Beyond hybridoma technology: production of monoclonal antibodies by phage display

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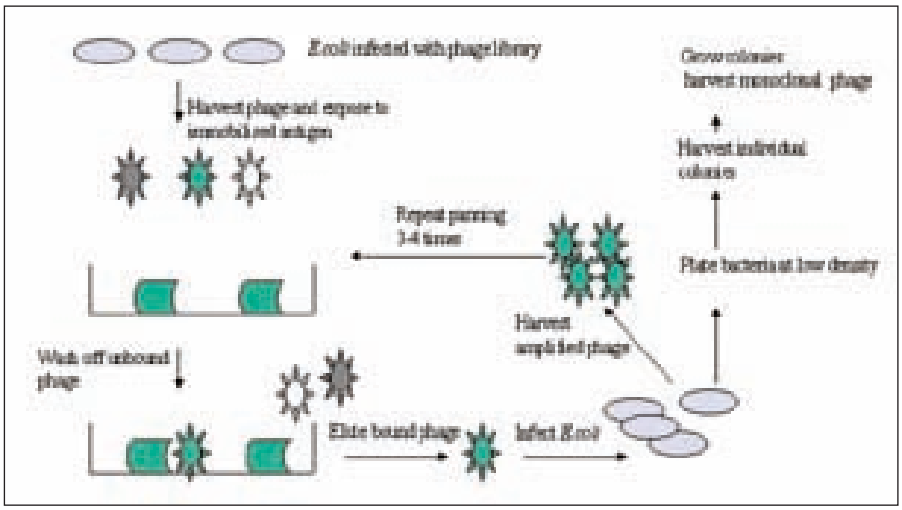
Pamela Greenwell and Sanjiv Rughooputh of the Molecular and Microbial Glycobiology Research Group at the School of Biosciences, University of Westminster, hope to run workshops in this new method of monoclonal antibody production.

Phage display technology using filamentous bacteriophage offers a new way of selecting specific antibodies from an antibody library. A library of large diversity can be constructed using a human naïve repertoire, circumventing the need for immunisation. This method allows the isolation of monoclonal antibody fragments against a large range of different antigens, including peptides, proteoglycans, proteins, nucleic acids and sugars and even cell surface antigens/receptors of organisms. Phage display is a quick and inexpensive method of antibody production. Since no immunisation is required, it overcomes the problem of having an animal house and a Home Office licence. The availability of libraries comprising human antibody encoding genes implies that fragments isolated may have not only diagnostic but also therapeutic uses. Antibody production using traditional hybridoma technology takes a minimum of 5-6 months; phage display on the other hand allows isolation of antibodies within three weeks. More interesting from our point of view is the ability to isolate various antibodies from a 'single pot' and having similar selection running simultaneously to act as control.

Preparation of a Phage Library

Blood is collected from a pool of healthy individuals, lymphocytes are harvested and mRNA is extracted from them. Reverse transcriptase PCR (RT-PCR) using specific primers then amplifies the specific antibody variable regions. Kits are available to enable these fragments to be manipulated and subcloned into 'phage display vectors' to produce a library. Each phage molecule will carry DNA encoding a light and heavy chain fragment. The system is designed so that expression of the antibody genes results in the production, in the Tg1 strain of *Escherichia coli*, of antibody fragments fused to the pIII protein of the bacteriophage.



Panning for antibodies

Panning methodology for antigen selection

Since the library is designed so that each phage expresses an individual antibody fragment, we need to select for those expressing antibody of interest. As the pIII protein is expressed on the surface of the phage itself, we can select for phage expressing antibody of interest using a simple panning technique.

The *E coli* is infected with phage and the broth culture is incubated in a shaking waterbath to allow the phage to infect and multiply in *E coli*. The phage is then harvested purified and added to immobilised antigen of interest and incubated for several hours. After incubation, the suspension is poured off, and the antigen washed to remove the unbound phage. The bound phage, expressing the antibody of interest, is then eluted and amplified by growing in *E coli*.

The process of panning is repeated three to four times. In the final round of panning, the phage is used to infect *E coli* and then these infected cells are plated at low density to allow individual clones to be harvested: one colony contains the progeny of one bacterium which in turn carries one phage and hence one antibody type – a monoclonal antibody. These isolated colonies can be used to mass produce the phage display antibodies which may be used directly in antigen detection. However, the presence of the phage can render these fragments sticky and, therefore, soluble antigen may be more desirable.

Production of soluble antibodies

The library is constructed to carry an amber mutation; that is, instead of reading through the antibody sequence immediately into pIII to form a fusion protein, the translation stops at the amber mutation provided specific bacterial hosts are used. It is therefore possible to make secreted fragments; these are soluble antibodies. The product has a myc tail to aid detection; antibodies to myc are available for use in immunoassay.

Avidity

The antibodies produced in un-immunised humans are immature and may not

be as avid as those derived from an immunised individual. However, once the phage of interest has been isolated, Taq polymerase without proof-reading capacity can be used in the polymerase chain reaction to randomly introduce base pair changes into the antibody sequence. The PCR products can be re-cloned into the phage, amplified, purified and selected by panning. Individual phages can then harvested, their antibody products being made as soluble fragments and tested for avidity and affinity. The most appropriate antibody may then be selected and grown in bulk.

Achievements and scope

We have already isolated monoclonal antibodies recognising cell surface antigens of several organisms involved in sexually transmitted disease. A colleague from the French West Indies isolated 14 antibodies recognising a clam-specific protein in one month spent in our laboratories. We are now working to produce a robust system with a view to running practical workshops in phage display technology. We would envisage participants arriving with their antigen and two weeks later returning to their laboratory with antibodies. Have we whetted your appetite? Then why not contact us for more information by emailing greenwp@wmin.ac.uk. ■

References

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