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The design, construction and function of a new chimeric anti-CD20 antibody

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Abstract

A novel murine IgM-type anti-human CD20 monoclonal antibody (mAb) 1–28 was prepared in our Lab, which can induce apoptosis and inhibit proliferation of Daudi and Raji cells. However, the efficacy of 1–28 mAb in human cancer therapy is likely to be limited by human anti-mouse antibody responses. A chimeric antibody, C1–28, containing 1–28 mAb variable region genes fused to human constant region genes (gamma 1, kappa) was constructed. However, C1–28 lost the antigen-binding activity. Here, using sequence similarity and known 3D structure of antibody variable regions as template, the spatial conformations of 1–28 variable regions (i.e. V_H and V_L) were analyzed with computer-guided homology modeling methods. According to the surface electrostatic distribution and interaction free energy analysis, the relationship between structure and stability of 1–28 variable regions was studied theoretically and a new chimeric anti-CD20 antibody scFv-Ig named 5S was designed. Expression level of 5S in the culture supernatant was determined to be around 50 μg/mL using sandwich ELISA method with chimeric antibody Rituxan as reference. 5S retained its murine counterpart's binding activity by fluorescence-activated cell-sorting analysis. Furthermore, it could kill CD20 positive Daudi and Raji cells by complement-dependent cytotoxicity. For binding affinity often decreased even lost when IgM antibody was constructed into chimeric IgG1 form, our success give a hint about how to construct a IgG1-type chimeric antibody from IgM-type murine antibody to preserve its binding activity.

Keywords: Computer-guided homology modeling; Chimeric antibody; Complement-dependent cytotoxicity; Single chain Fv

1. Introduction

A novel murine IgM-type anti-human CD20 mAb 1–28 was obtained in our Lab previously (Li et al., 2002), which can induce apoptosis and inhibit proliferation of Daudi and Raji cells. Murine antibody would not be chosen as a therapeutic mAb due to human anti-mouse antibody (HAMA) responses in the clinical use (Miller et al., 1983; Schroff et al., 1985). So far, a partial solution to the problem is to construct chimeric antibodies by coupling the murine antigen-binding variable domains to human constant domains (Boulianne et al., 1984; Morrison et al., 1984; Neuberger et al., 1985). In our previous study, a chimeric antibody C1–28, which contained murine antibody 1–28 variable domains and human IgG1, *k* constant domains, was constructed. However, the chimeric antibody C1–28 lost its binding activity (Wang et al., 2006). In 1994, Zebedee et al. had been described that mouse-human IgG1 chimeric antibody (ch2D10) reconstructed from IgM-type antibody had significantly lower binding affinity than its parent murine antibody (m2D10), presumably for loss of binding avidity during switching from IgM to IgG (Zebedee et al., 1994). The reasons for decreasing of C1–28 binding activity are not clear, but the enhanced dissociation tendency of Fv when constant region changed may be one of them. So, how to humanize a murine IgM antibody into a functional antibody was discussed here.

A series of experiment results have been suggested that many native Fv antibody fragments have insufficient stability because the V_H and V_L domains can rapidly dissociate, which is usually accompanied by aggregation (Glockshuber et al., 1990). Therefore, different strategies have been developed to stabilize the

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association of these domains (Glockshuber et al., 1990). The commonly used solution is to connect the V_H and V_L domains by a short peptide linker between the carboxyl-terminus of one domain and the amino-terminus of the other to form a single-chain Fv (scFv) antibody fragment (Bird et al., 1988; Huston et al., 1988). The improved stability of scFv antibody molecules compared with Fv fragments is mainly achieved by preventing free dissociation of the V_H and V_L domains and thus increasing their local concentrations during the association reaction rather than by a structural effect (Schmiedl et al., 2000). Linkage of murine scFv molecules through a hinge region with human constant domains has been initially described by Hu and colleagues (Hu et al., 1996). Pioneering work demonstrated the proof of priniciple that such molecules retain functional properties (Hu et al., 1996; Shan et al., 1999; Hombach et al., 2000; Li et al., 2001). In the present study, based on the computer-guided homology modeling and molecular docking method, the 3D complex structure of Fv fragment from IgM-type murine antibody 1–28 was analyzed theoretically. The relationship between structure and stability of 1–28 variable regions was studied theoretically and a new chimeric anti-CD20 antibody scFv-Ig named 5S was designed. The 5S antibody was produced using PCR and recombinant DNA technologies, and its activity was determined.

