Lec(3) Advanced Serology

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**MONOCLONAL ANTIBODY**

The knowledge that B cells are genetically preprogrammed to synthesize very specific antibody has been used in developing antibodies for diagnostic testing known as **monoclonal antibodies.** Normally, the response to an antigen is heterogeneous,because even a purified antigen has multiple epitopes that stimulate a variety of B-cell clones. In 1975,Georges Kohler and Cesar Milstein discovered a technique to produce antibody arising from a single B cell,which has evolutionized serological testing. For their pioneering research, they were awarded the Nobel Prize in 1984.Kohler and Milstein’s technique fuses an activated B cell with a myeloma cell that can be grown indefinitely in the laboratory. Myeloma cells are cancerous plasma cells.Normally, plasma cells produce antibody, so a particular cell line that is not capable of producing antibody is chosen. In addition, this cell line has a deficiency of the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) that

renders it incapable of synthesizing nucleotides from hypoxanthine and thymidine, which are needed for DNA synthesis.

**Hybridoma Production**

A mouse is immunized with a certain antigen, and after a time, spleen cells are harvested. Spleen cells are combined with myeloma cells in the presence of polyethylene glycol(PEG), a surfactant. The PEG brings about fusion of plasma

cells with myeloma cells, producing a **hybridoma.** Only a small percentage of cells actually fuse, and some of these are like cells—that is, two myeloma cells or two spleen cells.

After fusion, cells are placed in culture using a selective medium containing hypoxanthine, amino pterin, and thymidine (HAT). Culture in this medium is used to separate the hybridoma cells by allowing them to grow selectively .Myeloma cells are normally able to grow indefinitely in tissue culture, but in this case they cannot, because both pathways for the synthesis of nucleotides are blocked. One

pathway, which builds DNA from degradation of old nucleic acids, is blocked, because the myeloma cell line employed is deficient in the required enzymes HGPRT and thymidine kinase. The other pathway, which makes DNA from

new nucleotides, is blocked by the presence of amino pterin.Consequently, the myeloma cells die out. Normal B cells cannot be maintained continuously in cell culture, so these die out as well. This leaves only the fused hybridoma cells,

which have the ability (acquired from the myeloma cell) to reproduce indefinitely in culture and the ability (acquired from the normal B cell) to synthesize nucleotides by the HGPRT and thymidine kinase pathway **(Fig. 1).**

**Selection of Specific Antibody-Producing Clones**

The remaining hybridoma cells are diluted out and placed in microtiter wells, where they are allowed to grow. Each well, containing one clone, is then screened for the presence of the desired antibody by removing the supernatant.

Once identified, a hybridoma is capable of being maintained in cell culture indefinitely, and it produces a permanent and uniform supply of monoclonal antibody that reacts with a single epitope.



**Fig1:** Formation of a hybridoma in monoclonal antibody production. A mouse is immunized, and spleen cells are removed. These cells are fused with non secreting myeloma cells and then plated in a restrictive medium. Only the hybridoma cells will grow in this medium,where they synthesize and secrete a monoclonal immunoglobulin specific for a single determinant on an antigen.

**Clinical Applications**

Monoclonal antibodies were initially used for in vitro diagnostic testing. A familiar example is pregnancy testing, which uses antibody specific for the chain of human chorionic gonadotropin, thereby eliminating many false-positive reactions.

Other examples include detection of tumor antigens and measurement of hormone levels .Recently, however, there has been an emphasis on the use of monoclonal antibodies as therapeutic agents. One of the biggest success stories is in the treatment of two autoimmune diseases: rheumatoid arthritis and Crohn’s disease (a

progressive inflammatory colitis). Both of these diseases have been treated with a monoclonal antibody called inflixmabthat blocks the action of tumor necrosis factor-alpha.

Another tumor necrosis factor blocker, adalimumab (Humira), has also proven effective in decreasing symptoms of these two diseases .Monoclonal antibodies have also been used to treat various types of cancers. In the case of metastatic breast cancer ,trastuzumab (Herceptin), an antibody directed against HER-2/neu protein, which is present in large numbers on tumor cells, has been helpful in slowing the disease’s progress. Another example is rituximab (Rituxan), used to treat non-Hodgkin lymphoma. Other monoclonal antibodies approved by the FDA include cetuximab (Erbitux) to treat colorectal cancer and head and neck cancers and bevacizumab (Avastin) to treat colorectal, non-small lung, and breast cancers. Additionally, some monoclonal antibodies are conjugated with radioactive substances that are delivered directly to cancerous cells. Drugs in this category

include ibritumomab tiuxetan (Zevalin), for cancerous B lymphocytes, and to situmomab (Bexxar) to treat some non- Hodgkin lymphomas that no longer respond to rituximab. The fact that monoclonal antibodies can now be humanized by recombinant technology has cut down on reactions to the reagents themselves, which used to be of mouse origin. This area of research in pharmacology is

rapidly expanding and is likely to continue to grow in the future.

