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Comparison of Polyclonal Anti-CD147 Antibody Production Using DNA Based and Phage-Displayed CD147 Immunizations

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ABSTRACT: Immunization with two different immunogens, i.e., plasmid encoding CD147 (pCDM8-CD147) and phage-displayed CD147 were compared for producing polyclonal antibodies. Two mice were injected with pCDM8-CD147 or phage-displayed CD147 at bi-weekly intervals and antibody responses were determined by indirect ELISA and immunofluorescence. The anti-CD147 antibodies could be detected in the immunized sera after inoculating with either pCDM8-CD147 or phage-displayed CD147. However, the antibody response induced by phage-displayed CD147 was much higher than that by pCDM8-CD147. These results suggested the possibility of using the phage display technique for preparing hyperimmune serum when purified protein antigens are difficult to obtain.

KEYWORDS: DNA immunization, phage display technology, phage immunization, CD147, polyclonal antibody.

DNA immunization refers to the induction of immune responses to a protein expressed *in vivo*, subsequent to the introduction of its encoding DNA[1,2]. Several investigators have demonstrated the feasibility of using a direct injection of plasmid DNA for the induction of protective immunity against various pathogens and the production of specific antibodies[3-5]. Recently, phage display technology has been developed and has proven to be a very powerful technique for production of proteins or peptides. The proteins or peptides are expressed as fusion proteins with a range of phage structural proteins[6-8]. The possibility of using phage expressing protein for induction of immune responses was shown to be effective[9]. The phage display carrier system increases the half-life of the peptide in the circulation and provides T-cell help resulting in the induction of a strong antibody response, even without an adjuvant[7].

CD147 is a leukocyte surface protein broadly expressed on hemopoietic and non-hemopoietic cell lines[10]. Within peripheral blood cells, CD147 is expressed on all leukocytes, red blood cells, platelets and endothelial cells[10]. The function of CD147 molecule is not fully understood. It may be involved in signal transduction and cell adhesion [11]. Recently, CD147 has been found to be involved in T cell regulation[12].

In this report, we have evaluated the efficiency of antibody induction by using DNA immunization and the phage display carrier system. Production of anti-CD147 antibodies has been used as a study model. The plasmid DNA encoding CD147 and the phage-displayed CD147 molecules were constructed and used as immunizing agents for BALB/c mice. Anti-CD147 antibody responses were studied and compared. These findings will provide an alternative and efficient method for producing hyperimmune serum when purified protein targets are difficult to obtain. In addition, it may be applied for production of monoclonal antibody.

Preparation of cDNA Encoding CD147 Molecule

cDNA encoding CD147 membrane protein, named pCDM8-CD147, was generated in our department[13]. To produce a large amount of the plasmid, pCDM8-CD147 was .transformed into *E. col* MC1061/p3. The plasmid DNA was then isolated from transformed *E. coli* by QIAGEN chromatography columns. Plasmid DNA prepared from 4 transformed colonies were digested with *Xba*I restriction enzyme, followed by agarose gel electrophoresis. All digested plasmid DNA contained an inserted fragment of 1.8 kb (data not shown). The DNA pattern was identical to an original *Xba*I digested pCDM8-CD147[13], which was used as a

control. A bacterial colony harboring pCDM8-CD147 was selected and propagated. The large amounts of pCDM8-CD147 were then prepared by using QIAGEN chromatography columns.

The COS cell expression system was employed to validate that the pCDM8-CD147 obtained encodes CD147 protein. The isolated pCDM8-CD147 was transfected into COS cells which were then stained with anti-CD147 mAb and isotype-matched control anti-CD4 mAb by indirect immunofluorescent assay[13]. A strong fluorescent signal was observed on the surface of pCDM8-CD147 transfected COS cells probed with anti-CD147 mAb but not with anti-CD4 mAb. These results indicated that the prepared pCDM8-CD147 was able to express the CD147 molecule in mammalian cells.

Preparation of phage-displayed CD147

The phagemid expressing CD147 was constructed by inserting the *Sfi*I digested part of CD147 gene into the phagemid pComb3HSS vector as described previously[14]. The constructed product, named pComb3H-CD147, was transformed into *E. coli* TG-1. The pComb3H-CD147-transformed *E. coli* was infected with VCSM13 helper phage to produce recombinant bacteriophages. During the assembly of progeny viruses, the CD147-gpIII fusion proteins were simultaneously incorporated into phage particles. The phages, carrying the CD147 molecule, were released into culture supernatant and harvested for determination of CD147 by sandwich ELISA. The recombinant phages strongly reacted with anti-CD147 mAb and M6-1D4, but not to the control mAb, MT54 (Fig. 1). Phages carrying GFP protein were used as a negative control. The GFP-phage control showed negative reactivity with M6-1D4 (Fig. 1). The results indicate that the generated phages carry CD147 molecule on the surface.

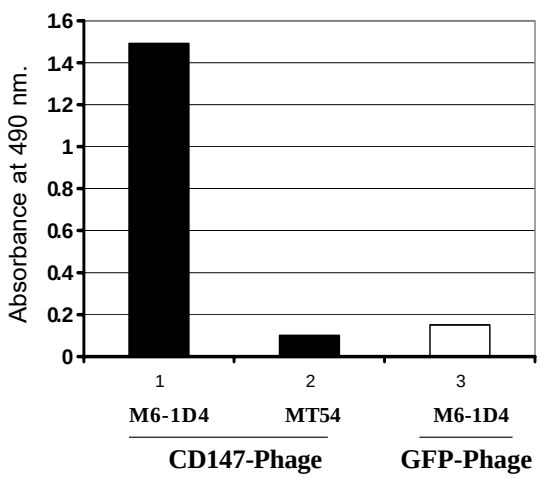

Fig 1. Sandwich ELISA for detection of phage bearing CD147. Solid phase was coated with M6-1D4 (anti-CD147 mAb) or isotype-matched MT54 (anti-CD54 mAb). The bound phages were traced with horseradish peroxidase-conjugated sheep anti-M13 monoclonal antibody.

