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Los Alamos
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Competency Development in Antibody Production in Cancer Cell Biology

Min S. Park*

Abstract

This is the final report of a three-year, Laboratory Directed Research and Development (LDRD) project at Los Alamos National Laboratory (LANL). The main objective of this project was to develop a rapid recombinant antibody production technology. To achieve the objective, we employed (1) production of recombinant antigens that are important for cell cycle regulation and DNA repair, (2) immunization and specific selection of antibody-producing lymphocytes using the flow cytometry and magnetic bead capturing procedure, (3) construction of single chain antibody library, (4) development of recombinant vectors that target, express, and regulate the expression of intracellular antibodies, and (5) specific inhibition of tumor cell growth in tissue culture. We have accomplished (1) optimization of a selection procedure to isolate antigen-specific lymphocytes, (2) optimization of the construction of a single-chain antibody library, and (3) development of a new antibody expression vector for intracellular immunization. The future direction of this research is to continue to test the potential use of the intracellular immunization procedure as a tool to study functions of biological molecules and as an immuo-cancer therapy procedure to inhibit the growth of cancer cells.

Background and Research Objectives

Antibodies provide human and other animals with immune defense against a wide range of antigenic substances, most notably viruses and bacteria. Since the turn of the century, biologists have exploited the natural immune response of laboratory animals to produce antibodies for use in the diagnosis and prophylactic/therapeutic management of numerous diseases. In the area of modern cell biology, antibodies also are being used to understand the role(s) proteins play in complex biological processes. Until recently, the production of antibodies has been limited to labor intensive, time-consuming, and expensive methods involving animal immunizations, the harvesting of immune cells, and the immortalization of antibody producing B cells (hybridoma cells). By using cutting-edge DNA recombinant and gene amplification technologies, it has now become possible to clone antibody genes into bacteria, which then serve as "factories" for the production of highly specific antibodies against proteins of interest. Such artificial "immortalizations" of

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antibody genes make it technically feasible to produce antibodies quickly in bacterial cultures, and to genetically manipulate their structures so that antibodies to any antigen may be constructed *in vitro*. Thus, the new technology has the potential power and versatility to mimic the features of immune diversity and selection that are hallmark features of humoral immunity *in vivo*, thereby rendering conventional approaches for antibody production obsolete. Furthermore, the use of an intracellular immunization procedure to study functions of given biomolecules opens a new door to study cell biology with convenience and power. The immuno-cancer therapy using the recombinant antibody technology will open a new door to cancer therapy. The main research objectives of the project were 1) to establish a rapid and effective production of recombinant antibodies, 2) develop a new method to express intracellular antibodies inside of cells, and 3) to inhibit the growth of tumor cells using the intracellular antibodies.

Importance to LANL's Science and Technology Base and National R&D Needs

LANL's core competency in bioscience and biotechnology brings together multi-disciplinary capabilities for the study of life processes, living organisms, and human health. Specific technologies and capabilities that contribute to this competency are: a) biomedical research and technology, b) cellular analysis, c) biomolecular structure, dynamics, and function, and d) genome analysis. The new competency in new antibody production technology will substantially strengthen the Laboratory's expertise in the areas of biomedical research and cellular analyses, and it will further enhance LANL's capabilities in all four of the above areas. For example, techniques used for producing antibodies, such as protein engineering, will directly benefit researchers involved in biomolecular structure, function, and dynamics by providing technical expertise in recombinant protein expression and purification. New, genetically engineered antibody molecules and their ligands will be available to theoreticians and biomedical researchers as potential model systems to predict molecular interactions and to rationally design antibodies for tumor therapy.

In our new "Cancer Risk Assessment" initiative, the development of multiparameter flow cytometric assays of cancer susceptibility gene products is completely dependent on the availability of large quantities of high quality antibodies for assessing the roles translated gene products play in determining cancer risk to environmental carcinogens. Due to the broad and crucial applications of antibodies to so many scientific areas here at LANL, the significance and relevance of a competency in antibody production cannot be over-

emphasized. Historically, the Life Sciences Division has made numerous contributions to understanding of fundamental alterations in cancer cells.