 **Uses of Monoclonal Antibodies**

The greatest impact of MAbs in immunology has been on the analysis of cell membrane antigens. Because they have a single specificity rather than the range of antibody molecules present in the serum, MAbs have multiple clinical applications, including the following:

• Identifying and quantifying hormones

• Typing tissue and blood

• Identifying infectious agents

• Identifying clusters of differentiation for the classification

of leukemias and lymphomas and follow-up therapy

• Identifying tumor antigens and autoantibodies

• Delivering immunotherapy .

**IMMUNOTHERAPY**

**Immunotherapy** : the another type for treatment of tumor immunology.

The possibility of stimulating the patient’s own immune system to respond to tumor-associated antigens has long intrigued scientists. Immunotherapeutic methods used can be separated into two types: passive or active immunotherapy.

 Passive immunotherapy involves transfer of antibody, cytokines, or cells to patients who may not be able to mount an immune response. With active immunotherapy, patients are treated in a manner that stimulates them to mount immune responses to their tumors.

**Passive Immunotherapy**

Passive transfer of allogeneic cellular immunity from one person to another to fight cancer has many barriers because of possible recipient rejection of foreign cells, graft-versus host disease (GVHD), and the fragility of live cells, Inducing a patient’s own cellular immunity is far more likely to be successful, However, a form of GVHD called **graft versus leukemia** has been demonstrated with transfer

of allogeneic T cells and is associated with improved patient prognosis. Therefore, successful passive transfer of anticancer T cells is theoretically possible .Adaptive T-cell therapy has been attempted using several models. For example, T cells from allogeneic donors can be immunized against tumors. After recipients are immunosuppressed to prevent rejection and to eliminate T suppressor mechanisms, they receive the T cells. One strategy in this model to treat GVHD is to genetically engineer the allogeneic T cells with a

 1-“suicide switch.” This gene makes the cells vulnerable to a drug that will immediately eliminate them in the event of GVHD.

Other strategies for adaptive T-cell therapy use autologous T cells.

2-Tumor-infiltrating lymphocytes (TILs) can be harvested and expanded in vitro using IL-2. The patient is then lympho depleted to remove T suppressor cells, and high

concentrations of TILs are transfused. Similarly, autologous T cells can be harvested, exposed to cancer antigens, expanded with IL-2, and then returned to the patient. Attempts have also been made to insert genetically engineered T-cell receptors into autologous T cells.

3-Passive transfer of antibody to treat cancer almost always employs monoclonal antibodies. “Naked” monoclonal antibodies

against cancer could induce antibody-dependent cell-mediated cytolysis (ADCC), complement-mediated lysis, or opsonization. If the antibodies are directed toward

particular receptors, they could trigger a desirable action in the cell such as inducing apoptosis or inhibiting growth signals

4. **Antibody conjugates,** or **immunotoxins,** are antibodies conjugated to toxins or radioisotopes on the premise that they can kill cancer cells while leaving adjacent cells intact. In order for these techniques to work, the following are required:

• Antibodies must be directed at a cell surface antigen with high density.

• The antigen must be present on the primary tumor and on all metastatic foci.

• Normal tissues must be free from the antigen or not susceptible to the toxin.

• The antibodies must have sufficient access to the tumor tissue (i.e., the tumor burden is not such that certain portions escape exposure to the antibodies).

The obstacles involved in immunotherapy with antibodies are:

• tumor heterogeneity with regard to antigenic expression;

• antigenic modulation or the loss of antigen from the tumor cells.

• failure of antibodies to penetrate tumor tissue.

• failure of antibody–toxin conjugates to internalize into the cell after binding and release toxin.

• toxic effects on other tissues, particularly the hematopoietic organs.

• the limited amount of toxin that can be linked to the antibody without destruction of binding activity.

• host immune response to the injected antibody.

• circulating antigen forming immune complexes with the antibodies, removing them from circulation.

 antibodies in patients receiving therapy is a significant interference. The chimeric antibodies splice animal (usually murine) variable Fab onto human FC for a final composition of about 25 percent animal and 75 percent human.“Humanized” antibodies splice the portion of animal antibody required for epitope binding to human antibody for a final composition of about 5 percent animal and 95 percent

human. This improves antibody half-life, which is naturally long in the absence of heterophile antibodies. Long antibody half-life is desirable for therapeutic effects, but it also prolongs negative effects. This is particularly a problem for antibodies conjugated to radioisotopes, which cause bone marrow toxicity and myelo suppression.