Comparison of the induction of specific antibody response by pCDM8-CD147 and phage-displayed CD147 immunization

Plasmid CDM8-CD147 or phage-displayed CD147 were injected into two BALB/c mice at bi-weekly intervals. For plasmid DNA immunization, 100 mg of pCDM8-CD147 were intramuscularly injected. For phage immunization, $4x10^{11}$ pfu of phage-displayed CD147 were immunized intraperitoneally using Freund's adjuvant. The anti-CD147 antibodies generated in the immunized mice were determined by indirect ELISA using soluble CD147-IgG fusion protein as antigen. By both immunization procedures, anti-CD147 antibodies could be detected after the 2nd immunization and increased significantly after the 3rd immunization (Fig. 2). However, the antibody titers induced by phage-displayed CD147 immunization were much higher (10-20 fold) and remained at the high level for a longer time than those obtained with pCDM8-CD147 immunization (Fig. 2). To confirm the specificity of the induced antibodies, immunofluorescent analysis was carried out using a CD147 expressing cell line. As shown in Figure 3, CD147 expressing BW5147 were positive with immunized mouse sera and negative with pre-immune sera. As predicted, immunization with phage-displayed CD147 gave stronger positive reactivity (fluorescent intensity) compared to the

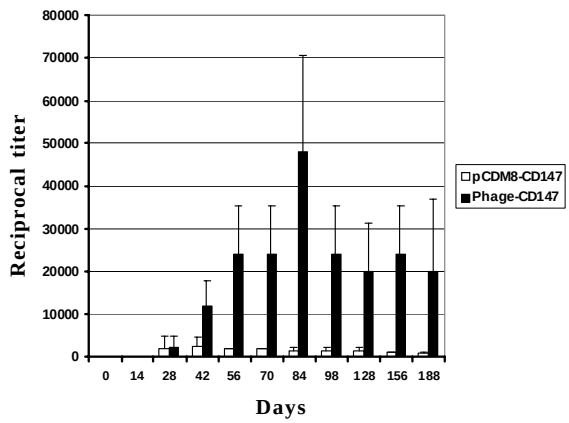

Fig 2. Detection of anti-CD147 antibodies in immunized sera by indirect ELISA. Mice were immunized with pCDM8-CD147 or phage-displayed CD147 at day 0, 14 and 28. Two mice were used for each type of immunization. Sera were collected before immunization (day 0) and at the indicated days. Mean values of antibody titers and SD are shown.

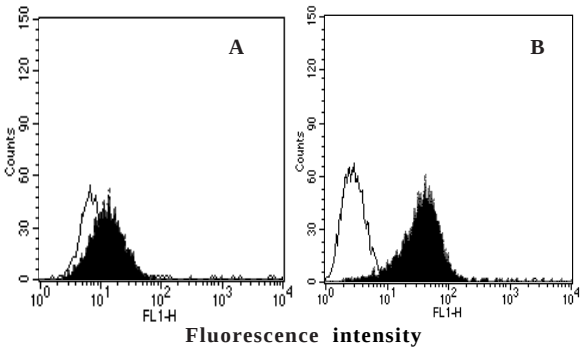

Fig 3. Flow cytometry analysis of anti-CD147 antibodies. CD147 expressing BW5147 cells were stained with pre-immunized sera or day 70 sera of mice immunized with pCDM8-CD147 (A) and phage-displayed CD147 (B). Open peaks represent the BW cells stained with pre-immune serum. Shaded peaks represent cells stained with day 70 immune mouse serum. Y-axis shows the relative cell number, X-axis shows log fluorescence intensity. Results are representative of the 2 mice studied.

pCDM8-CD147 immunization. These data correlated with the results obtained from the indirect ELISA assay indicating a higher CD147 antibody titer in sera of mice immunized with phage-displayed CD147.

The difference in antibody titers induced by DNA and phage-displayed polypeptide immunization may be due to the different mechanisms of antibody induction. By DNA immunization, naked DNA is introduced leading to *in vivo* expression of the gene encoding exogenous protein[1,2]. The quantity of foreign protein expressed in mice depends upon the effectiveness in uptake of the immunized plasmid DNA and the efficiency of *in vivo* transcription and translation in the cell type involved. In contrast, using the phage display system, the protein of interest is expressed *in vitro* before immunization. Thus, immunization of protein expressed on phage particle is comparable to the conventional protein immunization. Phage display carrier systems have been demonstrated to elicit strong immune responses as they can increase the half-life of the peptide in the circulation and provide T-cell help for the induction of a strong antibody response[7]. In addition, the large size of the phage particle increases the potential of immuno-surveillance to discover the displayed immunogen. These reasons may explain why the antibody responses observed in phage immunization were higher and sustained for a longer period of time than DNA immunization. The strategy of phage-displayed polypeptide immunization has high potential in producing antibodies against small peptide fragments regardless of chemical conjugation with the carrier proteins. This technique is useful and can be applied for the production of both polyclonal and monoclonal antibodies against molecules of interest.

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