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Construction, Purification, and Characterization of Anti-BAFF scFv–Fc Fusion Antibody Expressed in CHO/dhfr$^-$ Cells

Meng Cao · Peng Cao · Huaijiang Yan · Wuguang Lu · Fang Ren · Yunlong Hu · Shuangquan Zhang

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Abstract Elevated levels of B-cell-activating factor of the tumor necrosis factor family (BAFF) have been implicated in the pathogenesis of autoimmune diseases in human. In this study, we have constructed a vector for the expression of a novel compact antibody composed of anti-BAFF single-chain antibody fragment (scFv) and the Fc region (the hinge region, CH2, and CH3 domains) of human IgG1 in Chinese hamster ovary cells. The scFv–Fc fusion protein, showing spontaneous Fc fragment-mediated homodimerization via disulfide bridges, was affinity-purified on protein A Sepharose from culture supernatant. The scFv–Fc antibody was demonstrated to retain high binding affinity to antigen and prolonged clearance time in blood and to possess some human IgG crystallizable fragment effector functions such as protein A binding and antibody-dependent cellular cytotoxicity. These results suggest that this recombinant antibody may have therapeutic applications in the therapy of autoimmune disorders mediated by BAFF.

Keywords BAFF · CHO cells · Recombinant antibody · scFv–Fc · Stable expression

M. Cao · H. Yan · W. Lu · F. Ren · Y. Hu · S. Zhang (✉)
Jiangsu Province Key Laboratory for Molecular and Medical Biotechnology, Life Sciences College, Nanjing Normal University, Nanjing, Jiangsu 210046, People's Republic of China
e-mail: zhangshuangquan1640@yahoo.com.cn

P. Cao
Laboratory of Molecular Medicine, Jiangsu Province Institute of Traditional Chinese Medicine, Nanjing, Jiangsu 210028, People's Republic of China

S. Zhang
Jiangsu Province Engineering Research Center for Biomedical Function Materials, Life Sciences College, Nanjing Normal University, Nanjing, Jiangsu 210046, People's Republic of China

M. Cao
Department of Microbiology, Parasitology and Immunology, College of Basic Medical Science, Southeast University, Nanjing, Jiangsu 210009, People's Republic of China

Abbreviations

BLyS	B lymphocyte stimulator
BAFF	B-cell activating factor belonging to the TNF family
BSA	Bovine serum albumin
FBS	Fetal bovine serum
Ab	Antibody
TALL-1	TNF- and ApoL-related leukocyte-expressed ligand 1
THANK	TNF homologue that activates apoptosis, nuclear factor-kB, and c-Jun NH2 terminal kinase
TNF	Tumor necrosis factor
Fc	Crystallizing fragment, consists of CH2 and CH3, glycosylation site, not antigen binding, but responsible for effector functions
G418	Neomycin
VH	Immunoglobulin heavy-chain variable region
VL	Immunoglobulin light-chain variable region
CH2 and CH3	Immunoglobulin heavy-chain constant regions 2 and 3
HRP	Horseradish peroxidase
mAb	Monoclonal antibody
TBST	Tris-buffered saline Tween-20

Introduction

B-cell-activating factor belonging to the tumor necrosis factor (TNF) family (BAFF), also known as BLyS, TALL-1, THANK, and TNFSF13B, is a novel member of the TNF ligand family, which plays a critical role in B lymphocyte maturation, survival, and differentiation [1–5]. BAFF is a type II trans-membrane protein, which can be expressed as a surface membrane-bound molecule or secreted from cells as a soluble ligand. Several lines of evidence suggest that elevated levels of BAFF may be involved in the pathogenesis of B-cell-mediated autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis, and Sjögern's syndrome [6–9]. BAFF has been proposed to be a biomarker for SLE disease activity [10]. In this respect, development and production of antibodies specific for BAFF would be useful for therapeutic applications in autoimmune diseases and for immunodiagnostic prediction.

We recently isolated a novel neutralizing human anti-BAFF single-chain antibody fragment (scFv) from a phage antibody library [11]. The small size of scFv, its rapid clearance from blood, and its tumor penetration properties make it the format of choice for tumor targeting and for radioimmunoimaging applications [12]. However, for other uses, it would be desirable to transfer the antigen-binding properties of the scFv onto a full-length IgG to take advantage of avidity effects, effector functions, and the prolonged serum half-life of an immunoglobulin. One approach is to directly engineer a phage displayed scFv into a full-length IgG and to express it in mammalian cells [13]. However, this approach requires separate cloning steps for the VH and VL domains. An alternative approach is to engineer the scFv into an IgG-like structure [14], which can be accomplished in a single cloning step and which can be expressed in mammalian cells such as Chinese hamster ovary (CHO) cells [15, 16]. By the use of the dihydrofolate reductase (dhfr)-mediated gene amplification system, the exogenous genes can be amplified in dhfr-deficient CHO cells for the high-level expression of recombinant proteins [17–19].

