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INTRODUCTION

Epidemiological evidence suggests that pregnancy at an early age and multiparity provide protection against the development of breast cancer. However, the mechanism(s) of this protection remains unclear. Endocrinological factors have been proposed to play a role. In addition, a few studies have suggested that immunological factors may be involved. We are interested in these latter immunological factors. Our overall objective is to answer the question: Does pregnancy immunize against breast cancer and can this explain, at least in part, the protective effect of pregnancy on breast cancer? Our hypothesis is that immune responses generated against normal breast tissue antigens during pregnancy/lactation protect against subsequent development of breast cancer by targeting the same antigens expressed on breast cancer cells. Our long range goals are to determine how pregnancy might immunize against breast cancer, identify the antigens involved, and use this information for developing novel diagnostic and therapeutic strategies. Identification of these antigens will aid in dissecting the role of immunological factors in the link between pregnancy and breast cancer risk and will also provide novel targets for immunodiagnosics and immunotherapies. If pregnancy is immunizing against breast cancer, then the antigens identified in this study may be ideal candidates for preventative vaccines.

BODY

The specific aim of this project was to identify breast cancer antigens recognized by sera from multiparous women using a novel methodology, SEREX/PhD. Sahin et al. (1997) described a technique for identifying human tumor antigens recognized by autologous serum antibodies. The method, serological analysis of recombinant cDNA expression libraries (SEREX), involves constructing a cDNA expression library from tumor tissue and screening this library with autologous patient serum. The result is a panel of cDNA sequences coding for tumor antigens that are immunogenic in the autologous host.

We have recently developed a modified SEREX technique, SEREX/PhD, that utilizes cDNA libraries displayed on the surface of filamentous bacteriophage (see Figure 1). Our phage display libraries were constructed using the phagemid p66-12, kindly provided by Dr. Nuria Assa-Munt. This vector contains two synthetic anti-parallel leucine zippers to link the protein encoded by the cloned cDNA to one of the phage capsid proteins. We first modified this phagemid to obtain two other phagemids that contained EcoRI, PstI, and XhoI sites in different reading frames (with respect to the leucine zipper that becomes tagged to the protein coded for by the cDNA insert). These two phagemids, along with the p66-12 phagemid, enabled us to obtain libraries in all three reading frames.

We constructed three individual phage display libraries from the RNA obtained from 3 breast cancer cell lines: MDA-MB-231, MCF-7, and SK-BR-3. We initially encountered some technical difficulties in constructing large libraries, but with several modifications of our procedure, we were able to obtain libraries of $2-5 \times 10^6$ independent clones for each cell line. cDNA was prepared using a random RT-PCR technique to amplify RNA obtained from each cell line. The amplified cDNA was digested with either EcoRI, PstI, or XhoI and ligated into the three phagemids. Each ligation mixture was used to transform *E. coli* TG1 cells in five separate electroporations. These were pooled to yield individual libraries from each cell line. Then, aliquots of each cell line library were pooled together to create a master library of $\sim 5 \times 10^6$ transformants that was used for subsequent biopanning.

Our biopanning procedure is depicted in Figure 2. We pooled 8 nulliparous sera together and pre-panned the phage library on IgG captured from this sera pool. This was done to remove any phage in the library that bind to normal human IgG, specifically or non-specifically, as well as phage that might bind to the coating anti-IgG monoclonal antibody. Thus, autoantigens reactive with IgG antibodies found in both multiparous and nulliparous women will be eliminated and any antigens identified after panning on multiparous sera should discriminate the two. The pre-panned phage were then selected on IgG from a pool of 12 multiparous sera. Six rounds of pre-panning on nulliparous IgG and selection on multiparous IgG were performed. Individual phage clones obtained after rounds 4, 5, and 6 were tested in an ELISA for reactivity with multiparous or nulliparous sera. None of the clones obtained showed specific reactivity with the multiparous sera.

Recently, we obtained a commercially available breast cancer phage display library (Novagen). This library was made using a T7 phage system, as opposed to M13 phage used in the library we constructed. We have prepanned this library on nulliparous sera and then selected on multiparous sera, and preliminary results after 4 rounds of selection suggest that we may be obtaining some phage that show specific reactivity with the multiparous sera and not the nulliparous sera.

KEY RESEARCH ACCOMPLISHMENTS

- construction of phage display cDNA libraries from several breast cancer cell lines
- possible identification of specific antigens recognized by multiparous sera but not nulliparous sera using a T7 phage system

REPORTABLE OUTCOMES:

- phage display cDNA libraries from several breast cancer cell lines

CONCLUSIONS:

Although we did not identify any specific antigens from our initial panning with the libraries we constructed, we are continuing to test this library using other sera. On the other hand, we have recently obtained some phage from a T7-phage display system, that show specificity for multiparous sera versus nulliparous sera and we will continue the analysis of these clones.

