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The synthesis of a panel of human monoclonal antibodies with neutralizing activities against viral infectious diseases

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Overall Procedures For Constructing Phage Display Library And Isolation Of Monoclonal Human Fabs

In the technique of phage display, which was first demonstrated in the 1990s, foreign proteins are expressed at the tip end of a phage developed for effective screening of precious peptides with biological activities in various libraries [9]. The technique consists of several steps which mimic a series of events for the selection of a useful antibody *in vivo*. Potentially therapeutic antibodies against important viral pathogens have been isolated from humans and non-human primates utilizing this method [4-6]. An outline of procedures for constructing human Fab libraries on phagemid vector pComb3H and subsequent selection of phages displaying promising human Fab have been summarized elsewhere [1, 2, 6, 7]. Briefly, lymphocytes from peripheral blood or bone marrow are separated and mRNA is reverse-transcribed into cDNA using oligo (dT) as a primer. The κ light-chain DNA product is amplified from cDNA by PCR with seven pairs of human κ light-chain family-specific 5' primers and a 3' primer in the constant domain. The V_HC_H1 heavy-chain DNA product is amplified using nine human V_HC_H1 heavy-chain-family-specific 5' primers plus a human V_HC_H1-specific 3' primer. Amplified κ light-chain DNA fragments are pooled and digested with restriction enzymes and then cloned into pComb3H phagemid vector. A phagemid containing the κ light-chain DNA inserts is prepared from electrocompetent *Escherichia coli* XL-1 Blue transformants and then cleaved with enzymes for insertion with amplified V_HC_H1 heavy-chain DNA fragments. The phagemid containing both the light-chain and heavy-chain DNA inserts is used for the transformation of *E. coli* XL-1 Blue by electroporation. The bacterial culture is infected with helper phage VSCM13 to generate the phage library. The phage library is then panned by affinity binding on various antigens in order to enrich phage clones displaying Fab which specifically bind to particular antigens. Following several rounds of panning, the selected phage mixture is used to infect *E. coli* XL-1 Blue to produce soluble human Fabs. The human Fab molecules produced from *E. coli* transformants are tested for biological activity by ELISA and/or neutralizing assay. Overall procedures necessary for the technique are summarized and depicted in the Figure.

A Series Of Human Monoclonal Antibodies With Neutralizing Potencies Against Several Viruses

A series of human monoclonal antibodies with virus-neutralizing potencies, principally Fab molecules, has been isolated in our group.

1) Human monoclonal Fabs with neutralizing activities against rabies virus.

A combinatorial human Fab library was constructed using RNAs from peripheral blood lymphocytes (PBLs) obtained from rabies virus hyper-immune volunteers on pComb3H phagemid vector. The Fab preparation EP5G3 exhibited neutralizing activity against strain CVS with an infected cell count reduction of 76% at dilution of 1:2, and of 20% at a dilution of 1:4. The Fab preparation GD2D12

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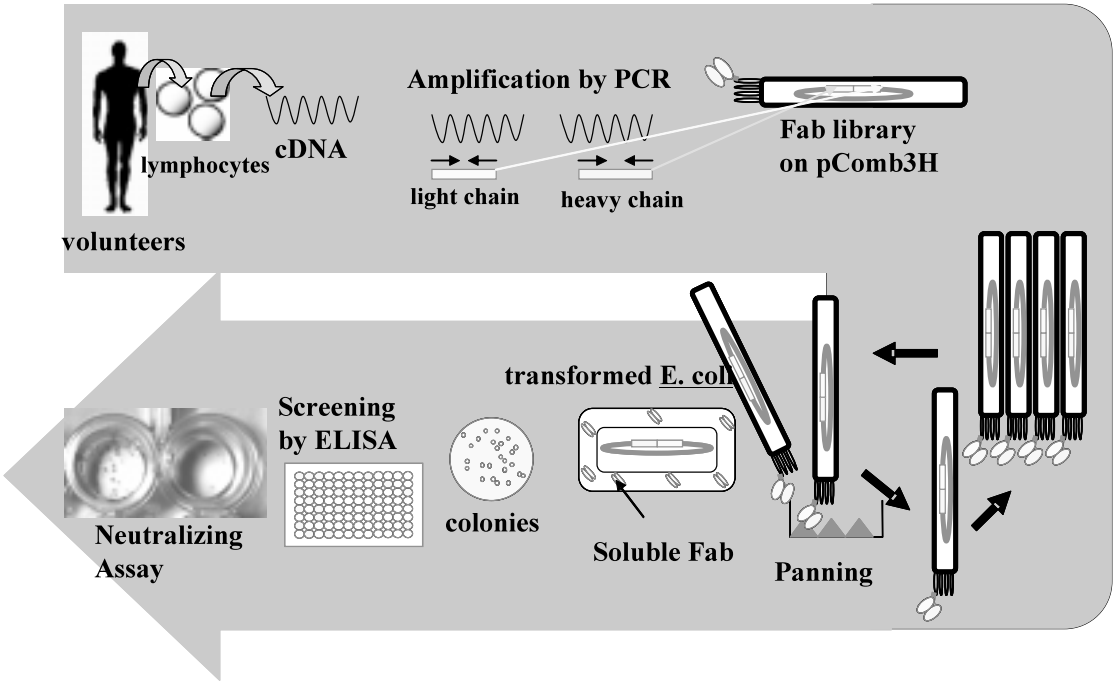