In the present study, we describe the construction, expression in dhfr-deficient CHO cells, purification, and characterization of an anti-BAFF scFv–Fc fusion (where the anti-BAFF scFv is fused to the hinge, CH2, CH3, domains of human IgG1). The results reveal that the recombinant antibody combined the affinity and specificity of the scFv with Fc-mediated bivalency, prolonged serum half-life, and antibody-dependent cellular cytotoxicity (ADCC).

Materials and Methods

Construction of Expression Vector

In a previous paper [11], we reported the isolation of a novel human anti-BAFF scFv selected from the Griffin.1 phage antibody library. The cDNA coding for the anti-BAFF scFv was amplified from the vector pHEN2 by polymerase chain reaction (PCR). The Fc fragment (hinge, CH2, and CH3; AF150959, NCBI Nucleotide) of human IgG1 was amplified from a baculovirus expression plasmid (provided by Dr. Zhinan Xia, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, USA). Fusion of gene segments, encoding the anti-BAFF scFv and the human IgG1 Fc, were produced by overlap PCR. Subsequently, to achieve high-level antibody secretion, the signal peptide sequence from a murine kappa light chain was linked to the N-terminus of scFv–Fc by primer extension PCR [20]. The resulting PCR product was then digested with *Hin*dIII and *Eco*RV (Takara, Japan) and inserted, in frame, into the *Hin*dIII and *Eco*RV sites of pcDNA3.0 expression vector (Invitrogen, USA). Finally, plasmid integrity was confirmed by endonuclease restriction digestion assays and further confirmed by DNA sequencing. The resulting plasmid was named pcDNA3–scFv–Fc (Fig. 1).

Cell Line, Cell Culture, and Transfection

CHO/dhfr$^-$ cells (CRL-9096, ATCC) were kindly supplied by Dr. Hu (Nanjing Chuanbo Biotechnology Incorporation, China). Prior to transfection, CHO/dhfr$^-$ cells were grown in α-MEM supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified

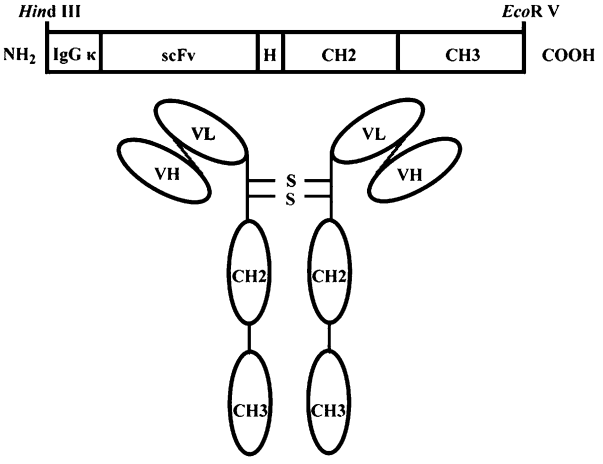

Fig. 1 Schematic diagram of the scFv–Fc fusion protein. *VH* and *VL* Heavy- and light-chain variable domain, respectively, derived from a scFv fusion-phage clone; *H* hinge region of a human IgG1 containing two disulfide bridges; *CH2* and *CH3* second and third constant domains of a human IgG1, respectively

atmosphere of 5% CO_2 and split when they reached 70–80% confluency by trypsinization. To express the anti-BAFF antibody, CHO/dhfr$^-$ cells were co-transfected with pcDNA3–scFv–Fc (20 μg) and pSV2–dhfr (37146, ATCC; 5 μg), which encodes the wild-type dhfr gene (provided by Dr. Hu), using Lipofectamine 2000 (Invitrogen, USA), according to the manufacturer's protocol.

Following transfection, cells were cultured in non-selective medium for 3 days, and then the culture supernatant was tested by western blot analysis. Drug selection was carried out by seeding 10^5 cells per well in six-well tissue culture plates (Corning, USA) containing α-MEM, lacking ribonucleosides and deoxyribonucleosides, 10% dialyzed FBS and G418 (500 μg/ml; all from Invitrogen). G418 was used only in this first selection. After 2 weeks, transformed colonies were isolated using cloning cylinders and subjected to increasing levels of methotrexate (MTX; Sigma, USA), 0.005, 0.02, 0.08, 0.32, 1.0, 4.0, and 10 μM. The culture supernatant from MTX-resistant colonies was screened for the secretion of the fusion antibody by enzyme-linked immunosorbent assay (ELISA) using 96-well plates coated with hsBAFF [21]. The highest-producing clone was chosen for laboratory production.