The results of these continuing studies will be a panel of antigens that elicit immune responses during pregnancy and that are also expressed on breast cancer cells. These antigens will aid in dissecting the role of immunological factors in the link between pregnancy and breast cancer risk and will also provide novel targets for immunodiagnostics and immunotherapies. If pregnancy is immunizing against breast cancer, then the antigens identified in this study may be ideal candidates for preventative vaccines.

REFERENCES:

Sahin, U., Tureci, O., and Pfreundschuh, M. Serological identification of human tumor antigens. *Curr. Opin. Immunol.* 9: 709-716, 1997.

PERSONNEL

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Lonnele Ball, M.S.

Figure 1. Phage display vector for cDNA library construction

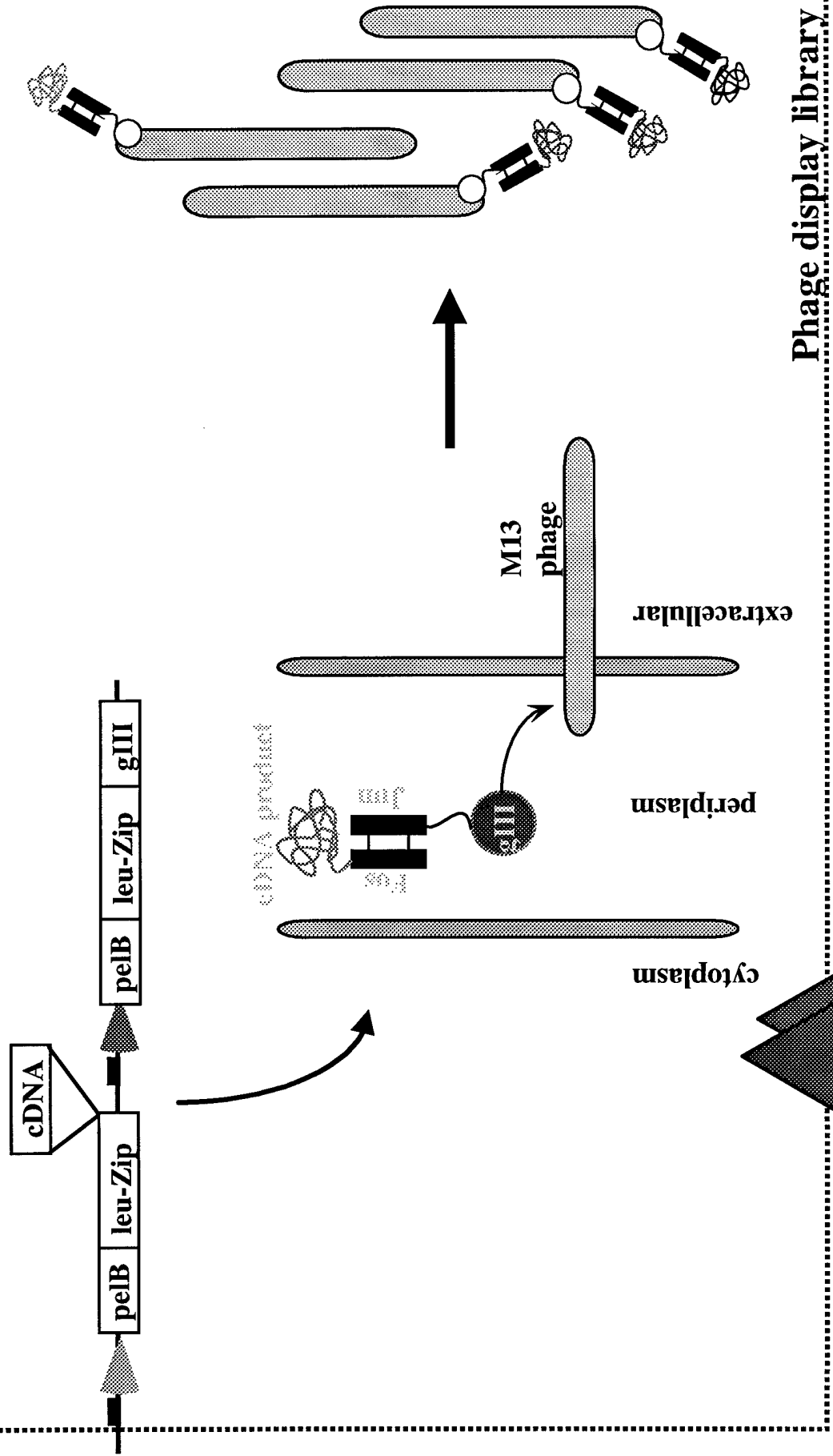


Figure 2. SEREX Phage Display (SEREX/PhD). Prepanning phage library of tumor antigen cDNAs on nulliparous sera (SEREX/PhD). Prepanning phage library of tumor antigen cDNAs on nulliparous sera IgG followed by selection on multiparous IgG.