2. Materials and methods

2.1. Computer-guided molecular modeling

Models of murine mAb 1–28 V_H and V_L domains were constructed using known Fab crystal structures from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) based on computer-guided homology modeling method (InsightII 2000 program, Biosym Technologies, San Diego). The structure was optimized using Discover program (Biosym Technologies, San Diego) under the CVFF forcefield. Considering the surface electrostatic potential distribution and structural character, the 3D complex structure of 1–28 Fv fragment was constructed using Docking method (InsightII 2000 program, Biosym Technologies, San Diego). On the basis of the homology modeling method, the 3D structure of murine 1–28 ScFv fragment was constructed and optimized under CVFF forcefield.

2.2. Construction of scFv-Fc

The variable domain genes of murine mAb 1–28 were cloned into the pGEM-T vector (Promega) and sequenced as described before (Wang et al., 2005). V_H and V_L were ligated with linker peptide $(Gly_4Ser)_3$. The V_H gene was generated with an upstream *Pvu* II cleavage site with primer P-1 and a downstream *BstE* II cleavage site with primer P-2. The $(Gly_4Ser)_3$-V_L gene was generated with upstream primer P-3 and downstream primer P-4. P-3 was used to introduce a *BstE* II cleavage site and an *EcoRV* cleavage site with nucleotide sequence encoding $(Gly_4Ser)_3$ among them at the amino terminus of V_L. P-4 was designed to give *Xho* I and *Eco*47 III at the carboxyl terminus of V_L. Primers used above were displayed in the Table 1. V_H product was digested with *Pvu* II and *Bst*E II ligated into the pCMV-V_H plasmid and transformed to the *E. coli* strain JM109. The sequence of the construct was confirmed by DNA sequence analysis. And then V_L product was cleaved with *BstE* II and *Eco*47 III, ligated into pCMV-V_H plasmid containing V_H gene and transformed to *E. coli* strain JM109. And the sequence of the construct was confirmed as above (Fig. 1).

Table 1
Primer used in this study

P-1	gaggtg*cagctg*caggagtcaggacctagc
P-2	caagggactct*ggtcacc*gt
P-3	ct*ggtcacc*gtctcctctggcggaggtggaagcggtggtggcggttccggaggcggaggatca-*gatatc*gttctcacccagtc
P-4	aggcagg*agcgct*gagcctggcttccagcagacaccctccctccctgtgctggcctctcacctttgat*ctcgag*cttggtc

Restriction sites were indicated as italic letters; Nucleotide sequence underlined encoding $(Gly_4Ser)_3$ linker peptide.

2.3. Transient transfection in 293Tcells

The 293T human embryonic kidney cells (American Type Culture Collection, USA) were seeded at a density of 3×10^5 cells per 3 cm diameter culture dish 36 h before transfection without penicillin and streptomycin added. For each dish, 4 μg plasmid DNA and 4 μg Lipofectamine™ Reagent (Invitrogen, USA) were diluted into 250 μL serum-free DMEM respectively, and mixed together within 5 min, incubated for another 20 min. Removed the culture medium from dishes and washed gently with serum-free DMEM once and then added in the mixed solution containing DNA and Lipofectamine™ Reagent. Six hours later, removed the solution from the transfected cells and DMEM containing 10% fetal bovine serum (FBS) was added. Twenty-four hours later, the culture medium was replaced with serum-free medium (Hyclone). Incubation was continued for 2 days at 37 °C. The supernatant was collected.

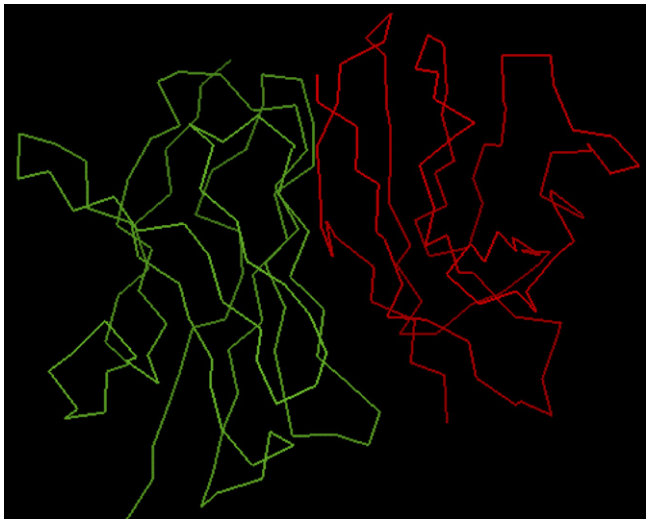

Fig. 1. The spatial conformation of anti-CD20 antibody variable domains derived from homology, mechanism optimum and molecular docking based on model proteins, wherein the green denoted the heavy chain and the red denoted the light chain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)