Purification and Characterization of Anti-BAFF Antibody

After screening the clones for antibody production, the highest-producing clone was transferred into CHO–S–SFM II serum-free medium (Invitrogen, USA), supplemented with 3% dialyzed FBS. When the cells reached the logarithmic growth phase, the culture medium was changed to the same medium from which serum had been omitted. The supernatant was harvested and centrifuged at 7,000×*g* to remove dead cells, and the pH of the supernatant was adjusted to 8.0 with 1 M Tris–HCl, pH 8.8. The supernatant was applied to protein A Sepharose columns (GE Healthcare, Sweden) that had been previously equilibrated with binding buffer (0.025 M Tris–HCl, 0.15 M NaCl, pH 8.0). The column was washed with binding buffer, and protein was then eluted with eluting buffer (0.1 M glycine–HCl, pH 3.5). The eluted fractions were collected in tubes containing neutralizing buffer (1 M Tris–HCl, pH 8.8) to adjust the pH to approximately 7.0. Finally, purified samples were dialyzed against phosphate-buffered saline (PBS) and concentrated fivefold with a Centricon 30 (Millipore, USA).

Purity of the eluted antibody fraction was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 12% gels under reducing or non-reducing conditions. Bands were visualized by Coomassie brilliant blue staining.

The purified antibody was also transferred to nitrocellulose membrane (GE Healthcare, Sweden) for 1.5 h at 300 mA in 50 mM Tris–HCl (pH 8.0), 150 mM glycine. After blocking for 1 h at 30 °C with Tris-buffered saline Tween-20 (TBST; 25 mM Tris–HCl, 125 mM NaCl, 0.1% Tween-20, pH 8.0) containing 5% skimmed milk, membrane was then incubated directly with a 1:1,000 dilution of HRP-conjugated goat anti-human IgG1 Fc Ab (KPL, USA) for 1 h. Specific binding was detected with 3,3′,5,5′-tetramethyl benzedrine chemiluminescence (Promega, USA).

Binding Activity of scFv–Fc Detected by ELISA

The reactivity of scFv–Fc to soluble BAFF antigen was determined by ELISA. Flat-bottom microtiter plates (Corning, USA) were coated overnight at 4 °C with soluble BAFF at a fixed concentration of 5 μg/ml or at various concentrations of 0.04–100 μg/ml in 50 mM carbonate bicarbonate buffer (100 μl/well) at pH 9.6. After washing in TBST and blocking

with 5 % BSA, the plates were incubated for 1 h with varying concentrations, 0.04–100 μg/ml or with a fixed concentration of 5 μg/ml of the scFv–Fc or scFv antibodies prepared according to the procedure of Cao et al. [11], or with human IgG1 or BSA (negative control; all from Sigma, USA) diluted in TBST at room temperature. After washing five times with TBST, the plates were incubated for 1 h with HRP-conjugated monoclonal Ab, diluted 1:1,000 in TBST [HRP-conjugated goat anti-human IgG Fc Ab (KPL Cat. 04-10-20, USA) for scFv–Fc and human IgG1, HRP-conjugated mouse anti-c-myc Ab for scFv (Santa Cruz Biotechnology, USA)]. The unbound conjugates were removed by washing with TBST, and then, 100 μl/well *o*-phenylenediamine (0.4 mg/ml in 0.05 M phosphate-citrate buffer, pH 5.0) was added to each well. The reaction was stopped using 1 M H_2SO_4, and the absorbance at 490 nm was measured by an ELx808 Microplate Reader (Bio-Tek, USA).

Flow Cytometric Analysis

To determine if the scFv–Fc can specially recognize membrane-bound BAFF, a cell line, K-562 (CCL-243, ATCC), expressing membrane-bound BAFF [22], and a negative control line, HEK-293 (CRL-1573, ATCC), were used. K-562 cells were grown in RPMI 1640 supplemented with 10% FBS. HEK-293 cells were maintained in Dulbecco's modified eagle's medium containing 10% FBS. All cells were cultured at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Approximately 5×10^5 cells in tissue culture plates (Corning, USA) were incubated with purified scFv–Fc or with human IgG1 Ab (5 μg/ml final concentration) for 30 min on ice. After washing three times with PBS, bound protein was detected using fluorescein isothiocyanate-conjugated goat anti-human IgG Fc antibody (KPL, USA). The fluorescence exhibited by stained cells was measured using a BD FACSCalibur (Becton Dickson, USA).