Figure 1 Lymphocytes are separated and mRNA is reverse-transcribed into cDNA. The light- and heavy-chain DNA are amplified from cDNA by PCR. Amplified light- and heavy-chain DNA fragments are pooled and digested with enzymes and then cloned into pComb3H phagemid vector. The phagemid is used for the transformation of *E. coli* by electroporation. The bacterial culture is infected with helper phage to generate the phage library. The phage library then is panned on various antigens in order to enrich phage clones displaying Fab specifically binds to particular antigens. Following several rounds of panning, the selected phage mixture is used to infect *E. coli* to produce soluble human Fab. The human Fab molecules are tested for biological activities.

also exhibited neutralizing activity with a 57% reduction at 1:2 and 41% at 1:4, when assayed by the rapid fluorescent focus inhibition test [1].

2) Human monoclonal Fabs with neutralizing activities against Japanese encephalitis virus.

Using RNAs from peripheral blood lymphocytes, we obtained PBLs from Japanese encephalitis virus (JEV) hyper-immune volunteers, and a combinatorial human Fab library was constructed on pComb3H phagemid vector. The library was panned 3 times on the purified JEV virion or on the JEV vaccine, and phage clones displaying JEV antigen-specific Fab were enriched. One purified Fab molecule isolated from the library showed the 50% focus reduction endpoint at the concentration ca. 50 μg/ml against JEV strain Nakayama (unpublished data). The Fab molecule recognized E or PrM protein of JEV strain Nakayama. One molecule isolated from the same library demonstrated the 50% focus reduction endpoint at a concentration much lower than the first one against JEV strain Nakayama (unpublished data). The second molecule recognized the E protein of JEV strain Nakayama.

3) A human monoclonal Fab with neutralizing activities against Influenza virus type A strain.

RNAs from PBLs of a volunteer who had recovered from influenza were used for Fab library construction. One Fab molecule isolated from the library showed a neutralizing activity against an influenza A (H3N2) strain with 50% plaque reduction neutralization test titer at much less than 1.0 μg/ml. However, it failed to show any neutralizing activities against an influenza virus A (H1N1) strain or an influenza virus B strain (unpublished data).

4) Human monoclonal Fabs with neutralizing activities against Dengue virus type 3 strain.

PBLs obtained from volunteers who had recovered from severe dengue virus infection were used for library construction. Two Fab molecules isolated from the library showed neutralizing activities against dengue virus type 3 strain with 50% focus reduction neutralization test titer at ca. 10 μg/ml and at a lower concentration (unpublished data).

THE CONCEPT OF HUMAN MONOCLONAL ANTIBODY BANK

Phage display is a molecular technique in which the

use of bacteriophage displaying a library of Fab or scFv antibody fragments on the surface has been proven efficient for the isolation of a diverse set of human monoclonal antibodies from immune or non-immune volunteers to a variety of infectious diseases [3, 8]. Potentially therapeutic or shortterm prophylactic antibodies against important viral pathogens for emerging and re-emerging infectious diseases (ERID) will be made available using the technique. The concept for establishing a human monoclonal antibody bank proposed by our group calls for the compilation of a series of human monoclonal antibodies with neutralizing potencies against several viruses and their critical DNA sequences. One molecule among the molecules to be compiled in the bank may prove valuable for passive immunoprophylaxis or therapy for humans with ERID.

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