Characterization of a Panel of Mouse Single-Chain Antibodies Against Human Recombinant Interferon β1b

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Abstract—A panel of single-chain Fv-antibodies (ScFv’s) against recombinant human interferon beta 1b (rhIFN-β1b) has been obtained from immune and naïve combinatorial cDNA libraries of the mouse variable immunoglobulin genes. ScFv’s were expressed in Escherichia coli cells. For producers isolated from the immune library a difference in production yield of ScFv’s in periplasm and incubation medium as well as their expression and storage stability have been demonstrated. After sequencing of target DNA the multiple align-ment and structural analysis of ScFv’s sequences with different primary structures were carried out and significant difference in both complementarity-determining (CDR) and framework (FR) regions of theirs variable domains has been shown. For the ScFv’s isolated from the immune library, specificity of their binding with native and denatured rhIFN-β1b in ELISA and Western-blotting as well as their high storage stability have been shown. The affinity constants for each representatives of the ScFv’s panel were in the range from $1.96 \times 10^{-8}$ to $1.69 \times 10^{-9}$ M.

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INTRODUCTION

The construction of large combinatorial libraries of cDNA of human and animal variable immunoglobulin genes as well as the possibility of isolating from these libraries immunoreagents with required antigen-binding properties has revealed essentially new possibilities for the use of monoclonal antibodies in biology and medicine [1, 2]. Single-chain antibodies (ScFv’s), obtained by translation of variable domains of heavy and light chains of immunoglobulins combined into a single gene, are the most common format for the construction of recombinant antibodies. Because of the features of its structure, ScFv’s retain the conformation of the active center of the natural antibody, assuring a high degree of specificity of their interaction with the antigen [3]. The basic advantage of ScFv’s derives from the fact that they can be produced in prokaryotic cells at relatively low cost.

The quality of the ScFv’s that are produced is determined by many characteristics, the most important of which are specificity, solubility, affinity, stability, and level of expression by the producer. Using the approaches of genetic and protein engineering, it is possible to obtain ScFv’s that recognize homologous proteins or different isoforms of the same protein with a high degree of selectivity. In combination with well-known methods of in vitro selection of combinatorial cDNA libraries, such as phage display or ribosome display, directed selection of ScFv’s against a particular antigenic determinant becomes possible. This is a very important step for determining the characteristics of biological molecules as well as for obtaining antibodies of diagnostic and therapeutic importance [1, 4].

In most cases, in selecting combinatorial cDNA libraries the principal objective is to produce ScFv’s with high affinity, specificity, and stability, particularly when further use of the ScFv’s in vivo is envisaged [1, 3]. If it is necessary to recognize several epitopes of the same antibody or different epitopes of closely adjacent antigens, an increase in affinity is achieved through the construction of multivalent forms of recombinant antibodies that possess identical or different antigen-binding centers in their structure, i.e., mini-antibodies or hetero-antibodies, respectively [5]. In a number of cases it becomes necessary to obtain ScFv’s which specifically recognize different epitopes or different conformational states of a target antigen and which are also characterized by high stability and by different binding characteristics. This makes it possible to significantly expand the potential range of application of ScFv’s, from chromatographic purification of a target antigen to its effective inactivation in vivo (in the case of viral or tumor antigens).

However, it is necessary to take into account the possibility of effective production of the resulting ScFv’s in prokaryotic cells, a process that is determined by the features of the primary structure of antibodies [3]. With this in mind, an important stage is to obtain
from a combinatorial library a panel of ScFv’s against a given antigen for the purpose of further selection of candidate molecules that will satisfy all the necessary requirements.

Human recombinant interferon β1b is an analog of a natural cytokine that has been approved by the United States Food and Drug Administration for treatment of multiple sclerosis and viral diseases [6] and in the treatment of certain forms of cancer [7]. The creation of panels of high-specificity ScFv’s against rhIFN-β1b would be an important step in the study of the properties of this antigen as well as for monitoring its concentration in vivo.

In a preceding study we created and described a combinatorial cDNA library of immunoglobulin V-genes from mice immunized with rhIFN-β1b [8]. The present study is concerned with generation a panel of ScFv’s against rhIFN-β1b from immune and non-immune cDNA libraries and determining their characteristics.