Measurement of the Binding Affinity of scFv–Fc for BAFF

The dissociation constants of anti-BAFF mAb (ABL-1) from hybridoma and scFv–Fc were determined by competition ELISA [23]. The equilibrium dissociation constants (K_D) were calculated from the Scatchard plot.

In Vivo Stability of scFv–Fc

Male CD-1 (ICR) BR mice (6–8 weeks old, four animals per group, obtained from the Laboratory Animal Center of Nanjing Medical University) were kept under SPF conditions and provided with standard rodent chow and water ad libitum at least 2 weeks before starting the experiments. All mice were maintained and cared for according to the guidelines of the Institutional Animal Care and Use Committee. Animals were injected intravenously with 1 mg/kg of purified scFv–Fc or scFv, respectively. Blood samples were collected at 3, 6, 24, 48, and 72 h after antibody administration, and the scFv–Fc or scFv serum concentration was measured by ELISA, as described above.

Effector Function Assay

Human effector cells, peripheral blood mononuclear cells (PBMCs), were separated from the blood of healthy adult humans on Ficoll density gradients. Target K-562 cells (1×10^4) in 50 μl of complete medium per well were co-cultured with various numbers of effector

PBMCs in 100 μl of medium per well. Different concentrations of the scFv–Fc fusion antibody were added to each well (final volume per well, 200 μl). Cultures were performed in triplicates, and plates were incubated for 4 h at 37 °C, after which plates were centrifuged, and 100 μl of supernatant was carefully removed from each well and transferred to corresponding wells of a flat-bottom microtiter plate. The lactate dehydrogenase (LDH) activity was determined using a CytoTox 96 cytotoxicity kit (Promega, USA), according to manufacturer's instructions. The absorbance of the supernatants was measured at 490 nm, and the percent cytotoxicity was calculated as follows: percent cytotoxicity $=[(A-B)/(C-B)] \times 100$, where A is the mean absorbance of the supernatants from the test cultures, B is the mean absorbance of the supernatants from cultures containing only target cells (spontaneous LDH release), and C is the mean absorbance of supernatants from cultures containing target cells cultured in the presence of 2% Triton (maximum LDH release).

Results

Construction, Transfection, and Purification

We constructed a compact antibody generated by joining anti-BAFF scFv with the human IgG1 Fc fragment at the hinge region (Fig. 1a). The predicted structure of this antibody is shown schematically in Fig. 1b. It is composed of two scFv–Fc monomers, and the molecular weight is approximately 110 kDa, as calculated by its amino acid sequence. Two monomers were joined together using three disulfide bonds in the hinge region of Fc.

CHO/dhfr$^-$ cells were co-transfected with pcDNA3/scFv–Fc and pSV–dhfr by lipofection. Transfectants were first analyzed for secreted protein in the supernatant by western blot analysis. In order to generate stable, highly expressing, recombinant scFv–Fc cell lines, the transfected cells were selected with G418 and MTX, and the culture supernatants of resistant clones were screened by specific ELISA. After drug selection, one clone showed significantly elevated antibody expression levels at approximately 18~19 μg/ml (Fig. 2). This clone was then grown in suspension culture, in serum-free medium, to produce scFv–Fc.

Since the recombinant antibody contained the human IgG1 Fc fragment, the antibody from the supernatant was successfully purified in one step by affinity chromatography using

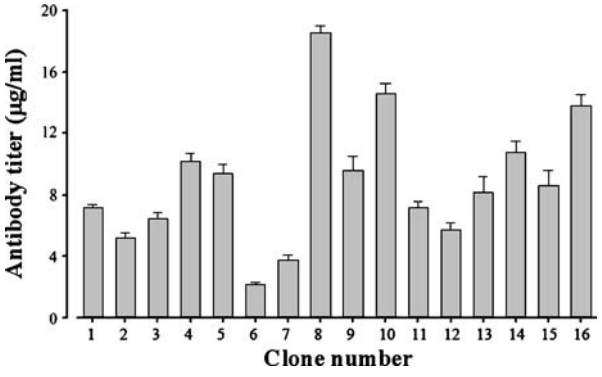

Fig. 2 Antibody titer of clones selected at various MTX concentrations. The recombinant antibody expression level in the supernatants was assayed by ELISA. Results are shown as mean ± SEM of triplicate determinations

