agitated at 1000 rpm. TACI-Ig (30 μg/mL) was loaded to saturation onto anti-human IgG capture biosensors, The loaded biosensors were washed in buffer for 120 s and transferred to the wells containing BAFF and its mutant proteins at concentrations of 900, 600, 300 μg/mL in buffer. We measured BAFF and its mutants' association and dissociation for 15 min, respectively. Kinetic parameters (K_{on} and K_{off}) and affinities (K_{D}) were calculated from a non-linear global fit of the data between antigen and antibody using the Octet software. Multiple independent measurements were performed.

2.8. Mouse splenocyte survival assay for BAFF bioactivity

B cells were isolated from the spleens of 6–8-week-old C57BL/6 mice. Briefly, cells were washed twice with medium (RPMI 1640, HyClone) and then separated through mouse B-Cell Isolation Kit (Miltenyi Biotech, Germany) according to the MiniMacs protocol (Miltenyi). At the end of the purification procedure, mouse B cells were resuspended at 1×10^6 cells/ml in RPMI1640 plus 10% FBS and dispensed into 96-well plates at 0.1 ml/well and incubated at 37 °C in a 5% CO_2 humidify incubator. After 2 h, serial dilutions of BAFF and mutants (10, 1 and 0.1 μg/ml) in the same culture medium were added and the cell suspensions were then incubated for 72 h at 37 °C in a 5% CO_2 humidify incubator. The same dilutions of trx plus cells were used as the negative controls. Three days later, 10 μl MTS (Promega Co., US) was added to all wells and incubation continued for a further 4 h. The MTS absorbance values were tested at 492 nm. These plots are representative of at least five experiments. The results were expressed as the stimulation index.