MATERIALS AND METHODS

A previously created cDNA immune library of mouse immunoglobulin V-genes [8] as well as a non-immune library kindly provided for the studies by A. Laman (Pushchino National Center, Russian Federation) were used to obtain the ScFv’s against rhIFN-β1b. Recombinant interferon β1b as well as lysate of cells producing rhIFN-β1b were obtained on PNDK from the firm of FarmBioteK (Kiev). The phage helper M13K07 and the strains E. coli TG1 and E. coli HB2151, Expression Module Recombination Phage Antibody System (GE Healthcare, United States) were used in the studies. The phagemid pCANTAB 5E and pHEN-1 were used for ScFv’s expression. Enzymes from the firm of Fermentas (Lithuania) were used in the molecular cloning procedures. Manipulation with DNA was performed in accordance with accepted methods [9]. The software, TotalLab 2.00 and OriginPro 7.0, were used to analyze the blots and for statistical processing.

Affinity selection of non-immune cDNA library of mouse V-genes. Four schemes were used to select ScFv’s against rhIFN-β1b. In schemes I–III, rhIFN-β1b (20 μg/ml) was immobilized in the wells of a polystyrene ELISA plate (Nunc, Denmark) and incubated for 14 h at 4°C. In Selection Scheme IV, rhIFN-β1b was presented for binding with the phages by means of immobilized polyclonal antibodies against rhIFN-β1b (Sigma, United States). Following blocking of the non-specific binding sites with a phosphate-buffered saline solution containing 0.1% Tween 20 (PBST), amplified recombinant phages (1011 cfu) were added to the PBST buffer and incubated for 2 h at 37°C. Elution of specifically binding phages was conducted by several different methods. Triethanolamine (0.1 M) was used in Scheme I, polyclonal antibodies against rhIFN-β1b in Scheme II, and rhIFN-β1b at a concentration of 50 μg/ml in Schemes III and IV.

The phages obtained were amplified in the E. coli TG1 strain and used for the subsequent four selection cycles according to a previously presented scheme.

The efficiency of affinity enrichment of the library was determined by means of Colony lift assay.

Immunoblotting of colony replicates. The phages obtained following affinity selection were used for infection of the E. coli TG1 strain, which was plated on the agar-contained medium 2YT containing 100 μg/ml ampicillin and 2% glucose (2YTAG). Immunochemical screening of the clones was conducted using a method we have described in a previous study (Colony lift assay) [8]. The monoclonal antibodies Anti-C-myc 9E10 (Sigma) were used as the primary antibodies. The immune complexes that formed detected by means of secondary antibodies conjugated with horseradish peroxidase, HRP/Anti Mouse Conjugate (IMTEK, Russian Federation); 4-chloro-1-naphthol (Sigma) was used as the substrate.

Cloning of ScFv-DNA in pCANTAB 5E. The pool of phagemids obtained following affinity selection of the non-immune library was used as a matrix for conducting the polymerase chain reaction (PCR) with the primers RS Primer Mix from Mouse Module Recombinant Phage Antibody System (GE Healthcare). The ScFv-DNA obtained (~750 bp) was hydrolyzed with the restrictases SfiI and NotI, ligated with the vector pCANTAB 5E, which after this was used for transformation of the E. coli TG1 strain.

Expression of ScFv’s in E. coli. E. coli clones that produce specific ScFv’s against rhIFN-β1b that had been isolated from the immune and non-immune libraries were grown at 30°C until A600 = 0.8. Induction of expression was conducted by the addition of isopropyl-β-D-thiogalactosid (IPTG) to a final concentration of 1 mM. Fermentation of ScFv’s was performed under conditions of intensive aeration at 30°C for 2–12 h. Following induction the cells were collected by centrifugation by means of centrifuging (10 min, 4000 g), supernatant was extracted, and a periplasm fraction was obtained for use in subsequent analysis in ELISA.

DNA sequencing. The nucleotide sequence of the ScFv’s was determined by Sanger’s method using the IBI Prism 3130 automatic sequenator (Applied Biosystems, United States). ScFv-DNA was obtained by PCR using the primers pCANTAB-R1 5’-d [CCATGAT-TACGCGAAGCTTGGAGCC]-3’ and pCANTAB-R2 5’-d [CGATCATAAGTTTGTCGTTTTCC]-3’ and recombinant phagemids of selected positive clones as matrix. The following primers were used to sequence the obtained PCR products (~1000 bp): pCANTAB 5-S3: 5’-d [GGTTCAGGCGGAGGTGGCTCTGG]-3’; pCANTAB 5-S4: 5’-d [CCAGGCCACCTCCGCT-GAACC]-3’; pCANTAB 5-S4: 5’-d [CAACGT-GAAATAATTATTTCCG]-3’; and pCANTAB 5-S6: 5’-d [GTAAATGATTTTCTGTATGAGG]-3’.

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