protein A Sepharose. The purified scFv–Fc was analyzed by SDS-PAGE and western blotting. Because the Fc region of human IgG1 introduced into the scFv–Fc fusion protein contained a hinge region, scFv–Fc is expected to form an internal S–S linked dimer. Therefore, scFv–Fc should show a single band of monomer size under reducing conditions and a band of dimer size under non-reducing conditions. As shown in Fig. 3, lane 1, SDS-PAGE analysis under reducing conditions (addition of 5% β-mercaptoethanol) revealed a single band of ~55 kDa. The molecular weight of the dimeric scFv–Fc under non-reducing conditions (Fig. 3, lane 2) was not strictly twice that of the monomer under reducing conditions. The gel migration of the homodimer was slower than expected. This is possibly due to high glycosylation of the antibody due to expression in CHO cells and/or a structural effect during electrophoresis. The dimeric nature of scFv–Fc was also confirmed by molecular weight analysis on Superdex S-200 gel filtration vs. molecular weight standards (data not shown). Therefore, the above results indicate that the S–S bonds of scFv–Fc were correctly constructed, as predicted from the sequence.

Detection of scFv–Fc Antigen Binding

The immunoreactivity of scFv–Fc against soluble BAFF was examined by ELISA. When plates were coated with a fixed concentration of soluble BAFF, scFv–Fc bound in a dose-dependent manner, similar to the binding of scFv to soluble BAFF (Fig. 4a). An additional ELISA test was performed in which a concentration gradient of soluble BAFF was titrated against a fixed concentration of scFv–Fc. A similar dose–response relationship was observed (Fig. 4b). In addition, scFv–Fc was also confirmed to bind to soluble BAFF by western blot analysis (data not shown).

The specificity of scFv–Fc to recognize membrane-bound BAFF on the surface of cells was examined by flow cytometry using the K-562 myelogenous leukemia cell line, which has been previously characterized to express membrane-bound BAFF [22]. As shown in

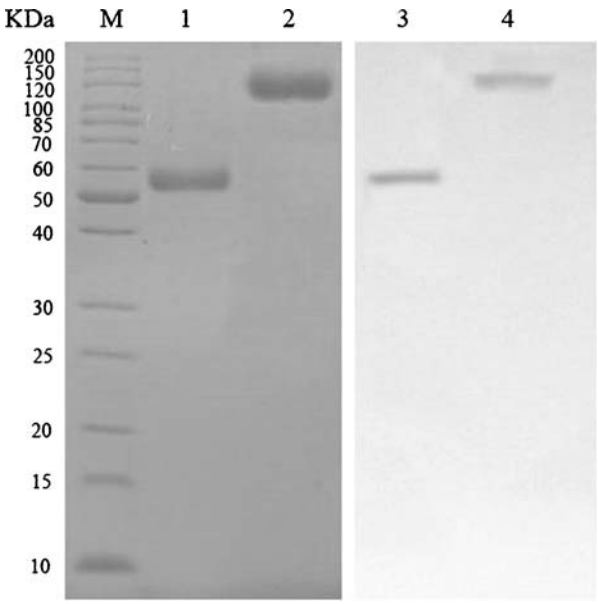

Fig. 3 SDS-PAGE analysis of purified scFv–Fc. Protein ladder is shown in the *left lane*, while scFv–Fc was run under reducing (*lane 1*) and non-reducing (*lane 2*) conditions. *Lanes 3 and 4* Western blot analysis of the sample run in *lanes 1* and *2* detected with HRP-conjugated anti-human IgG1 Fc Ab