The DOE has recently announced a restructuring of its Health Effects Research Program. Two major objectives of the restructured program are: 1) to elucidate the mechanisms by which radiation and other energy-related agents affect biological systems, and 2) to develop new approaches for understanding the basis of individual susceptibilities to diseases, especially cancer, caused by exposures to these agents. In order to optimally position LANL to pursue these objectives, the Cell Biology efforts in the LS Division are being redirected to develop biotechnological methods for assessing the risk of cancer development in individuals who have been or may be exposed to carcinogenic insults.

Inherent to our new programmatic direction is the need to develop a thorough understanding of fundamental mechanisms that underlie cellular stabilization and destabilization following exposures to environmental carcinogens, including mechanisms of DNA repair, cell cycle checkpoints, and cell cycle regulation. It is anticipated that this effort will lead to the development of mechanistically-based biomarker assays for human cancer risk assessment. As the Human Genome evolves beyond the determination of DNA sequences, antibodies will be required for structure/function analyses of newly discovered disease genes. Additionally, antibodies can be used as powerful reagents to determine the functions of genes identified by random sequence search.

Scientific Approach and Accomplishments

New recombinant antibody technology approaches rely on bacterial protein production systems in which antibody genes are expressed. Using the polymerase chain reaction (PCR), immunoglobulin genes are first amplified from antibody producing cells. The antibody genes are subsequently cloned in expression vectors and antibody proteins are produced in bacteria or other eukaryotes. This new technology, in conjunction with our advanced in-house capabilities in flow cytometry, overcomes several limitations of conventional antibody production technology. For example, antibodies can be more rapidly and easily cloned and screened. The cell fusion and extensive screening associated with conventional monoclonal antibody production is both time consuming and labor-intensive. Alternatively, specific antibody genes can be cloned directly from peripheral blood or spleen cells with rapid and direct gene amplification methods. With our advanced technology in flow cytometry, we have selectively identified cells producing correct antibodies from background populations. This was done first by directly conjugating a given antigen-protein with a fluorescent tag, and then identifying and sorting cells that are

producing the desired antibody for subsequent cloning. The time from isolation of spleen cells to established clones was reduced from 4 months to 6 weeks.

We have successfully cloned DNA repair genes and engineered them into appropriate protein expression vectors. From these recombinants, we have produced large quantities of several DNA repair and cell cycle proteins, including GADD45, Ku-70, RAD51, RAD52, XPG, and FEN-1. These proteins have been purified to near homogeniety and used to immunize mice. We were able to produce high quality mouse polyclonal antibodies specific to FEN-1, RAD51, XPG, and RAD52. We empirically determined and established a protocol/schedule to produce recombinant antibodies. It took only five weeks to isolate and purify antigen-specific B cell population and prepare RNA for antibody gene cloning. The antibody genes that encode heavy and light chains of FEN-1 and RAD52 antibody were produced and ready to be combined to produce recombinant antibodies.

In order to pursue a new approach for the immuno-cancer therapy, we have developed a new mammalian expression vector than can produce antibody molecules in cells. This vector provides a controllable expression of antibody molecules within the cell, which allows neutralization of biologically functional molecules in the cell. If we can produce antibody to block the proliferation of cancer cells by inhibiting an enzyme that is essential for replication or cell division, we should be able to block the growth of tumor. We have made progress in this direction by generating antibody gene librararies that are specific to human FEN-1, which is essential for replication. In the near future, we should be able to test the applicability of intracellular antibody technology as a new way of immunocancer therapy.

Future work

Areas to be pursued in the future include:

- Production of recombinant antibodies in bacteria.
- Initiation of protein engineering and modification of antibody molecules to develop strong catalytic antibodies.
- Development of controllable promoters and vectors for tight regulation of expression of antibody genes in tumor cells.
- Controlled expression of antibody genes specific to DNA repair and cell cycle control to regulate the DNA repair capacity and cell growth.
- Validation of the potential of antibody gene therapy for the control of tumor cells in proof-of-principle experiments; seek industrial partners interested in novel forms of cancer therapy.

- Employment of chromophore-conjugated antibodies for the development of "user friendly", flow cytometric biomarker assays to assess human cancer risk to carcinogens.
- Submission of a new DOE or NIH proposal in the area of new antibody technology development and/or antibody gene therapy.

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