Currently, immunotherapeutic antibodies are most effective against hematologic malignancies, small tumors, and micro metastases, not bulky tumors. Antibodies poorly penetrate tumor mass, because they are large molecules, and they bind to the first available antigen encountered on the outside of the tumor. Surgical removal or de bulking of a tumor may ideally precede the use of antibodies. Further,since there is usually more than one path for cell growth, antibodies are rarely, if ever, used alone. Combination is usually recommended .Radiolabeled antibodies are particularly effective because of the “bystander” or “crossfire” effect seen with high energy isotopes that can kill up to several hundred cells.This is particularly advantageous for tumors with heterogeneous antigen expression and could allow antibodies to kill cells that are not expressing antigen at all. An additional use for radiolabeled antibodies is imaging studies to locate foci of cancer. However, their use must be managed to minimize bone marrow toxicity, although this toxicity is generally reversible.

**Active Immunotherapy**

The goal of active immunotherapy is to have the patient develop an immune response that will help eliminate the tumor. Nonspecific stimulation by adjuvants such as Bacillus Calmette Guerin (BCG) was first attempted, and superficial

bladder cancer is still treated with BCG. Improved technology has allowed the production of novel adjuvants and selective use of stimulatory cytokines (TNF-α, IFN-γ,IL-1, IL-2, and so on) in immune competent patients to enhance the natural antitumor response and the artificial vaccine-induced response.

Other attempts at stimulating host immune systems have involved transfection of normal cells or isolated tumor cells with genes for cytokine production and injection of the modified cells into or around the tumor. This has been

done with many cytokines, including TNF-α, interferons,IL-2, and granulocyte monocyte–colony stimulating factor (GM-CSF). Of the cytokines transfected, GM-CSF has shown the most promise.

Cancer vaccines have been of great interest to researchers. When specific viruses are associated with a cancer, vaccine construction is relatively straight forward, since viral antigens are obviously foreign. The vaccine for human papillomavirus

(HPV) to prevent cervical cancer is an excellent example. It is important to note that many viruses have several serotypes ,not all of which may be associated with cancer, so vaccines must be protective against the appropriate epitopes. HPV vaccines, for example, are directed epitopes that prevent initial infection with carcinogenic serotypes but do not help treat established cervical cancer, as these epitopes are down regulated in cancer cells. Therefore, a distinction exists between prophylactic vaccines and therapeutic ones.

In the absence of a viral cause of a cancer, prophylactic vaccination is more difficult, since many of the antigens expressed on tumors, as stated previously, appear to some degree on normal tissue. High-affinity cytotoxic T lymphocytes

against tumor-associated antigens (TAA) are often deleted by tolerance pathways. Further, just the expression of an antigen on a tumor does not automatically make

it a suitable vaccine target, since co expression with MHC I and obligatory accessory molecules is required for adequate T-cell response. Vaccination with tumor lysates and peptide antigen vaccines have had limited success. This is at least partly due to the heterogeneity of antigens presented on tumors and the few “public antigens” common to all tumors and the many “private antigens” unique to an individuals tumor. Designing custom vaccines for each individual is

obviously more difficult than designing more universal vaccines.

Various strategies have been attempted to identify sufficiently

immunogenic antigens and how to present them to T cells. However ,an example is the strategy to prime dendritic cells (DCs),which are particularly potent antigen-presenting cells that can stimulate both humoral and cellular immunity and immune tolerance reactions. The general principle of this technique is that a DC line is cultured from the patient and then exposed to tumor antigen. Antigen exposure may be done using co culture with purified tumor antigens or tumor lysate, fusion of DCs with tumor cells, DC phagocytosis of dead tumor cells, or transfection of RNA. The DCs are reintroduced in the patient by various measures (intravenously,

sub cutaneously, and so on) as a vaccine. Preliminary research has demonstrated some success with these vaccines ,but the details of optimal antigens, DC priming, and modes of DC delivery are still being evaluated .It remains to be seen which, if any, of the numerous immunization protocols currently under study will work.

These protocols will be important adjuncts to traditional therapies in which tumors will first be de bulked and then the immune system will eradicate residual tumor and micro metastases. The increased understanding of tumor immunology in recent years has made this a field of active study and increased optimism.

Q1/ Compare and contrast passive versus active immunotherapy, describing

common techniques used in each.