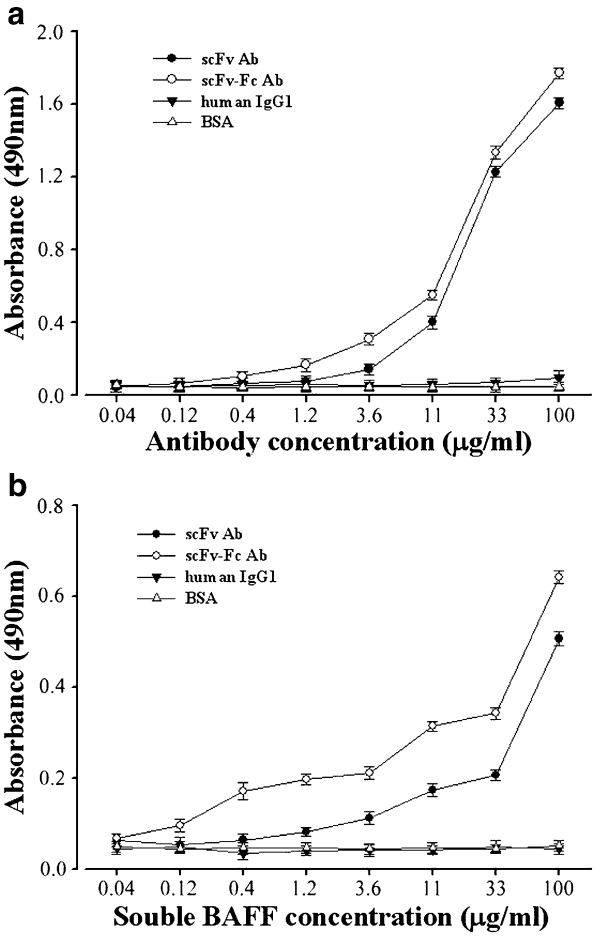

Fig. 4 Antigen-binding analysis by ELISA. **a** Various concentrations of each antibody were added to 96-well plates coated with 5 μg/ml of soluble BAFF. **b** Five micrograms per milliliter of each antibody was added to 96-well plates coated with various concentrations of soluble BAFF. Binding was detected with HRP-conjugated anti-human IgG Fc (for scFv–Fc and human IgG1 Ab) and HRP-conjugated anti-c-myc (for scFv Ab). The binding activity was measured as absorbance at 490 nm produced by peroxidase. Data of each point are shown as mean ± SEM of triplicate determinations

Fig. 5, scFv–Fc specifically bound to the BAFF positive cells, but not to the negative control cell lines HEK-293.

Antibody Affinity Measurement

The quantitative affinity of scFv–Fc for recombinant BAFF was determined by competition ELISA [24]. The results for the binding of scFv–Fc to BAFF are shown in Fig. 6 according to the Scatchard equation. The K_D value of scFv–Fc was calculated to be 5.2×10^{-9} M. The calculated affinity was slightly inferior to that of the intact mAb from hybridoma (2.5×10^{-9} M).

In Vivo Stability of scFv–Fc

It is known that scFv has a very short clearance time in vivo compared with the whole antibody [14, 25]. Since Fc-fused scFv has a similar structure to that of the whole antibody, it may have a prolonged half-life in blood. For the measurement of the clearance of scFv–Fc and scFv in vivo, the proteins were injected into mice. Figure 7 shows the time courses of scFv concentrations in blood after injection. While the concentration of scFv decreased

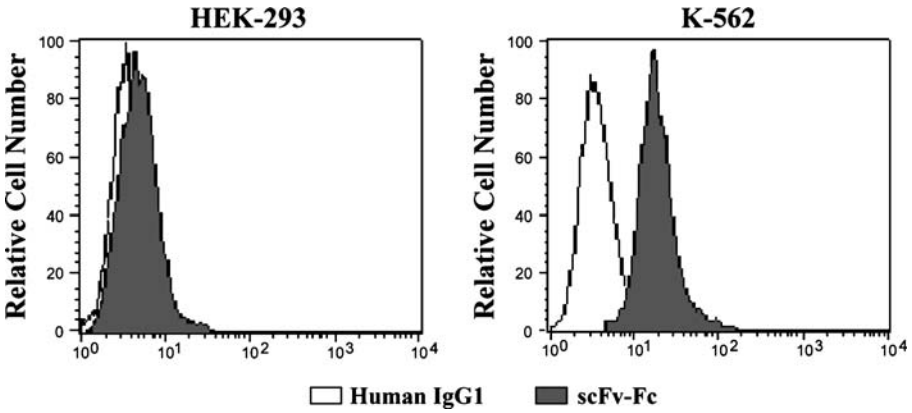

Fig. 5 Flow cytometry analysis of scFv–Fc Ab binding to membrane-bound BAFF. HEK-293 cells or K-562 cells were incubated with scFv–Fc or with human IgG1, followed by detection with FITC-conjugated goat anti-human IgG Fc. *Unshaded areas* Cells incubated with human IgG1. *Shaded areas* Cells incubated with scFv–Fc

rapidly in the serum within 24 h, scFv–Fc could be detected in the serum 72 h after injection. It is concluded that scFv–Fc was partially stabilized in blood compared with scFv.

Effector Function of scFv–Fc

The scFv–Fc fusion protein was assayed to determine if the Fc domain of the fusion was functionally able to direct ADCC toward antigen expressing target cells. The results of the cytotoxicity effect of the Fc fragment of the fusion antibody are shown in Fig. 8. The killing of the target cells occurred in a dose-dependent manner and reached a maximum at the antibody concentration of 1 μg/ml.

Discussion

In a previous study, we reported on a human single-chain antibody fragment against BAFF [11]. Single-chain antibody fragment is best suited to tumor targeting due to superior tissue

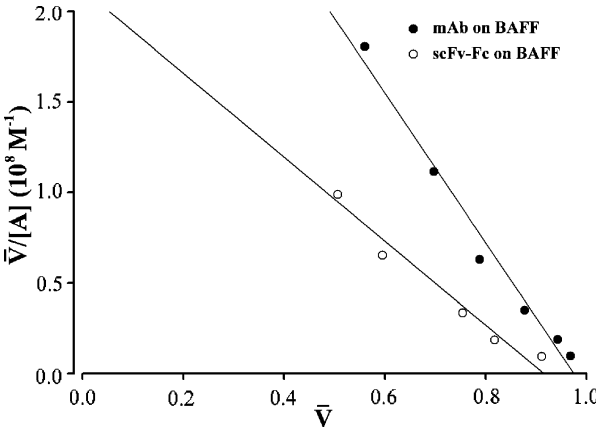

Fig. 6 Scatchard plots of the binding of the scFv–Fc or ABL-1 mAb to recombinant BAFF measured by the competitive ELISA. *V* is the fraction of bound antibody and *[A]* (10^{-8} M) is the concentration of free antigen at equilibrium. Each experiment was repeated, and all samples were analyzed at least triplicate. Equilibrium was achieved at 28 °C

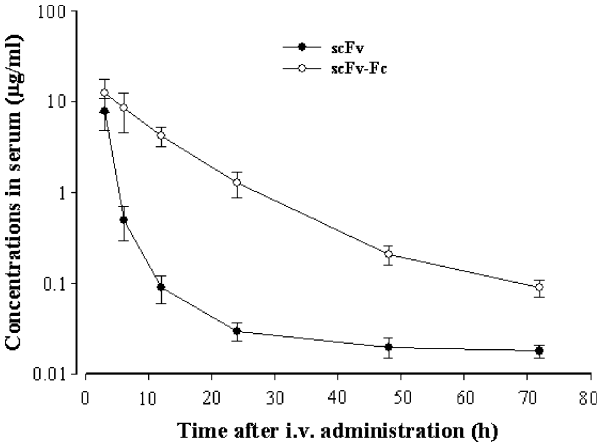

Fig. 7 Clearance pattern of antibody from mouse blood. Groups of four mice were bled at different time points, and antibody serum concentrations were quantified by specific ELISA. Data of each point are shown as mean ± SEM, n=4

penetration [26, 27]. However, for other uses, the scFv fragment retains a number of limitations. Fc fragments have, therefore, frequently been utilized as fusion partners to provide chimeric construction for a variety of applications. There are several advantages in producing recombinant proteins as fusion proteins with the Fc region of human IgG. Firstly, the Fc region increases the stability of the fused protein through its prolonged serum half-life, which is particularly important for efficient in vivo antigen neutralization. In addition, the proteins produced are usually in the form of an antibody-like dimeric molecule. Since affinity to an antigen is strengthened by the bivalency of antibodies, a similar effect can be expected for the Fc-fused proteins. Finally, another interesting feature of Fc-fused proteins is that they have strong affinity to staphylococcal protein A or streptococcal protein G and are, therefore, easily purified using affinity procedures [25, 28, 29].

The efficacy of a therapeutic antibody is critically dependent on appropriate post-translational modifications (PTM), in particular, glycosylation [30]. Glycosylation is the most common PTM of proteins in eukaryotic cells and has profound effects on antibody effector function and pharmacokinetics [16]. Thus, for the production of therapeutic recombinant antibody, mammalian expression systems are preferred to bacterial expression systems [31]. Currently, the most widely used mammalian expression system is the gene amplification procedure using dhfr-deficient CHO cells [32, 33]. In this system, the

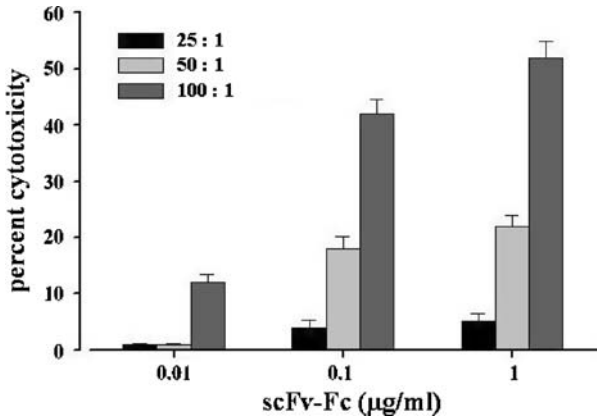

Fig. 8 ADCC assays of the scFv–Fc fusion protein toward target cells. The K-562 target cells were co-incubated with peripheral blood mononuclear effector cells at 25:1, 50:1, and 100:1 ratios in the presence of scFv–Fc at 0.01, 0.1, and 1 μg/ml. Percent cytotoxicity of the fusion antibody was measured as described in the "Materials and Methods". Results are shown as mean ± SEM of triplicate determinations

antibody genes are transfected on plasmids bearing the G418 resistance gene as a selectable marker. A plasmid bearing the dhfr gene is co-transfected. After selection of plasmid bearing genes with G418, the gene copy number is amplified by culturing the cells under increasing concentrations of MTX. Only cells that produce increased amounts of dhfr will survive this treatment. When the dhfr genes are amplified, other neighboring genes are often co-amplified so that after each round of amplification, cells are subcloned to search for clones with increased antibody production rates. In this work, we constructed a vector for the expression of scFv fused to human IgG1 Fc (hinge, CH2, and CH3) to produce scFv–Fc recombinant antibody in CHO/dhfr$^-$ cells. The recombinant antibody was isolated from the culture supernatant by protein A affinity chromatography and was analyzed. The results of SDS-PAGE and western blot analysis showed that the product was a monomer under reducing conditions (Fig. 3 lane 1) but formed homodimers under non-reduced conditions, using disulfide bonds in the hinge region (Fig. 3, lane 2). These results suggest that scFv–Fc is a dimeric antibody, similar to whole antibody molecules. This could be an important factor for antigen-binding activity and for structural stability of scFv–Fc.

The in vitro binding characteristics of scFv–Fc to soluble BAFF were assayed by ELISA. scFv–Fc exhibited strong binding activity toward soluble BAFF, indicating that the dimeric structure of scFv–Fc may contribute to strong antigen affinity. Recently, a fully human monoclonal antibody against BAFF (LymphoStat-B) has entered clinical trials [34]. LymphoStat-B was generated from a native human Ig library and can only recognize soluble BAFF. Flow cytometry results showed that scFv–Fc can recognize not only soluble BAFF but also the membrane-bound BAFF. In fact, the extract nature of membrane-bound BAFF is not fully understood, so it would be of interest to determine in vivo activity by using scFv–Fc. At the same time, affinity test (Fig. 6) showed that the scFv–Fc exhibited the same specificity and affinity to BAFF as the IgG ABL-1 mAb from hybridoma. The quantitative affinity of scFv–Fc for recombinant BAFF was determined by competitive ELISA in the Scatchard analysis. The calculated affinity of scFv–Fc was slightly inferior to that of the mAb; this might be due to the amino acid sequence difference in the scFv–Fc and ABL-1 mAb. However, our fully human antibody would avoid inducing the human anti-mouse antibody response when used in humans. As shown in Fig. 7, scFv rapidly disappeared from serum within 24 h, but scFv–Fc was detected in serum 72 h after injection. Previously, Huston et al. [26] indicated that a typical scFv had a short serum half-life $t_{1/2}$ of the beta phase of clearance of only 3.5 h in mice. Powers et al. [14] showed that scFv–Fc produced by a methylotrophic yeast had a prolonged serum half-life $\left(t_{1/2} = 37 - 93\,\mathrm{h}\right)$ in mice. In our stability assay, scFv–Fc had a significantly improved clearance pattern compared to that of the scFv. The stability in vivo may be attributable to the bivalent structure, and the prolonged in vivo serum half-life of scFv–Fc is an important characteristic for therapeutic use.

We were also interested in determining whether the scFv–Fc Ab retained immune effector functions mediated by Fc domains. ADCC is considered to be one of the major effector functions of therapeutic antibodies [35]. Our results demonstrate that scFv–Fc is capable of mediating ADCC, using human peripheral blood mononuclear cells as effectors. This finding suggests that the Fc region must be close in structure to the native profile. In summary, a human anti-BAFF antibody was used as an example of the production of scFv fused with human IgG1 Fc region in mammalian cells. The Fc-mediated dimerization of the anti-BAFF fusion antibody can strengthen antigen affinity, prolong serum half-life, and obtain the effector functions of native antibody such as ADCC. Therefore, these CHO cell-produced scFv–Fc fusion antibodies may be useful for the development of diagnostic and therapeutic applications.

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